GENE REGULATORY NETWORK UNDERLYING FUNGAL DEVELOPMENT AND METABOLISM

By

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DISSERTATION ABSTRACT

Fungi are of great importance in human lives and environments, largely because of their diverse roles in the medical area (human pathogens and antibiotics producers), food industries (fermentation and process), agricultural fields (pathogens and growth aids), and environmental recycling. Most filamentous fungi mainly reproduce through asexual sporulation, which generates multicellular reproductive organs and non-motile spores. Interestingly, in some fungi, this main reproductive system is tightly coupled with secondary metabolite production. Several studies in *Aspergillus* species have reported that developmental mutants defective in sexual and/or asexual development coincidentally exhibited a loss of ability to produce some secondary metabolites. This type of genetic link between development and metabolism has been observed in a variety of fungal species, but their full elucidation has not been established yet due to the complexity of gene regulatory networks.

In the most ubiquitous fungi Aspergilli, few regulators govern the development and secondary metabolism at a bona fide upstream molecular level. Among these so-called global regulators, VeA, LaeA, and NsdD are extensively studied in *Aspergillus* species. Despite the pivotal regulatory roles of VeA, LaeA, and NsdD in fungal biology, the detailed molecular mechanisms underlying how these upstream regulators govern fungal development and metabolism simultaneously are not clearly understood yet. This dissertation provides general information about the main upstream regulators of development and secondary metabolism in *Aspergillus* fungi (Chapter 1) and unveils the regulatory roles and mechanisms of NsdD in *A. nidulans* and *A. flavus* (Chapter 2) and the gene regulatory networks of VeA and LaeA in *A. nidulans* (Chapter 3). Overall, this work provides an advance in the knowledge of gene regulatory mechanisms of key upstream regulators governing development and metabolism in *Aspergillus* species.

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"That which does not destroy me, makes me stronger" - *Friedrich Nietzsche*

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Chapter 1: Main Upstream Regulators of Development and Secondary Metabolism in *Aspergillus* Fungi

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1.1 Introduction

Fungi are of crucial importance in human lives and natural environments, largely because of their various roles in medicine production, environmental recycling, food process, and agriculture. Due to these deep influences of fungi, researchers started to investigate metabolic mutants in the filamentous fungus *Neurospora crassa* in 1941 for the first time. Since then, fungi serve as wonderful model organisms for the eukaryotic genetic studies in that fungi are tractable for performing various experiments and abundant genetic research resources are easily accessible (Casselton and Zolan, 2002). Among multitudinous fungi, the genus *Aspergillus* is the most common and ubiquitous fungi, with more than 340 filamentous fungal species have been identified in this genus (Bennett, 2010). Moreover, due to their relevance to the various areas in human health (*A. fumigatus*), industry (*A. niger* and *A. oryzae*), agriculture (*A. flavus*), and genetic research (*A. nidulans*), extensive genetic studies on *Aspergillus* species have been conducted. Based on the previous findings, we herein reviewed regulators act at a bona fide upstream molecular level in governing growth, development, and metabolism of *Aspergillus* species.

1.2 Heterotrimeric G Protein Signaling Governs Development and Metabolism

Sensing the external environment and adapting to surroundings are crucial for fungi to coordinate their growth and development accordingly. Fungi are responsive to environmental clues through the heterotrimeric G protein signaling pathway. G protein-coupled receptors (GPCRs) relay signals from the extracellular environment inside the cell by existing across a cell membrane. These receptors react to a large spectrum of external signals including light, hormones, neurotransmitters, cytokines, growth factors, cell adhesion molecules, and nutrients such as sugars, amino acids, and nitrogen sources (Maller, 2003). Binding to extracellular molecules causes a conformational change in GPCRs and this alteration then activates the interaction between the GPCR and a nearby G protein. When the G protein becomes active by the interaction with GTP, it can trigger the production of thousands of second messenger molecules such as cyclic AMP (cAMP), diacylglycerol (DAG), and inositol 1, 4, 5-triphosphate (IP3) which initiate and coordinate further intracellular signaling pathways (Xue *et al*., 2008). In fungi, G protein-mediated signaling pathways include the cAMP-dependent protein kinase (PKA) and the mitogen-activated protein kinase (MAPK) pathways. Through these sequences of events, fungi regulate their growth, development, morphogenesis, mating, metabolism, virulence, and mycotoxin biosynthesis according to the environments where they are situated (Lengeler *et al*., 2000; Rispail *et al*., 2009).

1.2.1 G Protein-Coupled Receptors (GPCRs)

G protein-coupled receptors (GPCRs) are transmembrane proteins and the largest class of cell surface receptors in fungi. GPCRs are plasma-membrane-localized proteins that communicate changes in the environment to intracellular heterotrimeric G proteins (Xue *et al*., 2008). GPCRs contain seven transmembrane (7-TM) helices connected by intracellular and extracellular loops, with an extracellular amino-terminus (N-terminus) and the carboxyl-terminus (C-terminus) extending into the cytoplasm. Although the majority of GPCRs consist of 7-TM helices, some GPCRs are containing 5- or 6-TM helices such as GprB, GprG, GprN, and NopA. According to the previous studies, *Aspergillus* GPCRs are classified into ten different classes. Eighteen GPCRs named GprA to GprS and NopA belong to Class I to IX and Class X GPCRs are Pth11-like receptors, which promote fungal-plant pathogenic interactions in *A. nidulans* (Table 1-1). GprN was specifically identified in *A. nidulans,* and GprR and GprS were exclusively identified in *A. flavus*, which contain the regulator of G protein signaling (RGS) domain and PQ-loop repeat domain, respectively (Affeldt *et al*., 2014). Despite their prevalence and fundamental roles in fungi, only a few GPCRs have been identified and functionally characterized. In recent years, the rapid development and evolution of Next-Generation Sequencing technology have boosted GPCR studies by enabling accessible large-scale whole genome sequencing, which led to figuring out putative GPCRs in genome based on structural similarities and putative activating ligands. These studies have revealed that the *A. nidulans* genome encodes 86 putative GPCRs, which can be divided into sixteen GPCRs in nine categories (Class I to IX) and 70 class X Pth11-like receptors (Li *et al*., 2007; Brown *et al*., 2018; DeZwaan *et al*., 1999; Dilks *et al*., 2019). The major producer of aflatoxins *A. flavus*' genome encodes fifteen putative GPCRs in nine categories (Class I to IX), and Class X GPCRs still remain to be identified (reviewed in Affeldt *et al*., 2014). In the opportunistic human pathogen *A. fumigatus*, the genome encodes fifteen putative classical GPCRs (Class I to IX), yet only five of them (GprC, GprD, GprK, GprM, and GprJ) have been characterized (Gehrke *et al*., 2010; Jung *et al*., 2016; Filho *et al*., 2020). Functional studies on GPCRs have unveiled that they play significant roles in overall fungal biology relating to nutrient sensing, fungal development, pheromone response, fruiting body formation, mycotoxin production, and pathogenesis. According to functional characteristics, GPCRs can be categorized into 10 groups: pheromone (classes I and II), carbon (III), nitrogen (IV), cAMP receptor-like (V), RGS (Regulator of G protein signaling, VI), MG00532-like (VII), mPR-like (VIII), microbial opsin (IX), Pth11-like (X) receptors.

The pheromone receptors were firstly identified in *Saccharomyces cerevisiae*. Two different pheromone receptors, Ste2p (α-factor receptor) and Ste3p (a-factor receptor), presented in the cell membranes of opposite haploid mating types (MATα and MATa). When yeast cells are exposed to the pheromone secreted by the opposite mating type, their pheromone receptors are activated and initiate G protein-mediated signaling pathway leading to the eventual fusion with the mating partner (Bardwell, 2004). Carbon-sensing receptors regulate the response to carbon sources in fungi. In the filamentous fungus *N. crassa*, GPR-4 (G-protein coupled receptor 4) physically interacts with the Gα (GNA-1) to regulate carbon source-dependent growth and development. The gpr-4 null mutants displayed less mass accumulation compared to the WT in carbon-limited conditions and no transient increase in cAMP levels upon a nutrient shift from carbon-limited to glucose-rich media, which was normally observed in WT (Li and Borkovich, 2006). Nitrogen-sensing receptors act in a very similar way to carbon-sensing ones. In. *Schizosaccharomyces pombe*, the Stm1 receptor, coupling with the Gα2 protein, is required for proper recognition of nitrogen starvation signals. Overexpression of Stm1 led to the inhibition of vegetative growth and the decrease in intracellular cAMP levels even under nutrient-rich conditions (Chung *et al*., 2001). The cAMP receptors (cARs) were firstly identified in *Dictyostelium discoideum* and then the sequences of cARs were used to predict cAMP receptor-like GPCRs (Crls) in *D. discoideum* and other fungal species including *N. crassa*. The cARs are known to play significant roles during divergent developmental stages and in distinct subsets of developing cells within multicellular aggregates by interacting with secreted cAMP (Johnson *et al*., 1992; Insall *et al*., 1992; Swaney *et al*., 2010). The *N. crassa* GPR-1, distantly related to the four cAMP receptors (cAR1 to cAR4) and three cAMP receptor-like GPCRs (CrlA to CrlC), was the first cAMP receptor-like GPCR characterized in ascomycete fungi. In *N. crassa*, GPR-1 is localized in female reproductive structures and regulates female sexual development (Krystofova and Borkovich, 2006). The regulator of G protein signaling was firstly discovered in *Arabidopsis thaliana*. The *A. thaliana* RGS, AtRGS1 protein negatively regulates the Gpa1 Gα subunit affecting cellular proliferation. Canonical GPCRs cause the conformational change of G protein triggering the GDP-GTP exchange, but instead, AtRGS1 interacts with the active Gα subunit resulting in hydrolysis of GTP, which in turn deactivates the G protein (Chen *et al*., 2003). This type of GPCR has been found in several species of filamentous fungi. In *Aspergillus* species, GprK containing both 7-TM and RGS domains is similar to AtRGS1 and involved in germination, development, and stress response (Jung *et al*., 2016). The MG00532 group was represented by a protein with weak homology to rat growth hormone-releasing factor. The mPR-like class of GPCR includes proteins related to the human membrane progesterone receptors (mPRs), which mediate an array of rapid, cell surface-initiated progesterone actions in the reproductive system involving activation of intracellular signaling pathways (Zhu *et al*., 2003; Thomas, 2008). The microbial opsins are a class of retinal-binding proteins with seven membrane-spanning domains that form rhodopsins by interacting with the retinal and function as light-responsive ion pumps or sensory receptors. The NOP-1 protein of *N. crassa*, closely related to archaeal opsins, was the first opsin characterized in filamentous fungi and known to bind all-*trans* retinal by using a Schiff base linkage and play a role in *N. crassa* photobiology (Bieszke *et* *al*., 1999). The PTH11 protein was firstly discovered in the plant pathogenic fungus *Magnaporthe grisea* and identified as an activator of appressorium differentiation in response to inductive surfaces. The aberration of *pth11* gene in *M. grisea* led to the defect in pathogenicity (DeZwaan *et al*., 1999). In *Aspergillus,* although a large number of Pth11-like GPCRs have been predicted, their exact functions remain heavily unknown (Lafon *et al*., 2006; Affeldt *et al*., 2014).

In the presence of extracellular signals, corresponding GPCRs recognize molecules and relay the signal inside the cell. The recognition of external cues by GPCRs provokes the conformational change of G protein, which in turn initiates the G protein signaling pathways including the cAMP-dependent protein kinase (PKA) and the mitogen-activated protein kinase (MAPK) pathways.

1.2.2 G Protein-Mediated Signaling Pathway

Heterotrimeric guanine nucleotide-binding proteins (G proteins), consisting of alpha, beta, and gamma subunits, had previously been characterized in diverse eukaryotic organisms and found to be involved in major signal transduction pathways in the responses of cells to extracellular stimuli. The G proteins, present in all eukaryotic cells, control metabolic and developmental pathways (reviewed in Simon *et al*., 1991). In filamentous fungi, the first G proteins, particularly α subunits, were discovered in *N. crassa* in the early 1990s. Thereafter, the G proteins FadA (α subunit), SfaD (β subunit), and GpgA (γ subunit) had been identified in *A. nidulans* (Yu *et al*., 1996; Rosén *et al*., 1999; Seo *et al*., 2005). The inactive G protein heterotrimer is composed of α, β, and γ subunits where the α and γ subunits are associated with the plasma membrane. Upon binding of specific ligands, GPCRs experience a conformational change and then physically interact with heterotrimeric G proteins. This physical interaction results in an exchange of GTP for GDP on the Gα subunit, which in turn leads to the dissociation of the heterotrimer into the GTP-Gα subunit and the Gβγ heterodimer. Once dissociated, the GTP-Gα and the Gβγ become active so that GTP-Gα, Gβγ, or both moieties can relay and amplify signals by regulating activities of downstream effector proteins in divergent signal transduction pathways. Then RGS proteins interact with an activated GTP-Gα subunit and increase its intrinsic GTPase activity. The GTP hydrolysis enables GDP-Gα subunit to reassociate with Gβγ heterodimer and plasma membrane, becoming into the inactive form of heterotrimeric G proteins again. In fungi, G protein-mediated signaling pathway is transmitted through one or more of the following pathways: 1) cAMP-dependent protein kinase (PKA), 2) mitogen-activated protein kinase (MAPK), and 3) Ca^{2+}- and DAG-dependent protein kinase C (PKC) (Fig. 1-1; Neves *et al*., 2002; McCudden *et al*., 2005).

The *Aspergillus* species possess three distinct groups of Gα proteins. Each group of Gα was assigned according to the amino acid sequence similarity with the *N. crassa* Gα proteins; Gna-1 (group I), Gna-2 (group II), and Gna-3 (group III). The group I Gα proteins possess a consensus sequence for myristoylation (MGXXXS) at the N-terminus and a site for ADP-ribosylation by pertussis toxin (CAAX) at the C-terminus. Most well-characterized filamentous fungi are known to possess a single group I Gα protein and its function has been well elucidated. The group III Gα proteins are also highly conserved and possess a myristoylation at the N-terminus. They are known to positively influence cAMP levels. However, the functions of group II Gα proteins are not as obvious as group I and III Gα proteins (reviewed in Li *et al*., 2007).

In *A. nidulans*, the first characterized group I Gα subunit was FadA showing 93% identity of AA sequence to *N. crassa* Gna-1 and thereafter GanA (group II) and GanB (group III) had been identified. FadA (<u>f</u>luffy <u>a</u>utolytic <u>d</u>ominant) was initially investigated for the fluffy autolytic phenotype, which was attributed to an uncontrolled vegetative growth followed by autolysis (Yu *et al*., 1996). The dominant activating mutations on FadA resulted in the expression of the fluffy autolytic phenotype and the inhibition of mycotoxin production, especially sterigmatocystin (ST), while dominant interfering FadA mutants displayed reduced vegetative growth, enhanced asexual sporulation, and precocious ST production (Yu *et al*., 1996; Hicks *et al*., 1997). Constitutively active dominant FadA mutants were presumed to maintain a longer period of the activated state of FadA-GTP due to the decreased intrinsic GTPase activity. Taken together, these results indicated that activated GTP-FadA (Gα) mediates signaling via cAMP-dependent protein kinase A (PKA) that promotes vegetative growth, which in turn suppresses asexual sporulation, sexual development, and mycotoxin production in *Aspergillus*.

The role of the group II Gα subunit, GanA has been well studied in *A. fumigatus*. GanA shares 46.3% and 44.3% identity with GpaA (group I, the homolog of FadA) and GpaB (group III, the homolog of GanB) in *A. fumigatus*, respectively. The mRNA level of *ganA* was highly expressed at both early (6 h) and later (48 h) time of asexual development and the deletion of *ganA* gene resulted in faster germination but decreased radial growth compared to those of WT on solid media, however, it did not show any significant impact on asexual development, unlike other Gα proteins. In addition, the Δ*ganA* strain displayed reduced mRNA level of gliotoxin biosynthesis transcription factor *gliZ* and decreased GT production compared to those of WT as well. Interestingly, the *ganA* null mutant exhibited the highest activity of PKA in conidia and PKC in mycelia among Gα mutants (Choi *et al*., 2020). These results demonstrated that GanA plays important roles in vegetative growth, asexual development, and mycotoxin production through PKA or PKC signaling pathway in a similar, but slightly different way from other groups of Gα proteins.

The group III Gα protein (GanB) is functionally well characterized in *A. nidulans* by Chang *et al.* (2004) and Lafon *et al.* (2005). They revealed that GanB positively regulates conidial germination but inhibits asexual sporulation by mediating a rapid and transient increase in cAMP levels in response to the presence of extracellular glucose during the early phase of germination. Moreover, Lafon *et al.* (2005) elucidated that GanB (Gα) and SfaD::GpgA (Gβγ) form a heterotrimeric complex. Collectively, G protein α subunits in *Aspergillus* mediate signaling that promotes vegetative growth and stress responses, which in turn inhibit fungal development and mycotoxin production (Fig. 1-2).

Most filamentous fungi are predicted to have a highly conserved single Gβ subunit (from 66 to 92% identical with *N. crassa* Gnb-1) and Gγ subunit (from 39 to 92% identical with N. crassa Gng-1) (Li *et al*., 2007). Previous studies on the Gβ and Gγ mutations have shown that mutational inactivation of genes encoding these proteins affected vegetative growth, conidiation, and sexual development in filamentous fungi (Kasahara and Nuss, 1997; Yang *et al*., 2002). Particularly, Krystofova and Borkovich (2005) demonstrated that the *gng-1* and *gnb-1* loss-of-function mutations displayed similar phenotypes such as female sterility, defective conidiation, low levels of intracellular cAMP, and a severe reduction in Gα protein levels in *N. crassa*. In addition, they proposed that Gng-1 (Gγ) physically interacts with Gnb-1 (Gβ) and forms the Gnb-1::Gng-1 (Gβγ) heterodimer during signaling pathways. In *Aspergillus*, the Gβ subunit SfaD and Gγ subunit GpgA were identified and well characterized. Rosén *et al*. (1999) isolated SfaD composed of 352 AA that shares 86% identity with *N. crassa* Gnb-1 and revealed that SfaD has a conserved Trp-Asp sequence, which is known as a WD40 domain. They revealed that SfaD plays crucial roles in vegetative growth, conidial sporulation, sexual development, and ST production in *A. nidulans*. Moreover, Seo *et al*. (2005) identified the Gγ subunit GpgA, which consists of 90

AA that shows 65% identity with *N. crassa* Gng-1. The *gpgA* loss-of-function mutation exhibited reduced vegetative growth, delayed conidiation, and no sexual fruiting body formation, similar to Δ*sfaD* mutants. Later, Lafon *et al*. (2005) revealed that the SfaD::GpgA (Gβγ) heterodimer is crucial for the proper activation of GanB (Gα), while GanB plays a primary role in the PKA signaling pathway in response to glucose.

Timely modulation of G protein-mediated signaling pathways is the key for fungi in sensing and responding to internal/external signals and various stress conditions. Upon the recognition of extracellular signals, cells need to activate G proteins as soon as possible so that they can translate diverse incoming signals into corresponding cellular responses opportunely. However, as we reviewed above, the prolonged activated state of GTP-Gα can cause various defects in fungal development and metabolism. Thus, the neutralization of activated GTP-Gα into the inactive form on time is as significant as the activation. These tight upstream regulations play crucial roles in vegetative growth, development, mycotoxin production, and virulence in fungi. There are three different types of regulators present in *Aspergillus*: phosducin-like proteins (PhLPs), regulators of G protein signaling (RGSs), and a GDP/GTP exchange factor (RicA).

Phosducin-like proteins are a group of evolutionarily conserved positive regulators of Gβγ heterodimer function. PhLPs act as molecular chaperones during Gβγ assembly by stabilizing the nascent Gβ subunit until it associates with the Gγ protein (Lukov *et al*., 2005; Lukov *et al*., 2006). In *A. nidulans*, three potential PhLPs (PhnA, PhnB, and PhnC) were identified based on the AA sequence similarity with Bdm-1, which is a known fungal Gβγ activator in the chestnut blight fungus *Cryphonectria parasitica* (Kasahara *et al*., 2000) and among them, the function of PhnA was firstly investigated by Seo and Yu (2006) due to its highest similarity with Bdm-1. Seo and Yu (2006) revealed that PhnA is required for proper

SfaD functionality, sexual reproduction, and mycotoxin biosynthesis showing consistent results with the roles of SfaD::GpgA heterodimer.

RGSs are a group of proteins containing a conserved ∼130 AA RGS box, which physically interact with an activated GTP-Gα and accelerate the intrinsic GTPase activity of the Gα subunit, resulting in the attenuation of G protein-mediated signaling pathways (Ross and Wilkie, 2000; McCudden *et al*., 2005). In *Aspergillus*, several RGSs have been identified including FlbA, RgsA, RgsB, RgsC, GprK, and Rax1. Among them, FlbA and RgsA are the most well-characterized RGSs (Fig. 1-2). The FlbA consists of 719 amino acids containing one RGS box and two DEP (Dishevelled, EGL-10, and Pleckstrin) domains. The DEP is a globular protein domain of ~80 AA commonly found in proteins involved in G-protein signaling, however, the repeated pattern of DEP is only observed in fungi (Han *et al*., 2004). Along with the GTPase-activating RGS domain, the DEP domain may play a role in guiding RGS proteins to the Golgi and plasma membranes (Burchett, 2000). The *flbA* loss-of-function exhibited the fluffy-autolytic phenotype, which was observed in activating dominant FadA (Gα) mutants. In addition, the *fadA* deletion mutants did not display the fluffy-autolytic phenotype caused by Δ*flbA* and restored asexual development and mycotoxin production. The primary role of FlbA is to attenuate G protein-mediated signaling by deactivating GTP-FadA (group I Gα) protein, whereas FadA, SfaD, and GpgA constitute the major G protein heterotrimer modulating growth, development, and secondary metabolism in *A. nidulans* (Yu *et al*., 1996; Hicks *et al*., 1997). The RgsA consists of 362 AA containing one RGS box in the N-terminal region. Unlike FlbA regulating group I Gα subunit, RgsA negatively regulates GanB (group III Gα) signaling, which promotes stress responses via the PKA pathway resulting in the inhibition of asexual development (Han *et al*., 2004).

The GDP/GTP exchange factor RicA is relatively recently discovered compared to other regulators of G proteins in *Aspergillus*. The *ricA* deletion mutants displayed severely reduced colony growth, and a total absence of asexual sporulation and sexual development in *A. nidulans* (Kwon *et al*., 2012). Kwon *et al*. (2012) introduced the *A. fumigatus ricA* gene (Af*ricA*) into *A. nidulans* Δ*ricA* and found that the overexpression of Af*ricA* in the ΔAn*ricA* mutant partially restored colony growth and asexual development. In addition, they revealed that the removal of only *rgsA*, not *sfgA*, *flbA*, *rgsB*, or *rgsC*, restored vegetative growth and conidiation in ΔAn*ricA* and that RicA can physically interact with GanB (Gα) in vitro in yeast. These results led Kwon *et al*. to conclude that RicA primarily activates the GanB → PKA signaling cascade in *A. nidulans*.

1.3 The Velvet Regulators and the Global Regulator of Secondary Metabolism LaeA

In filamentous fungi, fungal development and secondary metabolism are intimately associated via the activities of the fungal-specific *velvet* family regulatory proteins and the global regulator of secondary metabolism LaeA. Velvet regulators form various complexes that play divergent roles in fungal development. The VosA-VelB heterodimer inhibits conidial germination, but positively regulates trehalose synthesis and β-glucan biogenesis. Moreover, VeA bridges VelB and LaeA to form the VelB-VeA-LaeA (velvet) heterotrimeric complex in the absence of light and this velvet complex controls not only secondary metabolism but also the formation of Hülle cells, which nurse the nascent sexual fruiting bodies. This section summarizes the functions of Velvet proteins and LaeA in *Aspergillus*.

1.3.1 The Velvet Family Regulators

The first study on the Velvet family was performed on the *velvetA* mutant, which was renamed *veA* afterward because the colony of this mutant showed a flat and nonvelvety appearance regardless of the presence or absence of light (Mooney and Yager, 1990). The functions of *veA* gene have been extensively characterized in *Aspergillus* species. The *veA* gene is necessary for proper sexual development and secondary metabolism in *Aspergillus* (Kim *et al*., 2002; Kato *et al*., 2003; Calvo *et al*., 2004; Amaike and Keller, 2009; Dhingra *et al*., 2012; Park *et al*., 2012; Chang *et al*., 2013). In 2007, a major advancement in the Velvet family study was made by Ni and Yu. They demonstrated that the novel regulator VosA (<u>v</u>iability <u>o</u>f <u>s</u>pores) governs sporogenesis and trehalose biogenesis, which in turn determines the viability of spores in *A. nidulans*. In addition, they found three other proteins including VeA were similar to VosA so that two proteins were named VelB (<u>ve</u>lvet-<u>l</u>ike protein) and VelC, and these four proteins were designated as the Velvet family of regulators (Ni and Yu, 2007). The Velvet family proteins are highly conserved in Aspergilli and they all share a fungi-specific and highly conserved Velvet domain, which consists of approximately 170 - 300 AA sequences with three conserved motifs (Park and Yu, 2016).

Velvet regulators in *A. nidulans*

The **VeA** protein was the first member of the Velvet family regulators identified in the 1960s (Kafer, 1965). VeA is composed of 573 amino acids containing the Velvet domain in the N-terminal region (Fig. 1-3). In addition, in the N-terminal region of this regulator, a potential nuclear localization signal (NLS) and nuclear export signal (NES) domains were found, suggesting roles in the nuclear localization of VeA (Stinnett *et al*., 2007). Moreover, VeA contains a putative PEST (proline (P)-, glutamic acid (E)-, serine (S)-, and threonine (T)-rich) sequence in the C-terminal region (Kim *et al*., 2002), which is commonly found in rapidly degraded proteins (Rogers *et al*., 1986). VeA is a key light-dependent developmental regulator that positively regulates sexual development, which in turn suppresses asexual sporulation in *A. nidulans* (Timberlake, 1990; Yager, 1992). The *veA* loss-of-function mutations resulted in the complete absence of sexual fruiting body formation, even under sexual development-promoting conditions, whereas the overexpression of *veA* enhanced the production of cleistothecia but inhibited asexual sporulation (Kim *et al*., 2002). Furthermore, VeA is known to play crucial roles in secondary metabolism. VeA acts as an activator on sterigmatocystin production but inhibits penicillin biosynthesis (Kato *et al*., 2003; Sprote and Brakhage, 2007). Underlying the significant roles of VeA in fungal development and secondary metabolism, the nuclear localization of VeA is a vital factor. The VeA protein is constitutively expressed during the life cycle of *A. nidulans* but is mostly found in the cytoplasm under the presence of light (Kim *et al*., 2002; Stinnett *et al*., 2007; Sarikaya *et al*., 2015). On the other hand, in the dark, VeA enters the nucleus, forms VelB-

VeA-LaeA heterotrimeric complex, and controls sexual development and mycotoxin production (Stinnett *et al*., 2007; Bayram *et al*., 2008).

VosA consists of 430 AA containing the Velvet, NLS, and potential TAD (transcription activation) domains, indicating that it may function as a transcription factor. VosA protein is expressed during vegetative growth and the early stage of asexual and sexual development, however, primarily localized in the nucleus of mature conidia. Interestingly, the expression of *vosA* is regulated by AbaA. In phialides, AbaA binds to the promoter region of *vosA* and induces the accumulation of *vosA* mRNA in conidia during the late phase of asexual development (Ni and Yu, 2007; Park *et al*., 2012). VosA is a key regulator of conidiation and sexual development. The *vosA* null mutants produced asexual developmental structures (conidiophores) in the liquid submerged culture, where the wild type solely undergoes vegetative growth and produced fewer numbers of cleistothecia compared to the WT. In addition, the deletion of *vosA* resulted in the accumulation of high mRNA levels of the *brlA* gene, which is a key initiative factor of conidiation, indicating VosA is a key negative regulator of *brlA* (Ni and Yu, 2007). Moreover, VosA controls various biological processes including conidia wall integrity, spore viability, conidial germination, and focal trehalose biogenesis (Ahmed *et al*., 2013; Park *et al*., 2015).

VelB is a 369-AA protein containing the Velvet domain covering the entire protein. The *velB* gene is mostly expressed during the life cycle, but particularly high levels of *velB* mRNA are observed during vegetative growth and in the late phases of asexual and sexual development. Similarly, VelB protein is detectable during entire vegetative growth and in early developmental stages. VelB has divergent functions regulating vegetative growth, development, and secondary metabolism. VelB negatively regulates conidial germination but acts as an activator of asexual development. The *velB* deletion mutants showed increased conidial germination rates yet exhibited a reduced conidia production as well as decreased expression levels of asexual development-related genes such as *brlA* and *abaA*. In addition, the deletion of *velB* led to the enhanced production of brown pigments (Bayram *et al*., 2008; Park *et al*., 2012).

VelC is composed of 524 AA containing the Velvet and PEST domains in the C-terminal region. Unlike other Velvet family members, the mRNA of *velC* specifically accumulates during the early phase of sexual development. The aberration of *velC* gene led to the slightly enhanced conidia production and increased mRNA levels of all three central regulatory genes of conidiation, *brlA*, *abaA*, and *wetA* regardless of the presence or absence of light. In addition, the deletion of *velC* resulted in a decreased production of sexual fruiting bodies, while overexpression of this gene led to increased production of cleistothecia but a decreased number of conidia. These suggest that VelC plays a role in the activation of sexual development (Park and Yu, 2016).

Individual Velvet protein plays multifunctional roles in *Aspergillus* development and metabolism, however, the most significant trait of Velvet proteins is that they interact with partner proteins including themselves, and form complexes in multiple combinations, which govern the various processes of fungal biology. The formation of Velvet protein complexes occurs in a cell type- and/or timing-specific manners. Among all different Velvet protein complexes, the three most extensively studied complexes, VosA-VelB, VelB-VeA, and VelB-VeA-LaeA, are discussed here (Fig. 1-4). During germination, the VosA-VelB heterodimer inhibits conidial germination rates. Moreover, the VosA-VelB heterodimer controls spore viability, trehalose biogenesis, β-glucan synthesis, and tolerance of conidia to various stresses such as heat and oxidative stress. The VelB-VeA complex is a key participant in sexual development. Although the molecular mechanism of the VelB-VeA heterodimer formation has not been clearly identified yet, the VelB-VeA complex plays an important role in sexual development as an activator. Furthermore, as VeA bridges between the VelB-VeA heterodimer and LaeA, the VelB-VeA complex interacts with LaeA and forms the VelB-VeA-LaeA heterotrimeric complex in the nucleus. This VelB-VeA-LaeA complex regulates sterigmatocystin production and sexual development in the dark. In addition, it controls the expression of secondary metabolism-related genes at transcriptional or epigenetic levels (Bayram *et al*., 2008; Atoui *et al*., 2010; Reyes-Dominguez *et al*., 2010).

Velvet regulators in other *Aspergillus* species

Most *Aspergillus* species have four Velvet family regulators (VeA, VelB, VelC, and VosA), but a recent study newly identified the fifth member of the Velvet family VelD in *A. flavus* (Eom *et al*., 2018). Although the Velvet family proteins are highly conserved in Aspergilli, their functions might have been divergent depending on the species. In the plant pathogenic and mycotoxigenic fungus *A. flavus*, the VeA protein was first identified among the Velvet regulators and revealed to regulate asexual and sexual development as well as mycotoxin production including the most carcinogenic mycotoxin aflatoxin (Duran *et al*., 2007). The deletion of the *veA* gene decreased the production of conidia and completely blocked sclerotia formation. The *veA* null mutant was also unable to produce aflatoxins and aflatrem. Moreover, VeA regulates the mRNA expression of genes associated with various secondary metabolite production such as aflatoxin, aflatrem, and asparasone (Duran *et al*., 2007; Cary *et al*., 2014). Similar to *A. nidulans* VeA, *A. flavus* VeA interacts with VelB and LaeA to form the VelB-VeA-LaeA complex regulating sclerotia formation and aflatoxin production (Chang *et al*., 2013). Of note, the deletion of *veA* or *velB*, but not *laeA*, resulted in the impaired conidiation, implying the positive regulation of VeA-VelB on asexual development in *A. flavus* (Chang *et al*., 2013). The

VosA-VelB heterodimer is required for proper trehalose biosynthesis and tolerance of conidia to various stresses. The newly identified VelD plays a role in aflatoxin production as the Δ*velD* mutant showed no aflatoxin production (Eom *et al*., 2018). In the opportunistic human pathogenic fungus *A. fumigatus*, Velvet family regulators except VelC are required for proper asexual development. The *veA*, *velB*, or *vosA* null mutants exhibited asexual development even in the liquid submerged culture where only vegetative growth occurs for the WT and the accumulation of high *brlA* mRNA levels, indicating a repressive role of these Velvet regulators in conidiation. In addition, VeA positively regulates the production of gliotoxin, which is known to inhibit the human immune response. Unlike *A. nidulans* and *A. flavus*, the roles of the VelB-VeA-LaeA complex in *A. fumigatus* are not clear yet, however, the cross-species complementation analysis suggests that the VelB-VeA-LaeA complex of *A. fumigatus* plays a similar role with those of *A. nidulans* in that the introduction of the *A. nidulans veA* gene into the *A. fumigatus* Δ*veA* restored the normal phenotypes in *A. fumigatus*. The VosA-VelB complex is necessary for spore viability, trehalose biosynthesis, and tolerance of conidia to UV and oxidative stresses (Park *et al*., 2012).

1.3.2 LaeA, a Global Regulator of Secondary Metabolism

Secondary metabolism is inseparable from fungal growth and development. Secondary metabolites are often associated with developmental processes and have received much attention due to their broad spectrum of pharmaceutical and/or toxic properties: antibiotic, antiviral, antitumor, and immunosuppressive activities as well as phytotoxic and mycotoxic activities. (Demain and Fang, 2000; Calvo *et al*., 2002; Bok and Nancy, 2004). Two decades ago, Butchko *et al*. (1999) performed mutagenesis screening on 23 mutants that exhibited loss of sterigmatocystin (ST) production but normal asexual development in *A. nidulans* to reveal genes that are specific for the regulation of secondary metabolism. Thereafter, Bok and Keller investigated one of these mutants and identified a novel nuclear protein, LaeA, as a global regulator of secondary metabolism in *Aspergillus* (Fig. 1-5; Bok and Keller, 2004).

In *A. nidulans,* LaeA is required not only for the biosynthesis of a large array of secondary metabolites (SM) but also for the proper expression of corresponding SM biosynthetic gene clusters. The deletion of *laeA* inhibited the production of ST, the β-lactam antibiotics penicillin (PN), the anti-hypercholesterolaemic agent lovastatin, and the biosynthesis of mycelial pigments, which is a visually noticeable phenotype of Δ*laeA*. The *laeA* null mutant exhibited a near absent mRNA expression of *aflR* and *stcU* genes encoding a transcription factor and a biosynthetic enzyme required for ST production. Furthermore, the transcriptional profiling analysis of 26 genes consisting of the entire ST biosynthetic gene cluster elucidated that the transcriptional regulation of LaeA is ST cluster-specific as adjacent genes of the ST cluster in the genome were not affected. To understand the effect of LaeA in the production of LOV, Bok and Keller introduced the partial LOV cluster of *A. terreus* into the *A. nidulans* Δ*laeA*, producing the LOV intermediate monocolin J (MONJ). The Δ*laeA*/LOV^+ strain displayed reduced mRNA levels of both *lovE* (encoding a LOV-specific Zn2Cys6 transcription factor) and *lovC* (a LOV biosynthetic gene) and diminished MONJ production as well. Overexpression of *laeA* elevated the expression levels of genes required for PN, and LOV biosynthesis (*ipnA*, *lovE*, and *lovC*) and the production of corresponding secondary metabolites, while ST production was unaffected interestingly (Bok and Keller, 2004).

A. flavus LaeA exhibits mostly similar functions consistent with *A. nidulans* LaeA yet also plays distinct roles in growth and sexual development. The Δ*laeA* mutant lost the ability to produce many secondary metabolites including aflatoxin B1 and B2, cyclopiazonic acid, kojic acid (on YES media), and oryzachlorin (on DG18 media). On the other hand, overexpression of *laeA* led to the enhancement of some secondary metabolite productions, which are not typically observed in the WT. The sclerotia-specific metabolites Paspaline/paspalinine, aflatrem, and aflavinines were produced exclusively in the *OE::laeA*. This phenomenon is highly correlated with the increased sclerotia formation in the *OE::laeA* strain. Along with the effect in sclerotia production, LaeA affects seed colonization and lipase activity, closely related to the pathogenicity of *A. flavus* (Kale *et al*., 2008).

The opportunistic human pathogen *A. fumigatus* has been extensively studied due to its notorious virulence in humans constituting the majority of invasive aspergillosis in immunocompromised individuals (Latgé and Chamilos, 2019). Secondary metabolites including toxins and melanins have been recognized as virulence factors in invasive aspergillosis. Deletion of *laeA* suppressed not only the production of multiple secondary metabolites including the immunotoxin gliotoxin but also the expression of 13 SM biosynthetic gene clusters including *A. fumigatus*-specific mycotoxin clusters. The transcriptomic profiling analyses of WT, Δ*laeA*, and *C'laeA* strains revealed that LaeA positively controls the expression of up to 40% of major classes of SM biosynthetic genes such as nonribosomal peptide synthetases, polyketide synthases, and P450 monooxygenases (Perrin *et al*., 2007). Regarding the effect of LaeA on virulence, two *A. fumigatus* Δ*laeA* strains exhibited decreased virulence in the mouse pulmonary model, which is attributed to the reduced killing of neutrophil cells. These suggest a strong correlation between LaeA-mediated toxin production and invasive aspergillosis development by *A. fumigatus* (Bok *et al*., 2005; Sugui *et al*., 2007).

1.4 NsdD, a Key Regulator of Conidiation and Sexual Development

The general life cycle of *Aspergillus* begins with vegetative growth. Spores start to form small germ tubes (germlings) and these tubes elongate in a highly-polarized manner resulting in hyphal growth. Under certain favorable conditions, Aspergilli initiate asexual or sexual reproductive processes. *Aspergillus* species primarily reproduce through asexual sporulation (conidiation), while few of them can reproduce via sexual development.

Asexual development (conidiation) in Aspergilli takes place via orchestrated gene expression of multiple positive and negative regulators. In order to initiate conidiation, upstream activators induce the activation of *brlA*, which encodes C_2H_2 zinc finger TF (Adams *et al*., 1988; Chang and Timberlake, 1993), initiating the development of conidiophore and activating the expression of *abaA*. Then, the AbaA and WetA play crucial roles in conidiophore maturation during the middle and late stages of conidiation, respectively. This central regulatory pathway (*brlA* → *abaA* → *wetA*) acts in concert with other genes to control conidiation-specific gene expression and determine the order of gene activation during development and spore maturation (Park and Yu, 2012). During this asexual stage in the lifecycle, *Aspergillus* species produce multicellular reproductive organs, termed conidiophores, each of which produces multiple chains of non-motile conidia.

Previous studies revealed that the evolutionarily conserved GATA-type transcription factor (TF) NsdD acts as a key negative regulator of asexual development by downregulating the expression of *brlA* in *Aspergillus* (Fig. 1-6; Lee *et al*., 2014; Lee *et al*., 2016). The NsdD directly binds to the promoter regions of the *brlA* gene and represses *brlA* expression in concert with another repressor VosA. The deletion of *nsdD* resulted in accelerated and precocious activation of conidiation; the mutant even produced asexual developmental organs under liquid submerged cultures where conidiation never takes place in the WT and exhibited the increased production of conidia on solid media. NsdD also plays a significant role in conidiophore morphogenesis. Deletion of *nsdD* resulted in abnormal hyphal branching during vegetative growth (data not shown). The *nsdD* null mutant in *A. flavus* displayed the formation of approximately 10 times shorter and 4 times smaller conidiophores that resemble closer to those of *A. nidulans* WT (Cary *et al*., 2012; Lee *et al*., 2016). Moreover, NsdD regulated mycotoxin production including sterigmatocystin (ST) and aflatoxin (AF) in *A. nidulans* and *A. flavus*, respectively. Furthermore, previous studies have found that NsdD is required for proper sexual development. The deletion of *nsdD* resulted in no fruiting body formation, even under the sexual development promoting conditions. In contrast, overexpression of *nsdD* led to the increased formation of fruiting bodies and displayed resistance to certain inhibitory effects on sexual fruiting in *Aspergillus* (Han *et al*., 2001; Szewczyk and Krappmann, 2010; Cary *et al*., 2012). Although the pleiotropic characteristics of NsdD regarding the development and metabolism of the genus *Aspergillus* have been well studied for the last two decades, the regulatory mechanism underlying how the single GATA-type TF NsdD governs all distinct aspects of fungal biology remains to be investigated.

1.5 Conclusions and Prospects

In this chapter, we summarized the most important upstream regulators of growth, development, and secondary metabolism in *Aspergillus* species. Although researchers now have a better understanding of the biological roles of these regulators, the detailed mechanisms of how every single regulator exerts diverse effects on different aspects of *Aspergillus* biology have yet to be unveiled. Among these regulators, our group identified the *nsdD* gene for the first time and has revealed the molecular functions of NsdD in asexual sporulation, sexual development, and mycotoxin production. Of note, we figured out that it plays key roles in every situation, unlike other upstream regulatory proteins. Thus, we hypothesize that NsdD governs development and metabolism by forming a NsdD-mediated gene regulatory network. As it affects all distinct biological processes in fungal development and metabolism, the NsdD-mediated network will become a wonderful tool to unearth the general gene regulatory network ruling the *Aspergillus* biology.

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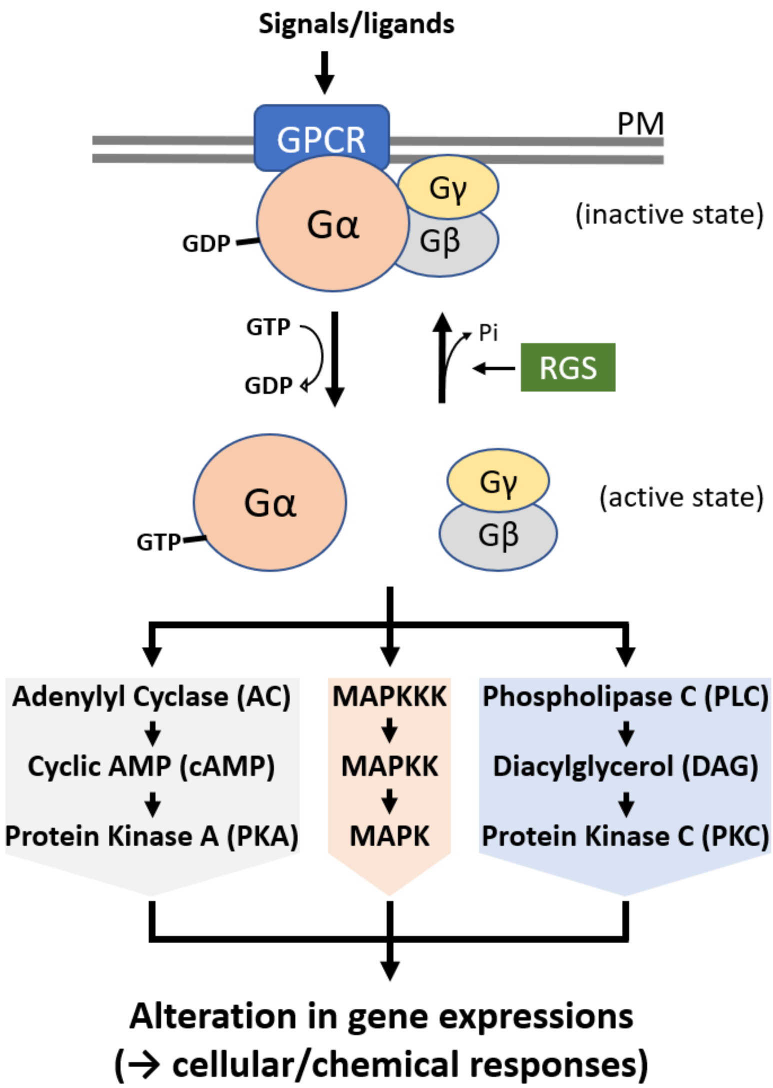

Figure 1-1. A schematic diagram of G protein-mediated signaling pathway. Upon the recognition of external ligands, G protein-coupled receptor (GPCR) is sensitized and interacts with a nearby G protein. Then G proteins become active by the interaction with GTP and initiate downstream signaling pathway(s): PKA, MAPK, and PKC. This signal transduction results in the differential expression of genes involved in growth, development, morphogenesis, mating, metabolism, virulence, and mycotoxin biosynthesis. The activated G proteins are then negatively controlled by regulators of G protein signaling (RGS) and become inactive again. PM: plasma membrane.

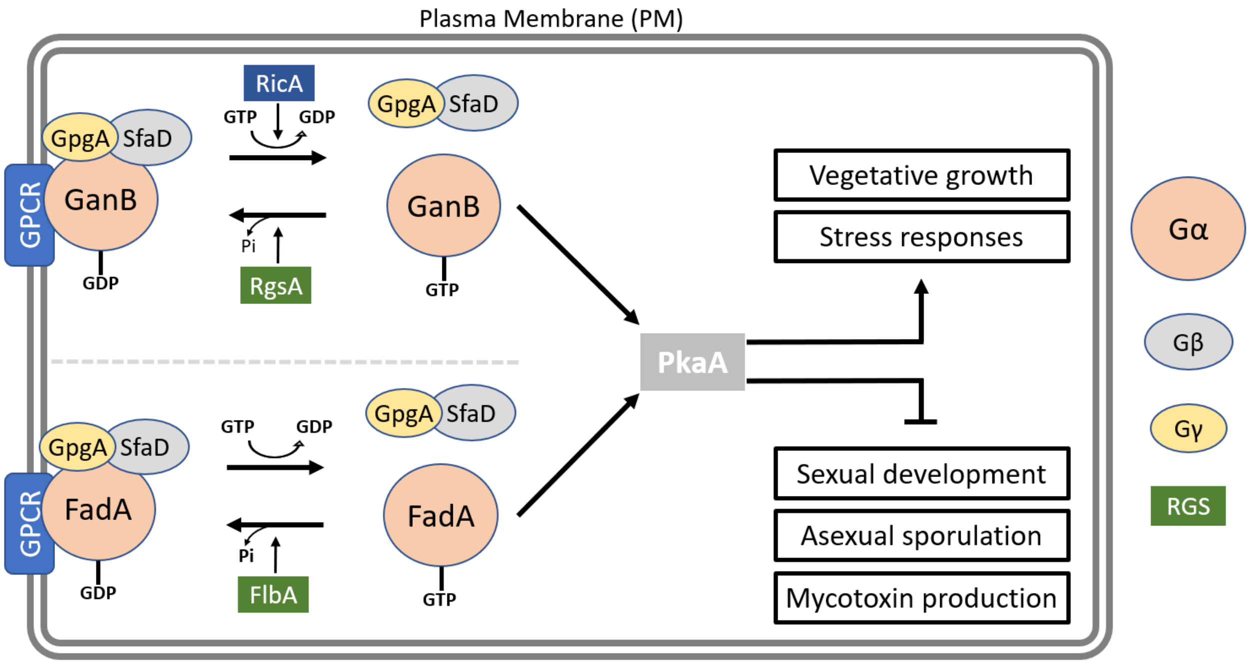

Figure 1-2. G protein-mediated signaling pathway in *A. nidulans*. In *A. nidulans*, two Gα subunits (FadA and GanB) regulate vegetative growth, development, and mycotoxin production via PKA pathway when they are in the activated state. The regulators of G protein signaling (RGS) FlbA and RgsA turn activated FadA and GanB back into the inactive state, respectively. RicA is a GDP/GTP exchange factor involved in the activation of G protein subunits.

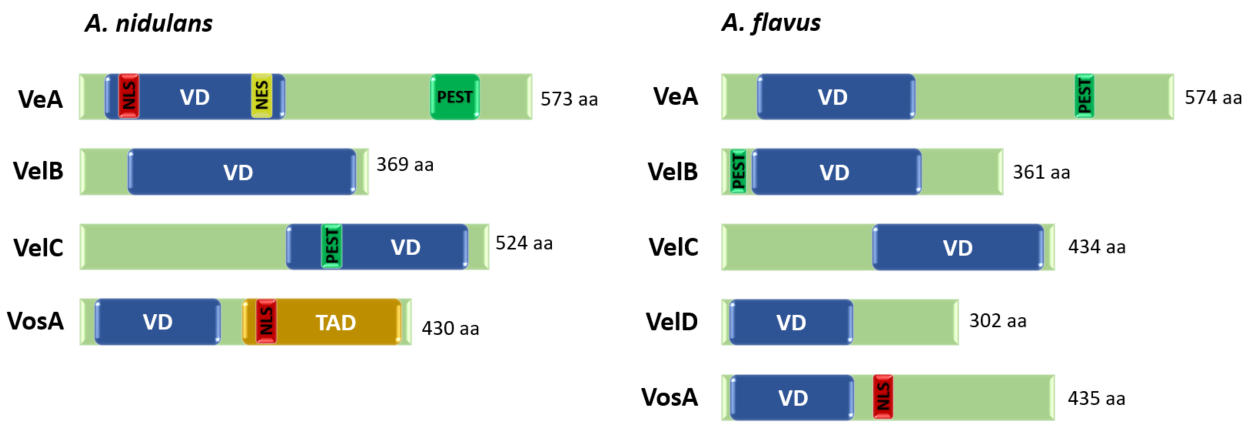

Figure 1-3. Domain architecture of the Velvet regulators in *A. nidulans* and *A. flavus*. VD: Velvet domain, NLS: Nuclear localization signal, NES: nuclear export signal, PEST: Proline (P)-, glutamic acid (E)-, serine (S)-, and threonine (T)-rich sequence, TAD: transcription activation domain, aa: amino acids.

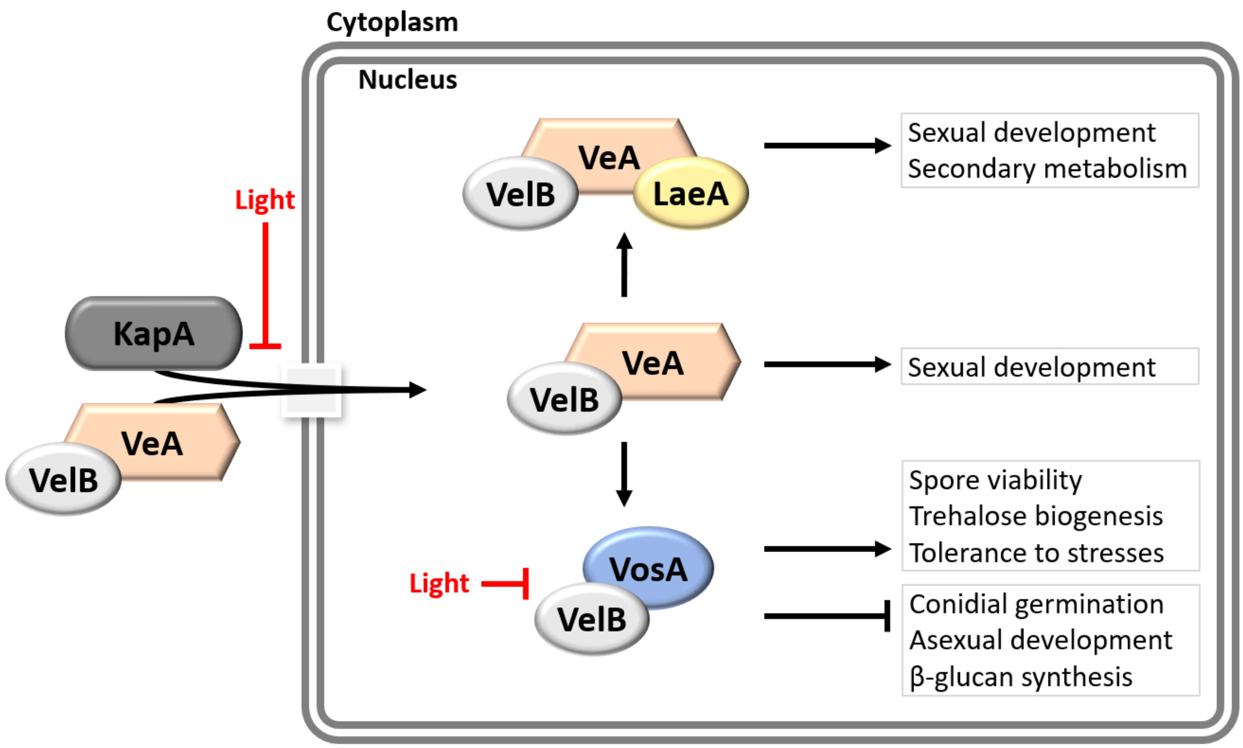

Figure 1-4. The functions of Velvet family proteins in *Aspergillus*. This model is adopted and modified from Bayram *et al*. 2010. VeA enters the nucleus together with VelB and the importin-α KapA in the dark. In the nucleus, Velvet family proteins and LaeA can form three different complexes depending on the presence or absence of light: VelB-VosA, VelB-VeA, and VelB-VeA-LaeA. These complexes regulate different biological processes.

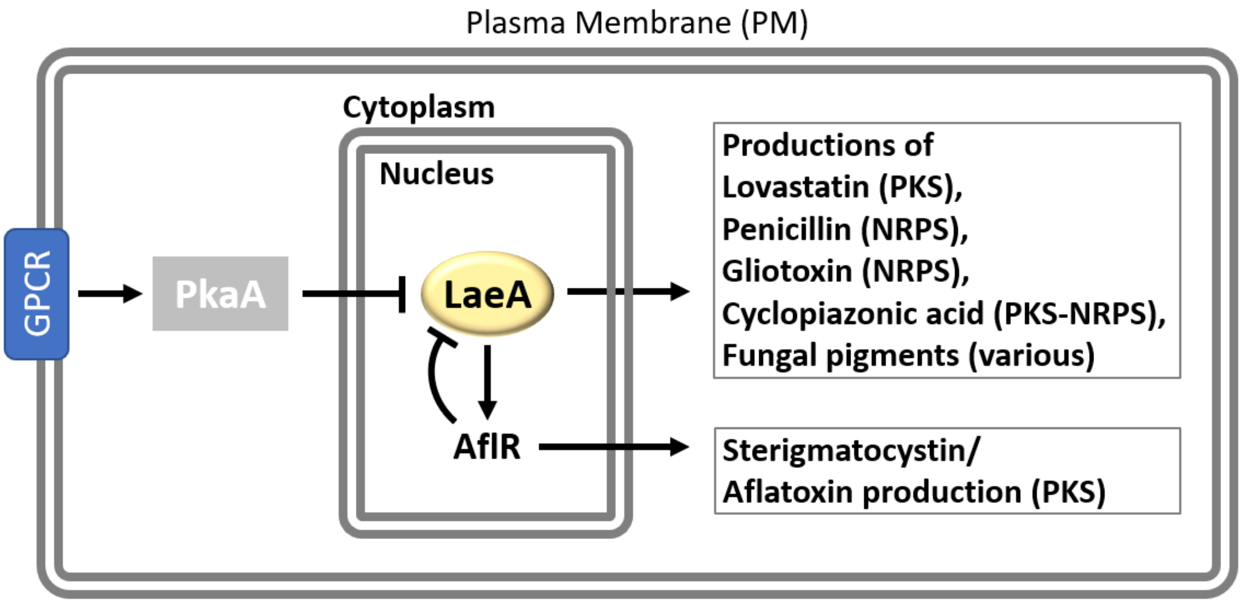

Figure 1-5. The functions of LaeA in *Aspergillus*. See the "LaeA, a Global Regulator of Secondary Metabolism" section.

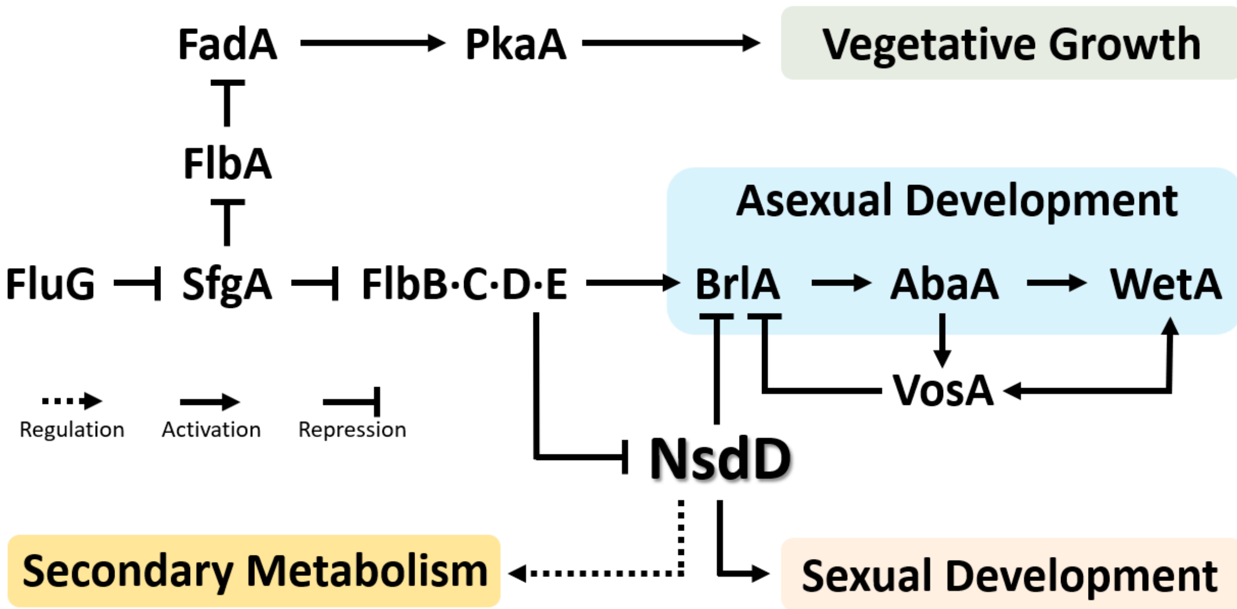

Figure 1-6. A genetic model for growth, developmental, and metabolic control in *Aspergillus*. An arrow with a solid line indicates a positive regulation (activation) in a relationship, an arrow with a dotted line indicates an unspecified regulation (can be activating or repressing), and a blunt-ended line indicates a repressive role in the relationship.

Table 1-1. G protein-coupled receptors in *Aspergillus* species

Gene name	Class	TM No.	Conserved Domains (note)	References
gprA	I	7-TM	Ste2; alpha-pheromone receptor (*S. cerevisiae* pheromone receptor)	Han *et al*., 2004
gprB	II	5-TM	Ste3; a-pheromone receptor (*S. cerevisiae* pheromone receptor)	Han *et al*., 2004
gprC	III	7-TM	Git3; Gpa2 C-term; Family 1-like (*S. pombe* glucose receptor)	Han *et al*., 2004
gprD	III	7-TM	Git3; Gpa2 C-term (*S. pombe* glucose receptor)	Han *et al*., 2004
gprE	III	7-TM	Git3; Gpa2 C-term (*S. pombe* glucose receptor)	Han *et al*., 2004
gprF	IV	7-TM	PQ-loop repeat (*S. pombe* nitrogen sensor)	Han *et al*., 2004
gprG	IV	5-TM	PQ-loop repeat (*S. pombe* nitrogen sensor)	Han *et al*., 2004
gprJ	IV	7-TM	PQ-loop repeat (*S. pombe* nitrogen sensor)	Lafon *et al*., 2006
gprS	IV	7-TM	PQ loop repeat (*S. pombe* nitrogen sensor)	Affeldt *et al*., 2014
gprH	V	7-TM	Secretin-like; cAR (*D. discoideum*); Family 2-like (signal through cAMP pathways)	Han *et al*., 2004
gprI	V	7-TM	cAR (*D. discoideum*); Family 2-like (signal through cAMP pathways)	Han *et al*., 2004
gprK	VI	7-TM	RGS (regulator of G protein signaling)	Lafon *et al*., 2006
gprR	VI	7-TM	RGS (regulator of G protein signaling)	Affeldt *et al*., 2014
gprM	VII	7-TM	MG00532-like	Lafon *et al*., 2006
gprN	VII	6-TM	MG00532-like	Lafon *et al*., 2006
gprO	VIII	7-TM	mPR-like; Hemolysin-III related (broad range of ligands)	Lafon *et al*., 2006
gprP	VIII	7-TM	mPR-like; Hemolysin-III related (broad range of ligands)	Lafon *et al*., 2006
gprQ	VIII	5-TM	mPR-like; Hemolysin-III related (broad range of ligands)	Lafon *et al*., 2006
nopA	IX	6-TM	Bacterial rhodopsin-like (photoreactive)	Lafon *et al*., 2006
***pth11* -like**	X	7-TM	Pth11-related group	Lafon *et al*., 2006

CHAPTER 2: The Master Regulator NsdD Governs Development and Metabolism in *Aspergillus*: Network-based Multi-omics Studies

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2.1 Abstract

The GATA-type transcription factor (TF) NsdD regulates sexual/asexual development and metabolism in *Aspergillus* species. To gain insight into the molecular and genomic bases of NadD-mediated regulation of cellular and metabolic developmental traits, we used network-based multi-omics approaches., i.e., transcriptome, protein-DNA interaction, primary, and secondary metabolism to construct and validate NsdD-mediated gene regulatory networks (GRNs). We intended to reveal not only the molecular function of NsdD but also the evolutionary transition of NsdD-mediated GRNs that result in the changes in development and metabolism in two distantly related species, *Aspergillus nidulans* and *Aspergillus flavus*.

NsdD contains a highly conserved GATA-type IVb DNA-binding domain (DBD). The deletion of *nsdD* in *Aspergillus* species leads to common features such as abnormal hyphal branching, hyper-conidiation, and no sexual development, but also distinct phenotypes including different morphology of asexual developmental structures as well as opposite regulation of the related mycotoxins sterigmatocystin (ST) in *A. nidulans* and aflatoxin (AF) in *A. flavus*. Particularly, NsdD inhibits biosynthesis of ST in *A. nidulans*, whereas it is required for proper AF production in *A. flavus*. These suggest that, despite the conserved DBD, the NsdD-mediated GRNs differ in each species.

To elaborate the NsdD-mediated GRNs in two species, transcriptomic profiling and chromatin immunoprecipitation (ChIP) assay followed by sequencing were performed. Three different cell types are selected for RNA-seq analyses to understand cell type-dependent effects of NsdD: vegetative cell (Vege), asexually developing cell (Asex), and conidia. The regulation pattern of NsdD was apparently divergent depending on species and cell types. To elucidate direct targets of NsdD, ChIP-seq analyses were performed in conidia and we identified 502 and

674 possible direct targets including well-known regulators of development and metabolism: *veA*, *flbD*, *laeA*, *kapA*, *rosA*, and *steA* in *A. nidulans* and *veA*, *flbA·C·D*, *vosA*, *brlA*, and *rosA* in *A. flavus*. Then, NsdD-associated peaks from ChIP-seq analyses were subjected to Multiple Em for Motif Elicitation (MEME) analysis, and the predicted NsdD response element (NRE) 5'-GATCT-3' was identified in both species. From metabolomic analyses in the Δ*nsdD* mutant, both species showed significant changes in the abundances of a large array of primary metabolites including some citric acids, amino acids, and energy-related metabolites, and secondary metabolites such as alternariol, austinol, asterriquinone, imizoquin D, and leporin B. In addition, the network analyses proposed species-specific NsdD networks and their respective core mechanisms as well. Then, the comparative network analyses have revealed the conserved and divergent roles of NsdD between the two species.

In summary, NsdD governs fungal development and metabolism via a species-specific NsdD-mediated gene regulatory network. Within the network, NsdD directly regulates not only crucial upstream developmental regulators, but also some important genes in development and metabolism, which in turn affects the expression of downstream genes, resulting in distinct cellular and metabolic developmental traits in the two distantly related *Aspergillus* species.

2.2 Introduction

Fungi are of vital importance to humankind, largely because of their relevance to medical purposes (pathogen and antibiotics producers), environmental recycling, food fermentation and spoilage, agricultural aid, and industrial production. Many filamentous fungi, especially those in the most diverse and species-rich phylum Ascomycota, reproduce primarily by forming asexual spores (conidia). These spores are easily dispersed by a gust of wind, water movement, or even by a mechanical effect so that they facilitate fungal survival, propagation, and infestation (Roper *et al.*, 2010; Dressaire *et al*., 2016). Of note, in some fungi, asexual development and secondary metabolite production are tightly associated. Several studies have shown that some mutants displaying a defect in asexual development are coincidentally unable to produce mycotoxins such as aflatoxin and sterigmatocystin (Calvo *et al*., 2002; Bennett and Klich, 2003; Yu and Keller, 2005). As asexual sporulation is particularly prevalent among fungi, it is a decent tool to investigate intraspecies genotype-phenotype relationships and gene regulatory network re-wiring underlying the evolution of fungal development and metabolism between species.

The genus *Aspergillus* is the most common and ubiquitous fungi, with more than 340 filamentous fungal species have been identified in this genus (Bennett, 2010). As Aspergilli encompass a broad spectrum of biological diversity and *Aspergillus nidulans* has served as an excellent model fungus for genetic study, they are a highly tractable system to elucidate GRN re-wiring resulting in distinct cellular and molecular phenotypes within *Aspergillus*. Asexual development (conidiation) in Aspergilli takes place via orchestrated gene expression of multiple positive and negative regulators. FluG (+), SfgA (-), and FLBs (+) as upstream regulators determine whether conidiation is initiated, and NsdD (-), VosA (-), and BrlA (+) govern the progress and completion of conidiation (Lee *et al.*, 2014). In order to initiate conidiation, the upstream activators induce the activation of *brlA*, which encodes C_2H_2 zinc finger TF (Adams *et al*., 1988; Chang and Timberlake, 1993), initiating the development of conidiophore while *abaA* and *wetA* act during the middle and late stages of conidiation, respectively. This central regulatory pathway (*brlA* → *abaA* → *wetA*) acts in concert with other genes to control conidiation-specific gene expression and determine the order of gene activation during development and spore maturation (Park and Yu, 2012). To establish proper asexual development, *brlA* expression must be preceded by the timely release of repressors of conidiation, NsdD and VosA. During this asexual stage in the lifecycle, *Aspergillus* species produce multicellular reproductive organs, termed conidiophores, each of which produces multiple chains of non-motile conidia. Conidiophore structure and conidia form are diversified depending on the species. The two distantly related species *A. nidulans* and the major producer of aflatoxin *Aspergillus flavus*, whose genomes are more divergent than those of human and chicken (Fedorova *et al*., 2008), form very distinctive conidiophores: some of *A. flavus* conidiophores are lack metula which connects vesicle and phialide in *A. nidulans*. About 36% of *Aspergillus* species are known to reproduce both asexually and sexually (Vries *et al*., 2017). During the sexual stage, they form sexual spores in the fruiting bodies. Sexual reproduction of *A. nidulans* can occur without the presence of a mating partner as they are homothallic (self-fertile). During this stage, mycelia are aggregated to form Hülle cells, which serve as a nuclear reservoir, developmental backup system, and nursing-structure for young fruiting bodies (Troppens *et al*., 2020) and sexual spore (ascospore)-bearing organs called cleistothecia are formed. On the other hand, *A. flavus* requires opposite mating types to reproduce sexually (heterothallic) and produces sclerotia as fruiting bodies. Without crossing with a partner, *A. flavus* is still capable of forming single strain-derived sclerotia (Horn *et al*., 2016). Several regulators of sexual development in *Aspergillus* species have been identified: NsdD

(+), VeA (+), SteA (+), RosA (-), and CryA (-). Furthermore, secondary metabolism is inseparable from fungal growth and development. Secondary metabolites are often associated with developmental processes and have received much attention due to their broad spectrum: antibiotic, antiviral, antitumor, and immunosuppressive activities as well as phytotoxic and mycotoxic activities. (Demain and Fang, 2000; Calvo *et al*., 2002; Bok and Nancy, 2004).

Previous studies have found that the evolutionarily conserved GATA-type transcription factor (TF) NsdD positively regulates sexual development. The deletion of *nsdD* resulted in lack of fruiting body formation, even under the sexual development promoting conditions; the overexpression of *nsdD* led to the increase in fruiting body formation and even resistance to certain inhibitory effects on sexual fruiting in *Aspergillus* (Han *et al*., 2001; Szewczyk and Krappmann, 2010; Cary *et al*., 2012). Furthermore, NsdD acts as a key negative regulator of asexual development by exerting its repressive role via downregulating *brlA* in *Aspergillus* (Lee *et al*., 2014; Lee *et al*., 2016). The GATA-type TF NsdD directly binds to the promoter regions of *brlA* gene, which is responsible for the initiation of conidiation, and represses *brlA* expression in concert with another repressor VosA. Disruption of *nsdD* causes accelerated and precocious activation of conidiation; the mutants even produce asexual developmental organs under liquid-submerged cultures where conidiation hardly takes place unless vegetative cells are exposed to air. NsdD also plays a significant role in altering conidiophore morphology. The *nsdD* null mutant in *A. flavus* resulted in the formation of 10 times shorter conidiophores that resemble closer to those of *A. nidulans* WT (Cary *et al*., 2012; Lee *et al*., 2016). Moreover, mycotoxin production including sterigmatocystin (ST) and aflatoxin (AF) is regulated by NsdD. Interestingly, while ST is a penultimate precursor of AF in AF biosynthetic pathway, the deletion of *nsdD* resulted in enhanced production of ST in *A. nidulans*, whereas it caused the near absence of AF production in *A. flavus*.

NsdD is one of the most well conserved key regulatory elements in the *Pezizomycotina*, which make up most of the *Ascomycota* fungi including Aspergilli (Ojeda-López *et al*., 2018). The NsdD protein is highly conserved in all *Aspergillus* species (Fig. S2-1) and other fungi including *Penicillium*, *Sclerotia*, *Coccidioides*, *Ajellomyces*, and *Fusarium*. To our knowledge, this is the first case where a single GATA-type TF governs all cellular and metabolic developmental traits in fungi. Evolutionary and regulatory mechanisms of NsdD-mediated gene regulatory networks (GRNs) governing cellular and chemical development still remain to be studied.

In the present study, we specifically aimed to elucidate not only the molecular functions of NsdD depending on cell types but also the basic structure of NsdD-mediated GRN using network-based multi-omics approaches in *A. nidulans* and *A. flavus*: transcriptomic and protein-DNA interaction analyses. Primary and secondary metabolite analyses were conducted to validate the genotype-phenotype relationship of the networks. In addition, the way that NsdD differentially forms GRNs between two species was investigated using comparative network analysis. The results propose the species-specific NsdD-mediated GRNs including each core section and the divergence of NsdD networks underlying the evolutionary changes in development and metabolism between *A. nidulans* and *A. flavus*.

2.3 Materials and Methods

2.3.1 *Aspergillus* strains and culture conditions

Aspergillus strains used in this study are listed in Table 2-1. The fungal strains were inoculated into liquid or on a solid 1% glucose minimal medium (GMM) with appropriate supplements and incubated at 37 °C for *A. nidulans* strains or 30 °C for *A. flavus strains*. If needed, 0.1 ~ 0.5% yeast extract (YE) was added to GMM (Pontecorvo *et al*., 1953; Kafer, 1977; Ni and Yu, 2007). To determine the numbers of conidia in *Aspergillus* strains, approximately 10^5 spores were spread onto solid GMM and incubated at 37 °C (*A. nidulans*) or 30 °C (*A. flavus*) for 2 days. The conidia were collected in phosphate-buffered saline (PBS) from the entire plate and counted using a hemocytometer. These 2-day-old conidia are used as an inoculation source and conidia sample. To collect vegetatively growing cells, 5×10^5 conidia$\cdot$ml^{-1} were inoculated into 100 ml liquid GMM and incubated for 36 hr at 37 °C (*A. nidulans*) or 30 °C (*A. flavus*), 220 rpm. To obtain asexually developing cells, postdevelopmental induction was performed as previously described in Seo *et al*. (2003). Briefly, 2×10^6 conidia$\cdot$ml^{-1} were inoculated into 100 ml liquid GMM and cultured for 18 hr at 37 °C (*A. nidulans*) or 30 °C (*A. flavus*). Cultured mycelia were harvested and transferred on fresh solid GMM. The plates were air exposed and grown for 24 hr for asexual developmental induction. For the secondary metabolomic study, 10^5 conidia of wild-type and mutant strains were spread onto solid GMM and placed in constant darkness at 37 °C (*A. nidulans*) or 30 °C (*A. flavus*) for 14 days. The *Escherichia coli* DH5α strain was grown in Luria–Bertani medium with ampicillin (50 mg/ml, Sigma-Aldrich) for plasmid amplification.

2.3.2 Generation of *nsdD* complemented strains in *A. flavus*

The oligonucleotides used in this study are listed in Table S2-1. To complement Δ*nsdD*, the wild-type (NRRL3357) *nsdD* fragment including its 1.8kb 5' untranslated region and coding sequence was amplified with the primer pair OMK718 & OMK719, digested with *EcoRI* and *NdeI*, and cloned into the pHS13 vector (Park *et al*., 2012), which contains a 3×FLAG tag and the *trpC* terminator. The *nsdD*-cloned plasmid pHM1 was confirmed by PCR followed by restriction enzyme digestion and its amino acid sequence is verified with genomic sequencing by using primers: OHM40, OHM42, and OHM39. Then pHM1 is introduced into the Δ*nsdD* mutant (LNJ11) and several THM5 transformants expressing the WT NsdD fused with the 3×FLAG tag at the C-terminus under its native promoter were isolated and confirmed.

2.3.3 Nucleic acid isolation and manipulation

Genomic DNA isolation was carried out as described in Lee *et al*. (2017). Briefly, a loopful of conidia (10^3-10^4/loop) from a solid culture were inoculated into 10 ml of liquid GMM on a sterile plate and incubated at 37 °C (*A. nidulans*) or 30 °C (*A. flavus*) for 12-15 hr. Then semi-transparent mycelial mat was collected, squeeze-dried, and freeze-dried. Freeze-dried fungal tissue was ground by using a motor-spatula tool until it gets into a fine powder and high-quality genomic DNA was isolated. Total RNA isolation and Northern blot analyses were carried out as described (Seo *et al*., 2003; Han *et al*., 2004).

2.3.4 Protein extraction and Western blot analysis

Western blot analysis of NsdD was performed as described in Jeong and Yu (2012). Briefly, 2-day-old conidia (2×10^8) of THM5 were collected and resuspended in the spore lysis buffer containing a 1× protease inhibitor cocktail. Then samples were homogenized by a mini-beadbeater with 0.5mm zirconia/silica beads. Protein concentrations were measured by the Bio-Rad Protein Assay (BioRad). Approximately 15 μg of total proteins per lane were separated on 4-15% gradient SDS-PAGE (BioRad) gel and transferred onto immobilon-P PVDF membrane (Millipore). The membrane was blocked with a blocking buffer, incubated with the monoclonal Anti-FLAG antibody produced in mouse (clone M2, Sigma-Aldrich), and then incubated with HRP-conjugated anti-mouse IgG (Millipore). The membrane was developed using Amersham enhanced chemiluminescence detection reagents (GE Healthcare).

2.3.5 Vegetative growth rate and hyphal branching analysis

For the vegetative growth rate test, 2-day-old conidia of strains were point inoculated onto solid GMM with supplements and cultured at 37 °C (*A. nidulans*) or 30 °C (*A. flavus*). Then samples were taken at 1-day intervals for up to 4 days of cultivation and colony diameter was measured. To analyze hyphal branching patterns between WTs and *nsdD* deletion mutants, samples were taken at 1-hour intervals for up to 21 hours of incubation and entire colonies were examined and photographed under a microscope.

2.3.6 Aflatoxin extraction and TLC analysis

For aflatoxin analysis, 10^5 conidia (2-day-old) samples were inoculated into 2 ml liquid GMM with 0.5% YE and stationary cultured at 30 °C for 5 days. AF was extracted by $CHCl_3$ and analyzed by thin-layer chromatography (TLC) as described in Cary *et al*. (2006). Briefly, each sample was loaded onto a TLC silica plate with a positive control, ~ 1 μg of AF standard (Sigma-Aldrich). The plate was then developed with toluene-ethyl acetate-formic acid (50:40:10, v/v/v) as a mobile phase, air-dried. Photographs of TLC plates were taken under UV light (365 nm).

2.3.7 RNA sequencing analysis

Total RNAs of individual samples were extracted and submitted to Novogene company (Beijing, China) for sample quality check, library preparation, and mRNA sequencing. The quality of total RNA was validated thoroughly in multiple experimental confirmations using 1% agarose gel electrophoresis, Qubit 3.0 fluorometer (Thermo Fisher), and Agilent 2100 Bioanalyzer. During this step, RNA concentration (≥ 20ng/μl), purity (OD260/280 > 2.0), and integrating number (RIN ≥ 6.3) were verified to proceed to the library preparation. A strand-specific library was prepared using an Illumina TruSeq strand-specific RNA sample preparation system. The DNA library of 250-300 bp insert size was constructed and sequenced using an Illumina NovaSeq 6000 platform with a 150-bp paired-end sequencing strategy. Over 3.9×10^7 high-quality reads with 5.7×10^9 clean bases and less than 0.03% base error rate for all samples were achieved. The genome and gene annotations were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/; GCF_000149205.2 for *A. nidulans*; GCF_000006275.2 for *A. flavus*).

Mapping of the clean reads to the genome was carried out using Tophat2 (version 2.0.12, Veg and Asex samples) and hisat2 (version 2.1, conidia samples). The default parameter settings were used for programs unless indicated specifically. > 95% of total reads of conidia RNA and > 84% of total reads in RNA samples of Veg and Asex were mapped to the genome. Gene expression level was processed using HTSeq (version 0.6.1, Veg and Asex samples) and FeatureCounts (version 1.5.0, conidia samples), and finally quantified as FPKM values with a coverage of 100% in each sample. For the differential expression analysis, the values were quantile-normalized using the edgeR package of R (version 4.1.2). We defined a gene as a differentially expressed gene when the expression level is significantly different between WTs and *nsdD* deletion mutants (two-tailed Student's t-test, $p < 0.05$), as well as showed more than 2-fold changes of increase or decrease in raw FPKM values. A fold change (FC) of a gene was calculated by the FPKM value in WT over the FPKM value in Δ*nsdD* and converted into the logarithm to the base 2 so that a gene with a negative $\log_2$ FC value is up-regulated in Δ*nsdD*, indicating NsdD actually represses this gene's expression. Positive $\log_2$ FC values work oppositely.

2.3.8 Chromatin immunoprecipitation sequencing analysis

Samples for chromatin immunoprecipitation sequencing (ChIP-seq) analysis were prepared as described (Ahmed *et al*., 2013; Park *et al*., 2015; Lee *et al*., 2016). Briefly, two-day-old conidia (2×10^9) of TMK13 and THM5 were cross-linked with 1% formaldehyde, resuspended in spore lysis buffer, and homogenized by a mini-beadbeater with 0.5mm zirconia/silica beads. The lysates were then sonicated for five to seven cycles (60 s on, 60 s off) with a sonifier (Jeong and Yu, 2012). After centrifugation, the lysates were diluted in ChIP dilution buffer and then were applied for ChIP assays according to the manufacturer's instructions using the MAGnify Chromatin Immunoprecipitation System (Invitrogen) with a modest modification. The diluted chromatin extracts were incubated with 1 μg of mouse monoclonal Anti-FLAG antibody (Sigma-Aldrich). As negative controls, the chromatin extract was reacted with 1 μg of Anti-rabbit IgG. Initial input DNAs before immunoprecipitation were used as positive controls. The enriched DNA fragments were retrieved and used as a template for ChIP-PCR and ChIP-seq. The primer pairs used for PCR are shown in Table S2-1.

A. nidulans ChIP DNA samples were sent to ProteinCT (Madison, WI) and *A. flavus* ChIP DNA samples were submitted to Novogene company (Beijing, China) for library preparation and sequencing. DNA libraries were prepared using TruSeq ChIP library Preparation

Kit (Illumina) and sequenced using an Illumina HiSeq2500 (*A. nidulans*) NovaSeq 6000 (*A. flavus*) platform. More than 8 million (*A. nidulans*) and 18 million (*A. flavus*) reads per sample were achieved and DNA fragment sizes of each sample were ranged between 50 to 150 bp. The read sequences were mapped to the genome using bowties2 (*A. nidulans*) and BWA (version 0.7.12, *A. flavus*) and Homer (*A. nidulans*, Langmead *et al*., 2009; 2012) and MACS2 (version 2.1.0, *A. flavus*) were utilized to call peaks. Identification of NsdD direct targets was done by selecting genes, in which NsdD peaks are found in their promoter regions within the 1.5kb upstream range from the translation start site (TSS). Then the NsdD response elements (NREs) were analyzed using the MEME-ChIP program as described in Wu *et al*. (2018). Briefly, all read sequences achieved from ChIP-seq were processed using MEME-ChIP software version 4.12.0 (Machanick and Bailey, 2011). For the MEME analysis, the default parameters were used except 10 for number of motifs and 5 to 21 bp for length.

2.3.9 Functional enrichment analysis

Gene Ontology enriched terms were identified using the tools available at FungiDB (Stajich *et al*., 2012). The parameters for this study were biological process for the ontology, no limit to GO Slim terms, and 0.05 p-value for the cutoff. Then enriched terms were sorted by p-value in ascending order or number of genes in descending order. Both approaches were considered together to determine the most functional enriched terms.

2.3.10 Trehalose quantification and primary metabolite analysis

Trehalose quantification was performed as described in Tao and Yu (2011). Briefly, 2-day-old conidia ($2{\times}10^8$) were collected, resuspended in 200 μl ddH_2O, and incubated at 95 °C for 20 min. Samples were centrifuged and the supernatant containing trehalose was collected. The supernatant was mixed with 50 µl 0.2 M sodium citrate (pH 5.5) and 3 mU trehalase (Sigma-Aldrich) and incubated at 37 °C for 8 h. Trehalase activity was assayed using a Glucose (GO) Assay kit (Sigma-Aldrich) following the manufacturer's instructions.

The samples for primary metabolite analysis were prepared as described in Wu *et al*. (2021). Briefly, 2-day-old conidia (2×10^8) of each sample were suspended with 500µl HPLC-grade acetonitrile-methanol-water (40:40:20, v/v) and 300µl beads, homogenized by using a mini-beadbeater with 0.5mm zirconia/silica beads and centrifuged. The supernatant was filtered using a 0.45-mm PTFE Mini-UniPrep filter (Agilent) and used for the analysis.

The samples were then analyzed as described (Wang *et al*., 2016; Ostrem *et al*., 2019). The HPLC-MS consisting of a Dionex ultra-high-performance liquid chromatography (UHPLC) instrument coupled by electrospray ionization (ESI) (negative mode) to a hybrid quadrupole–high-resolution mass spectrometer (Q Exactive orbitrap; Thermo Scientific) operated in full-scan mode was performed for the analysis. From the raw data of primary metabolism, differentially produced metabolites between wildtypes and Δ*nsdD* strains were identified if they meet the criteria: p-value less than 0.05, peak intensity more than 10^6, and a $\log_2$ fold change less than -1 or greater than 1. To identify the known metabolites, their exact masses were evaluated with an accuracy of three decimal digits and retention times were matched within the ±1.5 range to those of pure standards (Sigma-Aldrich).

2.3.11 Secondary metabolite analysis

For secondary metabolite analysis, WT and *nsdD* deletion mutants were point inoculated into solid GMM, thoroughly wrapped with Parafilm (Bemis), and incubated at 37 °C (*A. nidulans*) or 30 °C (*A. flavus*) for 14 days. Whole agar plates were blended in methanol (300 ml), sonicated for 60 min, and left for 24 hr at room temperature. The solid materials were removed using vacuum filtration and the organic phase was separated and dried over anhydrous magnesium sulfate. After additional filtration, each solvent was evaporated *in vacuo* to afford the organic extract. Each dried organic extract was measured in a 20 ml vial. The extracts were normalized to 10 mg/ml in acetonitrile and filtered through a 0.45 μm Acrodisc syringe filter with nylon membrane (Pall Corporation). The samples were analyzed using Ultra-high-performance high resolution mass spectrometry (UHPLC-HRMS) in both ESI positive and negative modes within the m/z range from 150 to 1500. UHPLC-HRMS data were acquired using Thermo Scientific Vanquish UHPLC system (Waltham) connected to a Thermo Scientific Q Exactive Orbitrap mass spectrometer (Waltham). A Zorbax Eclipse XDB-C18 column (2.1 × 150 mm, 1.8 μm) was used with a flow rate of 0.2 ml/min for all samples. Water with 0.05% formic acid and acetonitrile (MeCN) with 0.05% formic acid were used with the following gradient: 0 min, 80%aq. MeCN; 15 min, 2%aq. MeCN; 20 min, 2%aq. MeCN. XCMS online (version 3.7.1, Scripps Research Institute) was used for data acquisition and procession. For the identification of differentially produced metabolites between wild types and Δ*nsdD* strains, the same strategy from primary metabolism was applied except for matching metabolites with known standards. To identify the known secondary metabolites, their exact masses with an accuracy of three decimal digits were matched to the *A. nidulans* and *A. flavus* metabolite lists retrieved from the renowned microbial natural products database NPAtlas (version 2021_08, van Santen *et al*., 2022).

2.3.12 Gene regulatory network analysis

We defined a species-specific NsdD-mediated GRN as a protein-protein interaction network (PPIN), which consists of NsdD, direct targets of NsdD identified from ChIP-seq, and differentially expressed genes identified from transcriptome analyses in conidia samples. The known protein-protein interactions in *A. nidulans* and *A. flavus* were obtained from the renowned gene network database STRING (version 11.5, Franceschini *et al*., 2013), by matching the protein ID and gene ID using the 'protein.aliases' table provided by the database. We firstly remained only the edges supported by protein-protein interaction from the whole STRING network with a threshold for confidence score of 150. Then we selected out the edges which have both nodes belonging to either the direct target genes of NsdD and/or the DEGs. These nodes and edges were used as a backbone of our network. For visualizing the species-specific networks, NsdD was placed at the center of the networks, genes from the ChIP-seq analyses encircled NsdD, and genes from the RNA-seq analyses were laid out in the outer area. To investigate the fundamental and systematic pathways underlying our findings, we analyzed the species-specific networks based on the "guilt-by-association (GBA)" principle, which was fulfilled by including the first neighbors of the backbone networks found from the full set of PPINs and then we were able to extract the core sections of the species-specific networks. For the visualization of the core networks, the connections between *nsdD* gene and several extensively studied transcription factors or regulators identified from ChIP-seq were highlighted and the genes from RNA-seq were laid out in the bottom area. Different shapes containing gene names were used to indicate the source of a gene: rectangle for genes from ChIP-seq and ellipse for genes from RNA-seq. Then these shapes were colored depending on the predicted functional categories: vegetative growth (pale green), asexual development (green), sexual development (deep saffron), primary metabolism (blue), secondary metabolism (magenta), and transcription regulation (red). The network visualization was performed using Cytoscape software (version 3.9.1, Shannon *et al*., 2003).

For comparative network analysis between *A. nidulans* and *A. flavus*, conserved orthologous genes including the *nsdD* gene were identified using the ortholog list of those *Aspergillus* species available in Wu *et al*. (2018). Then we selected out the orthologs found from both species-specific networks and visualized the orthologs by locating them near the center of the network (NsdD) and plotting the other genes in the same manner used in the species-specific network construction manually

2.4 Results

2.4.1 Verification of *nsdD* complementation

The Δ*nsdD* mutant displayed lower radial colony growth, much shorter conidiophores, and no sexual fruiting body formation which are remarkable and recognizable phenotypes. The re-introduction of the *nsdD* gene fused with the 3×FLAG tag into the *nsdD* mutant restored these phenotypes. The THM5 strain showed similar radial growth and conidiophore morphology on solid GMM (Fig. 2-1A). under SM promoting conditions, it produced sclerotia (fruiting bodies) (Fig. 2-1C). In addition, the *nsdD* null mutant barely produced aflatoxins, but the *nsdD* complemented strain produced a substantial amount of aflatoxins showing a similar level compared to WT(Fig. 2-1D). These observations suggest that the re-introduced *nsdD* gene is able to express functionally operating NsdD::FLAG3X proteins and the FLAG tag does not interfere with the activity of the NsdD protein. To verify whether the *nsdD* gene fused with the FLAG tag is properly transcribed and translated in the THM5 strain, Northern blot and Western blot analyses were performed on vegetative and conidia cells. In vegetative cells (48 hr), *nsdD* transcript is barely observed in Δ*nsdD* as it's the K/O mutant, yet THM5 was able to express *nsdD* transcripts (Fig. 2-1F).

Our previous studies revealed that *A. nidulans nsdD* encodes two distinct transcripts, *nsdD*α and *nsdD*β and these transcripts encode the NsdDβ (461 aa) and NsdDα (424 aa) polypeptides. The levels of both NsdDα and NsdDβ remain high in vegetative and asexually developing cells, yet NsdDα becomes undetectable after developmental induction. Very low amounts of NsdDα and NsdDβ were detected in conidia. To figure out whether *A. flavus* transcripts were translated into two distinct active proteins, we carried out Western blot analysis employing THM5 strain expressing NsdD::3×FLAG ectopically in Δ*nsdD* with an anti-FLAG antibody. As THM5 expresses *nsdD* transcripts in Vege, Vege (24 hr) samples were used. NsdD protein was detected in THM5 with approximately 56 kDa size, which is predicted as NsdDβ (503 aa). Then we used 2-day-old conidia samples to confirm the presence of NsdD protein for ChIP sequencing. NsdD protein was observed at around 50 kDa size (Fig. 2-1G), which is predicted as NsdDα (453 aa). These findings indicate that *nsdD* complementation was successfully done as NsdD::3×FLAG protein was properly expressed and functioned like a native NsdD and that *A. flavus* similar to *A. nidulans* generates two different forms of NsdD: NsdDα and NsdDβ in conidia and vegetatively growing cells, respectively.

2.4.2 Roles of NsdD in *Aspergillus* morphogenesis

To understand the effect of NsdD in *Aspergillus* morphology, we performed analyses on hyphal branching, vegetative growth rate, and asexual structure morphology. First, we observed a morphological variation in conidia between *A. flavus* wild type and *nsdD* mutant (Fig. 2-2A). Disruption of *nsdD* did not change the conidial form in *A. nidulans*, but it induced *A. flavus* to produce bigger and variable sizes of conidia (Fig. 2-2B). Then trehalose quantification was performed to figure out whether these bigger spores contain more amounts of cellular/cytosolic compounds, particularly trehalose. Despite the increase in conidial size, no significant difference in the levels of trehalose amount was detected between WT and Δ*nsdD* (Fig. 2-2C). Subsequently, we investigate how these spores germinate, elongate, and form a colony. After 6 hours of incubation on solid media, conidia of *A. nidulans* WT and Δ*nsdD* strains started to germinate and there was no significant difference in germination timings observed. On the other hand, in *A. flavus*, *nsdD* null mutants germinated at 4 hours after inoculation, yet for the wild type, it took 5 hours repeatedly. Aberration of *nsdD* caused early germination in *A. flavus* with one hour gap, but not in *A. nidulans* (data not shown). After 12 hours of incubation, we found *nsdD* mutants of both species displayed different hyphal growth patterns compared to wild types (Fig. 2-2D). At that time point, hyphae of wild types were straightforward with almost no hyphal branching. In Δ*nsdD*, however, a zigzag pattern of hyphal elongation and abnormal hyphal branching were noticed. Then, we investigated conidiophore morphology. In *A. nidulans*, we did not discover any changes in conidiophore structure between WT and Δ*nsdD*, but a drastic alteration in morphology of asexual structures was monitored in *A. flavus*. *A. flavus* Δ*nsdD* mutant exhibited a dwarf phenotype on conidiophores: approximately 4 times smaller conidiophore in width from the top view, 10 times shorter stalks in length, and 3 times smaller vesicles in size compared to those of WT (Fig. 2-3). Of note, these dwarf conidiophores of *A. flavus* Δ*nsdD* became resemble closer to those of *A. nidulans* WT. These results suggest collectively that NsdD plays an important role in the general morphology of *Aspergillus*; especially it acts as a morphological determinant of asexual developmental structures in *A. flavus*.

2.4.3 Species-specific and cell type-dependent gene regulation of NsdD

To understand the regulatory roles of NsdD depending on different cell types or developmental stages in *A. nidulans* and *A. flavus*, we carried genome-wide gene expression analyses in WTs and null mutants vegetative cells (36 hr), asexually developing cells (24 hr), and conidia (2-day-old) using RNA-seq. We found that the regulatory effects of NsdD substantially vary among cell types as well as species; 23.03%, 42.99%, and 9.57% of total genes (10,988) are differentially regulated in *A. nidulans* Δ*nsdD* Vege, Asex, and Conidia, respectively, and 3.3%, 9.18%, and 14.56% of total genes (13,485) showed differential accumulation of mRNA in *A. flavus* Δ*nsdD* Vege, Asex, and Conidia, respectively (Fig. 2-4). Among the differentially expressed genes (DEGs), 40%, 77%, and 32% were up-regulated and 60%, 23%, and 68% were down-regulated in *A. nidulans* Δ*nsdD* Vege, Asex, and Conidia, respectively. In the case of *A. flavus* DEGs, 49%, 46%, and 68% were up-regulated and 51%, 54%, and 32% were down-regulated in Δ*nsdD* Vege, Asex, and Conidia, respectively. We further identified these DEGs whether they belong to core genes or lineage-specific genes between the two species. Our previous study revealed that two species share 7,630 orthologs; thus 3,358 genes (30.56%) in *A.nidulans* and 5,855 genes (43.42%) in *A.flavus* are lineage-specific (Wu *et al*., 2018). Regardless of up- or down-regulation pattern, *A. nidulans* Vege and *A. flavus* conidia DEGs somewhat followed the composition of core and lineage genes in species, whereas NsdD tended to affect more lineage-specific genes in *A. nidulans* conidia, *A. flavus* Vege and Asex and more core genes in *A. nidulans* Asex compared to the gene composition (Fig. 2-5). Put together, these results indicate that NsdD forms gene regulatory networks in species-specific and cell type-dependent manners.

To gain further understanding on the regulatory roles of NsdD-mediated GRNs, functional category enrichment analyses were performed in the two species and the three different cell types using Gene Ontology (GO) terms. First, in *A. nidulans* Vege and Asex, translation processes and protein metabolism-related processes were up-regulated in Δ*nsdD*, while transmembrane transport and cellular carbohydrate metabolic processes were down-regulated in Δ*nsdD*, especially secondary metabolic processes are also down-regulated in Δ*nsdD* Asex. In *A. nidulans* Δ*nsdD* conidia, amino acid catabolic and carbohydrate transmembrane transport processes were up-regulated, but secondary metabolic processes and fungal cell wall component (β-1,3-glucan) metabolism-related processes were down-regulated. These results suggest that *A. nidulans* NsdD activates the metabolism of cellular carbohydrates and secondary metabolites, yet represses protein metabolic processes from a translation during vegetative growth and asexual development. On the other hand, in conidia, NsdD activates metabolic processes of cell wall components and secondary metabolites and inhibits carbohydrate transmembrane transports and amino acid catabolic processes (Fig. S2-2). In *A. flavus* Vege and Asex, most of secondary metabolism-related processes were down-regulated in Δ*nsdD*. Macromolecule and peptidoglycan biosynthetic processes were up-regulated in Δ*nsdD* Vege and peptide metabolic process with translation and D-xylose metabolic processes were up-regulated in Δ*nsdD* Asex. Surprisingly in Δ*nsdD* conidia, amino acid catabolic and oxidation-reduction processes were down-regulated, while many process regulations were up-regulated (Fig. S2-3). These findings suggest that *A. flavus* NsdD activates secondary metabolism including mycotoxin production, but negatively regulates metabolism of cellular components including carbohydrates and proteins in Vege and Asex. Remarkably in Δ*nsdD* condia, NsdD negatively regulates many processes such as RNA biosynthesis and DNA-templated transcription from the transcription level but activates amino acid catabolic processes. The most up- or down-regulated 50 DEGs in *A. nidulans* Δ*nsdD* Vege, Asex, conidia, *A. flavus* Δ*nsdD* Vege, Asex, and conidia are listed in Table S2-2 to S2-13, respectively. In addition, the list of DEGs related to asexual development in *A. nidulans* and *A. flavus* is shown in Table S2-14 and Table S2-15, respectively, the list of DEGs related to G protein signaling pathway in *A. nidulans* and *A. flavus* is shown in Table S2-16 and S2-17, respectively, the list of DEGs predicted to encode transcription factors in *A. nidulans* and *A. flavus* is shown in Table S2-18 and Table S2-19, respectively, and the list of DEGs predicted to encode kinases in *A. nidulans* and *A. flavus* is shown in Table S2-20 and Table S2-21, respectively. Overall, functional category analyses indicate that NsdD regulates all aspects of fungal biology in *Aspergillus* species: transcription, translation, development, metabolism, and other biological processes and that NsdD-mediated GRNs might be partially or fully operated in accordance with the fungal developmental stages.

Our previous study elucidated that NsdD is a key repressor of conidiation, which physically binds to the promoter regions of *brlA* gene that is responsible for the initiation of conidiation and hampers the gene expression of *brlA* until suitable environments for asexual development are prepared. In addition to the *brlA* regulation of NsdD, we also proposed the genetic model for growth and developmental control in *Aspergillus* (Lee *et al*., 2014). To understand the regulatory impact of NsdD on this genetic model, we overlaid all element genes of the model with gene expression level changes identified from RNA-seq (Fig. 2-6). In *A. nidulans* Δ*nsdD* Asex, all three key regulators (*brlA*, *abaA*, and *wetA*) and the other key repressor of conidiation, *vosA* showed increased mRNA levels as we expected. Interestingly, a similar pattern was observed in *flbD* and *flbE*, which are direct upstream developmental activators (UDAs) of *brlA* even though their upstream repressor *sfgA*'s mRNA level was increased. These results suggest that NsdD might acts as a repressor of *flb* genes as well as *brlA*. Within the increased *sfgA* gene expression level, *flb* genes are supposed to be down-regulated including *flbA*, yet all *flb* genes are up-regulated except *flbB* and *flbC,* suggesting a possible negative role of NsdD in *flb* gene regulation. Unlike NsdD of *A. nidulans*, we noticed only a few statistically significant differences in the gene expression level of genes in *A. flavus*. The *brlA* and *abaA* were identified as not differentially expressed in Δ*nsdD* Vege and Asex as their p-values did not pass the cutoff value (less than 0.05), however, they showed 2 to 6 times higher expression levels compared to those in WT, suggesting that an analogous regulatory pattern of NsdD occurred in *A. flavus* as well. In *A. flavus* Δ*nsdD* Asex, both *wetA* and *vosA* genes are up-regulated as anticipated. The increased level of *vosA* mRNA is likely attributed to the positive feedback of WetA, however, the increased mRNA levels of *vosA* in Δ*nsdD* Vege was a novel finding. These results suggest that NsdD represses the expression of *vosA* gene and that the known role of VosA in *Aspergillus* asexual development may be slightly different in *A. flavus*. For sexual development, the known sexual development regulators *veA*, *rosA*, and *cryA* were downregulated in *A. nidulans* Δ*nsdD* Vege.

Next, we checked the expression levels of genes involved in sterigmatocystin (ST)/aflatoxin (AF) biosynthetic gene cluster (BGC) to understand genetic variations underlying the changes in ST/AF production. As shown in Figure 2-7, most of the genes (18/24) related to ST-BGC were downregulated in *A. nidulans* Δ*nsdD* Asex, whereas 70% (24/34) of AF-BGC genes were downregulated in *A. flavus* Δ*nsdD* Vege, although the BGC transcription factor *aflR*s' expression had not been changed. These results suggest that NsdD-mediated GRNs regulate sterigmatocystin/aflatoxin biosynthesis in a similar way between two species.

2.4.4 Identification of NsdD potential direct targets in conidia

We have utilized three different cell types, Vege, Asex, and conidia, to understand the broad regulatory roles of NsdD in *Aspergillus* biology, however, we decided to focus on conidia for further NsdD direct target identification and network analysis revealing the regulatory mechanism of NsdD-mediated GRNs. As conidia are unicellular, haploid, and isogenic to the haploid parent (Yu, 2010), these genetic traits provide practical advantages for NsdD-DNA interaction analysis; The ChIP experiment from sample preparation becomes more tractable and the result gets clearer as all genetic behavior is homogenous in conidia. To identify the direct target genes of NsdD, ChIP followed by high-throughput sequencing was performed using the FLAG epitope-tagged NsdD production strains TMK13 and THM5 in *A.nidulans* and *A. flavus* conidia, respectively. We identified totals of 502 (4.6% of 10,988 genes) and 674 (5% of 13,485 genes) putative direct target genes that their promoter regions were bound by NsdD in *A. nidulans* and *A. flavus*, respectively. Among them, we found some notable regulators known to govern asexual development and/or sexual development and/or secondary metabolism in *Aspergillus*; *veA*, *flbD*, *laeA*, *kapA*, *rosA*, and *steA* in *A. nidulans* as well as *veA*, *flbA·C·D*, *vosA*, *brlA*, and *rosA* in *A. flavus*. In addition, *nsdD* was found in both species, indicating the presence of its self-feedback loop. To explore the significance of NsdD in terms of transcription regulation, we sorted out genes predicted to encode transcription factors from all putative direct target gene lists. We found 24 and 41 TFs including putative ones in *A. nidulans* and *A. flavus* conidia, respectively. The most enriched 100 putative direct target genes of NsdD in *A. nidulans* and *A. flavus* conidia are listed in Table S2-22 and S2-23, respectively, and all direct targets predicted to encode transcription factors in *A. nidulans* and *A. flavus* conidia are listed in Table S2-24 and S-25, respectively. Then NsdD response element identification was conducted using Multiple Em for Motif Elicitation (MEME) analysis in both species. A very substantial degree of consensus on the NRE was recognized between the two species; the predicted NRE was 5’-GATCT-3’ (Fig. 2-8A).

To identify potential direct target genes of NsdD, we compared the results of the ChIP-seq and RNA-seq analyses. The 68 and 126 genes were identified as potential direct targets of NsdD, which are bound by NsdD in promoter regions and differentially expressed in the absence of NsdD (Fig. 2-8B). To investigate the functions of potential direct targets, the functional enrichment analyses were performed; in *A. nidulans*, the potential direct targets were involved in various types of transmembrane transportation and in *A. flavus*, the direct targets were related with the regulation of diverse biological processes including transcription. All potential NsdD direct targets in *A. nidulans* and *A. flavus* conidia are listed in Table S2-26 and S2-27, respectively. Taken together, these results suggest that NsdD forms distinct GRNs depending on species by directly regulating large numbers of TFs and genes resulting in the genome-wide extensive change of gene expressions.

2.4.5 The alteration of primary metabolite production in Δ*nsdD* conidia

From functional category enrichment analyses, we discovered that the deletion of *nsdD* affected the mRNA expression of many genes associated with general metabolic processes (peptide, carbohydrate, nitrogen compound, etc.) and metabolism of amino acids. To investigate whether these genetic shifts led to the actual alteration in the amount of primary metabolite production (genotype-phenotype relationship), the abundances of total primary metabolites including amino acids, citric acids involved in the citric acid cycle (CAC), and energy-related metabolites such as ATP, NAD, and NADP, were examined in WTs and *nsdD* mutants of *A. nidulans* and *A.flavus* conidia (Fig. 2-9). The production of 111 and 167 primary metabolites were significantly ($p < 0.05$ and $|\log_2 FC| > 1$) altered in *A. nidulans* and *A.flavus* Δ*nsdD* conidia, respectively. In *A. nidulans* Δ*nsdD* conidia, 59 metabolites out of 111 were decreased in the abundances, while 52 metabolites were increased in the amounts. In *A. flavus* Δ*nsdD* conidia, 97 primary metabolites out of 167 were increased in the amounts and the abundances of 70 metabolites were decreased. All primary metabolites affected in the abundances in *A. nidulans* Δ*nsdD* conidia and *A. flavus* Δ*nsdD* conidia are listed in Table S2-28 and S2-29, respectively.

The abundances of 12 amino acids (alanine, arginine, asparagine, glutamine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) and three citric acids (citrate, aconitate, and α-ketoglutarate) were affected in *A. nidulans* Δ*nsdD* conidia. In *A. flavus* Δ*nsdD* conidia, most amino acids (16/20) were affected in the abundances except asparagine, cysteine, glutamate, and histidine. Moreover, the abundances of 6 citric acids (acetyl-CoA, citrate, aconitate, α-ketoglutarate, succinate, and malate) were altered (Fig. 2-9A). In addition, of note, we found that most energy metabolism-related metabolites were produced in higher amounts from 21.3% to 357.9% increase in *A. flavus* Δ*nsdD* conidia compared to those of WT, while we only observed the changes in NAD^+ and $NADP^+$ amounts in *A. nidulans* Δ*nsdD* conidia (Fig. 2-9B). Put together, these results indicate that NsdD regulates overall primary metabolism in a species-specific manner and has a heavy impact on energy metabolism exclusively in *A. flavus*.

2.4.6 The regulatory roles of NsdD in secondary metabolism

Previous studies unveiled that NsdD plays a repressive role in sterigmatocystin production in *A. nidulans* but activates the biosynthesis of aflatoxin in *A. flavus*. Along with these findings, many DEGs in Δ*nsdD* were involved in secondary metabolic processes including mycotoxin production according to the GO term analyses. In addition, we checked an alteration in the mRNA expression level of multiple biosynthetic gene clusters encoding numerous secondary metabolites, including sterigmatocystin/aflatoxin, asperfuranone, aspernidine, asterriquinone, austinol, dehydroaustinol, imizoquin, monodictyphenone, penicillin, and ustiloxin (Table S2-30 and S2-31). To elucidate the regulatory effect of NsdD in secondary metabolism, secondary metabolites were extracted and subjected to UHPLC-HRMS analysis in the two species. The abundances of 555 and 195 secondary metabolites were significantly ($p < 0.05$ and $|\log_2 FC| > 1$) affected in *A. nidulans* and *A. flavus* Δ*nsdD*, respectively. In *A. nidulans* Δ*nsdD*, the production of 381 metabolites was decreased and the abundances of 174 metabolites were increased. Among them, interestingly, 196 metabolites were produced in WT, however, their productions were completely halted so that they were not detected in Δ*nsdD*. On the other hand, 9 metabolites were only found in Δ*nsdD*, not in WT. In *A. flavus* Δ*nsdD*, the abundances of 88 secondary metabolites were declined, yet the production of 107 metabolites was enhanced. Among them, 26 metabolites were present in WT but not found in Δ*nsdD*, while 21 metabolites were only found in Δ*nsdD*. The lists of secondary metabolites affected in the abundance in *A. nidulans* Δ*nsdD* and *A. flavus* Δ*nsdD* are in Table S2-32 and S2-33, respectively. These whole metabolomic analyses demonstrate that NsdD plays a vital role in the global regulation of secondary metabolism in *Aspergillus*.

Next, we focused on secondary metabolites of interest to obtain practical insights for the real application. Known secondary metabolites were identified matching the exact masses with an accuracy of three decimal digits from the whole metabolite list achieved from the secondary metabolite analyses to the *Aspergillus* natural products database. Totals of 25 and 5 known secondary metabolites were discovered in *A. nidulans* and *A. flavus*, respectively (Table 2-2). To further understand the correlation between the alteration in the mRNA expression of biosynthetic gene clusters and the production of their corresponding secondary metabolites, we checked whether BGCs of these known metabolites were affected by NsdD in any cell types. Interestingly, the abundances of alternariol, austinol, dehydroaustinol, asterriquinone, and emericellamide C/D were significantly changed and the backbone genes of these metabolites' BGCs were differentially expressed in *A. nidulans* Δ*nsdD*. In *A. flavus* Δ*nsdD*, the deletion of NsdD led to alterations in the production of imizoquin D and leporin B and the mRNA expression of corresponding genes as well (Fig. 2-10, Table S2-29, and Table S2-30). The backbone genes of these metabolite BGCs are predicted to encode a polyketide synthase (*pkgA*, *ausA*, and *easB*), non-ribosomal peptide synthetase (*tdiA*, *easA*, and *imqB*), and transcription factor (*lepB*). More importantly, the relative expression level of genes and the relative abundance of metabolites shared an analogous trend, sometimes almost identical e.g., asterriquinone and imizoquin D production, implying the genotypic alteration is tightly related to the metabolic production in secondary metabolism. Put together, these results prove that NsdD governs the gene expression of secondary metabolite BGCs and the production of their associated metabolites in *A. nidulans* and *A. flavus*.

2.4.7 NsdD-mediated gene regulatory networks in *Aspergillus*

To dissect the detailed regulatory mechanism of NsdD underlying cellular and chemical development of *Aspergillus*, the network analysis was performed integrating the ChIP-seq, the RNA-seq, and protein-protein interaction database. Totals of 1,486 and 2,512 genes were adopted from the ChIP seq (504 and 674 genes) and the RNA-seq (1,052 and 1,964 genes) in *A. nidulans* and *A. flavus* conidia, respectively. (Fig. 2-8A). Then we examined the recognized protein-protein interactions (PPIs) of these genes and NsdD among them from the database. NsdD was previously known to interact with 263 genes and 262 genes including itself in *A. nidulans* and *A. flavus*, respectively, however, we noticed that these numbers only constitute 1.1% to 4.7% of the NsdD's interactions that we proposed in this study (Fig. S2-4). Thus, these findings evidently suggest novel PPIs of NsdD in the two species, providing the basic knowledge on NsdD-mediated regulatory mechanisms.

Next, we constructed species-specific NsdD-mediated gene regulatory networks by integrating the genes from RNA- and ChIP-seq analyses and their corresponding PPI data. To facilitate further investigation on the regulatory mechanism of NsdD, the species-specific networks were visualized using the Cytoscape software. During the visualizing process, the source of genes was indicated with different shapes: rectangle for genes from ChIP-seq and ellipse for genes from RNA-seq and the interactions between NsdD and rectangles were highlighted as a thicker edge in that they are direct targets of NsdD (shown in Fig. S2-5 and Fig.

S2-6). Thereafter, to explore the core sections of the NsdD-mediated networks, the species-specific networks were analyzed by applying the “guilt-by-association (GBA)” principle to the important regulators of development and metabolism found in the ChIP-seq analyses. For the *A. nidulans* core network analysis, *veA*, *flbD*, *laeA*, and *steA* were selected and analyzed. In *A. flavus*, *veA*, *flbA·C·D*, *vosA*, *brlA*, and *mpkB* were selected for the core network analysis. The component number of the core networks was restricted to around 30 nodes for proposing network models as concise as possible while covering the details. The nodes were colored by their functional categories. The proposed NsdD core networks of each species are shown in Fig. 2-11 and all genes forming the core networks of *A. nidulans* and *A. flavus* are listed in Tables 2-3 and 2-4, respectively.

Within the *A. nidulans* NsdD core network, we found that the genes were related to asexual development, sexual development, primary metabolism, and secondary metabolism. Well-known asexual development regulators such as *flbD*, *brlA*, *abaA*, *lreA*, *lreB*, and *veA* appeared in the network. FlbD, a Myb transcription factor, coordinates the initiation of conidiation in *Aspergillus* by inducing the gene expression of *brlA* (Wieser and Adams, 1995). BrlA activates the expression of *abaA* and other genes during the early stage of conidiation (Adams *et al*., 1988; Adams *et al*., 1990), then AbaA regulates the expression of *wetA*, *vosA*, *velB*, and other genes (Andrianopoulos and Timberlake, 1991; 1994). The *rodA* gene encoding a protein component of the conidial hydrophobic (rodlet wall) layer is also transcriptionally regulated by BrlA (Stringer *et al*., 1991, Chang and Timberlake, 1993). We found this sequential transcriptional activation (FlbD→BrlA→AbaA/RodA) in the core network as well. Overlaying with the results of the RNA-seq analyses, the expression of *flbD*, *brlA*, *abaA*, and *rodA* was increased in Δ*nsdD* Asex simultaneously and we also observed that the gene expression pattern of *brlA* and *rodA* in Δ*nsdD* appeared to be consistent in all three cell types as both were all up-regulated in Vege and Asex, but down-regulated in conidia. For secondary metabolism of the core network, we found *laeA*, two TFs (*afoA* and *mtfA*) and other genes involved in secondary metabolite BGCs. LaeA serves as a global regulator of secondary metabolism in *Aspergillus* species (Bok and Keller, 2004). LaeA forms a heterotrimer with VeA and VelB and this velvet complex coordinates development and secondary metabolism in *A. nidulans* (Bayram *et al*., 2010; Eom *et al*., 2018). Along with the protein interaction, they showed the same gene expression pattern in Δ*nsdD*: downregulation in Vege and upregulation in Asex. Within the network, both *laeA* and *veA* interact with most of secondary metabolism-related genes. Their expression changes may be responsible for the global alterations of secondary metabolism in Δ*nsdD* mutants. Furthermore, LaeA positively regulates the expression of *tdiB*, which encodes a dimethylallyl-L-tryptophan synthase required for the terrequinone production in *A. nidulans* (Bok *et al*., 2006). The mRNA levels of both *laeA* and *tdiB* were up-regulated in Δ*nsdD* Asex; we also noticed that the production of asterriquinone and terrequinone A was enhanced in Δ*nsdD*. Particularly, terrequinone A was only detected in Δ*nsdD*, but not in WT. These results demonstrate that NsdD utilizes upstream regulators to form the NsdD-mediated gene regulatory network governing development and metabolism in *A. nidulans*.

Within the *A. flavus* NsdD core network, two additional functional categories were included compared to the *A. nidulans*' network: vegetative growth and transcription regulation. For asexual development, *brlA* gene and its several upstream regulators (FlbA·C·D (+) and VosA (-)) appeared in the network. The bZIP-type TF AtfA regulates asexual development by governing stress responses especially oxidative stress (Emri *et al*., 2021) In addition, unexpectedly, we found three putative forkhead box (FOX) genes including AFLA_048110, which is predicted to encode a TF and up-regulated in Δ*nsdD* conidia. The FOX genes have been well characterized in vertebrates and some fungi. The FOX proteins play important regulatory roles in cell proliferation, immunity, and metabolism; especially they are important for cell cycle control and morphogenesis in fungi (Ribár *et al*., 1999; Bulmer *et al*., 2004; Golson and Kaestner, 2016). In *A. nidulans*, six forkhead genes (*fkhA-F*) were identified and characterized. FkhA is a positive regulator of sexual development and other forkhead proteins affect asexual development (Lee *et al*., 2005; Park *et al*., 2009). The roles of *brlA* and its regulators in asexual development have been studied extensively, however, we still lack information on the molecular functions of FOX proteins in *Aspergillus flavus*. These results suggest the divergent role of *A. flavus* NsdD in the regulation of FOX genes affecting sexual and asexual development in comparison with *A. nidulans* NsdD. For primary metabolism, *areA* and *creA* may play important roles within the *A. flavus* NsdD-mediated GRN. AreA and CreA are involved in nitrogen and carbon metabolite repression, respectively. By repressing syntheses of enzymes and permeases involved in nutrient acquisition and utilization, these transcription factors enable fungi to utilize preferred nutrients such as ammonium and glucose (Kudla *et al*., 1990; Dowzer and Kelly, 1991). These results suggest that NsdD affects nitrogen and carbon metabolism by regulating the expression of *areA* and *creA* genes. Unlike the NsdD core network in *A. nidulans*, we observed that approximately one-third of the genes (11/31) in the *A. flavus* core network were involved in transcription regulation processes. The molecular functions of these 11 genes have not been characterized in *A. flavus* yet, however, based on the functional prediction, these genes are involved in various molecular processes altering transcriptional activities of genes: histidine phosphorylation during signal transduction (*cdk1*), histone methylation (*pasG* and AFLA_024110), DNA binding activity (*bdf1*, *cbf1*, and AFLA_026850), transcription machinery

(*TFIID*, *TFIIIA*, and *cyc8*), and translation initiation (*eIF2C4*). Among them, the seven genes (*cdk1*, *pasG*, *AFLA_024110*, *cbf1*, *TFIID*, *TFIIIA*, and *eIF2C4*) were up-regulated in the absence of NsdD protein, implying that NsdD affects the expression of a broad spectrum of genes including general transcription regulators in *A. flavus* conidia. Taken together, these results demonstrate that the *A. flavus* NsdD governs development and metabolism by regulating upstream regulators as well as genes especially involved in a broad range of transcription regulation.

To further dissect the conversed and divergent regulatory roles of NsdD, the comparative network analysis was performed comparing the species-specific NsdD-mediated GRNs of two species. First, we selected orthologs found in both networks and used them to connect the networks. A total of 253 orthologous genes were pulled out and defined as common targets of NsdD between *A.nidulans* and *A.flavus*. For visualizing the comparative network of NsdD, NsdD was placed at the center, the orthologs encircled NsdD, and the other genes filled up the outer space; genes were marked in red (*A. nidulans*) and blue (*A. flavus*) (illustrated in Fig. S2-7). Then, the functional enrichment analysis (GO terms) was carried out to investigate the regulatory roles of common targets of NsdD and the genes regulated in one species. The GO term analysis for 253 orthologous genes was performed with *A. nidulans* gene IDs as the functional annotation of *A. nidulans* provided more detailed functional categories than those of *A. flavus*: for 253 genes, 290 in *A. nidulans* but 99 in *A. flavus*. The most enriched GO terms of NsdD common targets were basically involved in all aspects of the *Aspergillus* biology: transmembrane transport, primary and secondary metabolic processes, and regulation of sporulation and sexual development. In contrast, a species-specific trend was noticed in the functional categories of genes regulated only in one species. In *A. nidulans*, they were mainly involved in cell structure-related functions such as phospholipid and alpha-glucan metabolic processes and secondary metabolism especially austinol biosynthesis, while genes regulated only in *A. flavus* displayed a variety of processual regulations mostly on nucleic acid-templated transcription activities and cellular metabolic processes (Table 2-5). Put together, these results suggest that conserved targets of NsdD govern the general biology of the two *Aspergillus* species, yet species-specific targets exhibited divergent functions, resulting in the cellular and chemical developmental heterogeneity between the two species.

2.5 Discussion

Sexual development, asexual development, and a variety of metabolic processes require a proper orchestration of multiple regulators and frontline genes in *Aspergillus* species (Ojeda-López *et al*., 2018). Despite the complexity of *Aspergillus* biology, NsdD serves as a master regulator previously identified as a key regulator of sexual and asexual development as well as secondary metabolism including mycotoxin production. In the last few decades, a broad spectrum of NsdD functions has been shed light on, however, a comprehensive understanding of how a single GATA-type TF governs all discrete biological processes in *Aspergillus* remains to be established. In this study, we aimed to elucidate not only the unexplored molecular functions of NsdD but also the regulatory mechanism of NsdD by utilizing network-based multi-omics approaches in *A. nidulans* and *A. flavus*.

The noticeable phenotype of *nsdD* mutants on solid media is a slow radial growth of a colony in *Aspergillus* species. This has been explained by the accelerated conidiation in Δ*nsdD*, which develops asexual developmental organs earlier than WT by several hours, regardless of environmental conditions (Han *et al*., 1998). Earlier asexual development initiates, shorter the time for vegetative growth is guaranteed. However, previous studies revealed that NsdD regulates hyphal growth in *A. nidulans* and *A. fumigatus*. Overexpression of *nsdD* resulted in the formation of elongated aerial hyphae and when *nsdD* was forced to express constitutively, conidiation was inhibited and coiled-hyphal structures were formed (Grosse and Krappmann, 2008). We observed hyper-hyphal branching in Δ*nsdD* mutants in *A. nidulans* and *A. flavus*. Their WTs formed mostly elongated hyphae, but the hyphae of deletion strains were more branched and shorter than those of WT post 12 hr inoculation. These results suggest that the retarded colonial growth of Δ*nsdD* is caused by the combination of the early conidiation commencement and the increased branching of hyphae. In addition to the conserved role of NsdD in fungal morphogenesis, we found the divergent function of NsdD in the formation of asexual developmental structures including conidia in *A. flavus*. Exclusively in *A. flavus*, the deletion of *nsdD* led to the occurrence of dwarf conidiophores displaying highly altered, but still functional vesicle and stalk structures. Furthermore, the structure of conidia produced from dwarf conidiophores was also altered; the size of Δ*nsdD* conidia was approximately 30% bigger than those of WT. These results suggest that the aberration of *nsdD* gene agitated the timely expression of several key regulators, which control spatial and temporal specificity of *Aspergillus* development, leading to abnormal morphological alterations (Mirabito *et al*., 1989). However, the detailed molecular mechanism of how NsdD acts as a morphological determinant remains to be studied.

By integrating ChIP-seq, transcriptomic, and functional enrichment analyses in conidia, 68 and 126 direct target genes of NsdD were identified and their enriched functional categories were transmembrane transport and regulation of diverse biological processes in *A. nidulans* and *A. flavus*, respectively. Fungal transmembrane transporters can be classified into two major types: solute transporters and ion channels (Gouaux and Mackinnon, 2005; Rudnick, 2013). Solute transporters physically bind substrates at one side of the membrane and transport the substrates to the other side of the membrane through its conformational changes. On the other hand, ion channels work as selective pores letting specific ions pass through. Regardless of their types, both transporters are indispensable for proper cellular functioning in that they govern the uptake and efflux of small molecules such as nutrients, metabolites including drugs, or ions across cellular membranes (Diallinas, 2016). Given the crucial, but broad roles of transmembrane transport and regulation of biological processes, we hypothesized that both bring a strong influence on overall fungal biology, however, the effect of these processes in certain circumstances is not yet clear and further studies will be needed to understand this.

The GATA-type transcription factor NsdD is a well-conserved key regulatory element in the *Pezizomycotina* consisting of most *Ascomycota* fungi including Aspergilli, suggesting similar developmental roles of NsdD among *Pezizomycotina* species (Ojeda-López *et al*., 2018). The NsdD polypeptide contains a highly conserved DNA-binding domain consisting of a type IV Cys_2-Cys_2 zinc-finger, which is particularly found in the GATA-type TFs (Teakle and Gilmartin, 1998; Han *et al*., 2001). The GATA family of TFs (GATA-1 to GATA-6) binds to the consensus binding sequence [(A/T)GATA(A/G)] through two zinc finger domains regulating transcription of their target genes (Urnov, 2002). Our NsdD-DNA interaction analyses in conidia followed by MEME analyses suggest that the consensus binding motif of NsdD is the 5'-GATCT-3' in both *Aspergillus* species. Previous studies have elucidated that GATA TFs bind to both GATA and GATC motifs in some animals, plants, and fungi (Newton *et al*., 2001; Sugimoto *et al*., 2003; Xu *et al*., 2017; Liu *et al*., 2022). Interestingly, GATC motifs are sometimes more crucial for the activation of a target gene than GATA motifs. In the plant *Catharanthus roseus*, two GATA and two GATC motifs were identified in the *D4H* promoter, which is highly activated by CrGATA1. The researchers mutated two GATA motifs and two GATC motifs separately to investigate which motifs are crucial for the activation of *D4H*. They found that mutation of GATA motifs did not affect, but mutation of GATC motifs significantly altered the D4H promoter's activation by CrGATA1 (Liu *et al*., 2019). Along with our previous study revealed that NsdD binds to the GATAA motifs of the *brlA* promoters in *A. nidulans*, these results suggest that NsdD directly binds to both GATA motifs and GATC motifs and regulates the expression of target genes in

Aspergillus. However, further studies will be needed to dissect the effect of NsdD-binding motif type on activation of a target promoter.

From primary metabolite analyses, we observed the apparent change in the production of most energy-related metabolites such as ATP, NADH, and NADPH in *A. flavus* Δ*nsdD* conidia. Adenosine triphosphate (ATP) is the main biological phosphate donor and protein kinases use ATPs to phosphorylate proteins by transferring the terminal phosphate group of ATP to the hydroxyl (OH) group of Serine (Ser), Threonine (Thr), or Tyrosin (Tyr) residues in substrate proteins (Hanks, 2003). Protein phosphorylation plays a crucial role in a large array of cellular processes in fungi, including cell cycle, metabolism, growth, signal transduction, and development by regulating the activity of enzymes, which are involved in metabolic homeostasis and cell signaling pathways (Oliveira *et al*., 2012; Albataineh and Kadosh, 2016). In addition, the NAD^+/NADH and $NADP^+$/NADPH redox couples are known to regulate various biological processes such as cellular redox state, energy metabolism, gene expression, and signal transduction pathways (Ying, 2008; Canto and Auwerx, 2011; Canto *et al*., 2015; Yang and Sauve, 2016). The functional enrichment analyses indicated that transmembrane transport and regulation of diverse biological processes were enhanced, whereas catabolic processes of small molecules and some acids including cellular amino acids were down-regulated in *A. flavus* Δ*nsdD* conidia. Moreover, the abundances of phosphoenolpyruvate, acetyl-CoA, three citric acids (α-ketoglutarate, succinate, and malate), and 11 amino acids were increased (Fig. 2-9), implying these metabolites were accumulated in Δ*nsdD* conidia due to the attenuation of acid catabolic processes. Given the crucial roles of ATP, NAD^+/NADH, and $NADP^+$/NADPH in energy metabolism, gene expression, and cell signaling pathways, we speculated that the high levels of energy-related metabolites were attributed to the increased molecular regulatory activities in Δ*nsdD* conidia.

Along with the effect of NsdD in primary metabolism, drastic alterations in the abundances of numerous secondary metabolites were observed in Δ*nsdD*. To provide insights into practical application, we selected out few known secondary metabolites, which have received worldwide attention due to their significant association with human health. Alternariol (AOH) is a mycotoxin produced by plant pathogenic *Alternaria* species and is frequently found in apple, cranberry, grape, raspberry, and some grain products. AOH shows adverse effects on humans and animals, reported to be associated with oesophageal cancer. This mycotoxin can induce the breakage of DNA by forming reactive oxygen species (ROS) and stimulating topoisomerases I, IIα, and IIβ to generate both single and double-strand DNA breaks (Fehr *et al*., 2009). In 2012, the alternariol production in *A. nidulans* was reported for the first time (Ahuja *et al*., 2012). We discovered that the core gene (*pkgA*) of alternariol production was down-regulated in *A. nidulans* Δ*nsdD* Vege and Asex and the abundance of alternariol was declined in 14 days culture of *A. nidulans* Δ*nsdD*. The declined pattern of both the gene expression and the abundance was almost synchronized, implying NsdD regulates the *pkgA* expression level affecting the actual alternariol production. These results suggest a novel positive regulation pathway of AOH biosynthesis in *A. nidulans*. Next, the production of asterriquinones (ARQ) has been reported in many *Aspergillus* species including *A. terreus* and *A. nidulans*. ARQs are tryptophan-derived indolyl benzoquinones exhibiting anti-tumor activity against transplantable animal tumors, Ehrlich ascites carcinoma, ascites hepatoma AH13, and mouse P388 leukemia (Shimizu *et al*., 1982). Moreover, ARQ and its analogues exhibited strong inhibitions on the activity of reverse transcriptase (RT) from human immunodeficiency virus type 1 (HIV-1) (Ono *et al*., 1991). In *A. nidulans*, these asterriquinones including terrequinone A are biosynthesized by the biosynthetic gene cluster consisting of *tdiA*-*tdiE*. These genes are predicted to encode a single-module nonribosomal peptide synthetase (NRPS, *tdiA*), indole prenyltransferase (*tdiB*), oxidoreductase (*tdiC*), aminotransferase (*tdiD*), and unknown protein (*tdiE*) (Bok *et al*., 2006). From the RNA-seq analyses in Δ*nsdD*, we found that the expression of *tdiB*, *tdiD*, and *tdiE* genes was up to 16 times increased in Asex and *tdiA*, *tdiB*, and *tdiD* genes were up-regulated in conidia up to 10 times. In accordance with the changes in the biosynthetic gene expression, the production of asterriquinone and terrequinone A was substantially enhanced; especially terrequinone A was not detected in WT, but it appeared in Δ*nsdD*. These results suggest that NsdD plays a crucial role in the ARQ biosynthesis in *A. nidulans*. Given the important roles of ARQs against tumors and HIV-1, these findings can be applied to the medical and pharmaceutical industries. In the pathogenic fungus *A. flavus*, we observed a huge gulf in the production of leporin B between WT and Δ*nsdD*. Leporins are 2-pyridone secondary metabolites initially isolated from *Aspergillus leporis*. Leporin B has specifically proven to be inducing the gene expression of Hexokinase II (HK2), the initial enzyme of glycolysis phosphorylating glucose to produce glucose-6-phosphate and trapping it in the cell. Due to this feature, leporin B is recognized as a potential treatment for type 2 diabetes. By increasing HK2 activities in cells, leporin B can lower the blood sugar level of type 2 diabetic patients so that they may maintain the appropriate blood sugar level (Zhang *et al*., 2003). Moreover, leporin B exhibited slight cytotoxicity against human tumor cell lines (MCF7, H460, and SF268), but showed strong antimicrobial activities against *Candida albicans* and *Staphylococcus aureus* (Sy-Cordero *et al*., 2015). Among all known secondary metabolites identified from secondary metabolism analyses in this study, the deletion of Δ*nsdD* resulted in the biggest change in the production of leporin B;

it was produced approximately 5.7 times higher than WT. Taken together, these findings suggest that *nsdD* mutants can serve as a reservoir of anti-tumor and anti-microbial secondary metabolites and NsdD is a possible target for further secondary metabolite studies in not only *Aspergillus* species, but also other *Pezizomycotina* fungi.

Our previous studies discovered the molecular mechanism of NsdD in asexual developmental regulation. NsdD directly binds to the multiple regions of *brlA* promoter and plays a repressive role in the expression of *brlA*; NsdD and VosA, another major negative regulator of *brlA*, together exert full repressive control on *brlA* expression and their timely take-off determines the developmental competence, which enables fungi to switch from vegetative growth to asexual development (Axelrod *et al*., 1973; Lee *et al*., 2016). By controlling the initial factor of conidiation, NsdD affects the whole downstream gene network in asexual development. Thus, we hypothesized that this type of upstream regulation obtained by NsdD occurs in different molecular processes as well. Our transcriptomic analyses revealed that the regulatory effect of NsdD was considerably different depending on the developmental stages and species. Taken together, we speculated that NsdD's upstream regulations resulted in the alterations of downstream genes' expressions, which in turn exhibited all cellular and chemical changes in Δ*nsdD*. To elaborate on this sequential regulatory mechanism of NsdD, we adopted network-based multi-omics analyses. The ChIP-seq analyses were used to identify the direct target genes of NsdD and the RNA-seq analyses were utilized to figure out the total list of differentially expressed genes in Δ*nsdD*, which can be considered as indirect targets of NsdD. The protein-protein interaction information of the direct and indirect target genes obtained from the STRING database enabled us to construct NsdD-mediated gene regulatory networks in *A. nidulans* and *A. flavus* conidia. The core network analyses elucidated the core regulatory mechanisms of NsdD in different developmental and biological processes. Most interestingly, many developmental and metabolic regulators including VeA and LaeA appeared in the core networks. VeA acts as a key light-dependent developmental regulator, promoting sexual development but suppressing asexual sporulation. In dark, it bridges VelB and LaeA to form the VelB-VeA-LaeA velvet complex that regulates secondary metabolism and sexual development (Timberlake, 1990; Yager, 1992). LaeA is a global regulator of secondary metabolism as it is required for the proper expression of various secondary metabolite (SM) biosynthetic gene clusters and their corresponding SM production including mycotoxins. In addition, LaeA affects growth and conidiation in *Aspergillus* species (Bok and Keller 2004; Stinnett *et al*., 2007; Bayram *et al*., 2008). The fact that the global regulators VeA and LaeA are direct targets of NsdD and several upstream regulators of development and metabolism are directly regulated by NsdD demonstrates that NsdD acts as a bona fide master regulator of development and metabolism in *Aspergillus* fungi. The unequivocal distinction was observed in the *A. flavus* core network compared to those of *A. nidulans*; three forkhead genes and several genes involved in transcription regulation appeared as core components. We speculated that these forkhead genes might affect the morphogenesis of conidiophore resulting in a dwarf phenotype in Δ*nsdD*, and the distinct transcriptional regulations of varied biological processes in *A. flavus* may contribute to the individual biological evolution of two distantly related species; *A. nidulans* and *A. flavus*. To further verify these, further experiments should be conducted to characterize the roles of all these putative genes in *A. flavus*. Then, we validated the core network models by overlaying the data from RNA-seq analyses and metabolite analyses. The modeling process of these networks was based on conidia samples, however, we found that they were still applicable to the cases of Vege and Asex and provided rational explanations for metabolic changes in Δ*nsdD* as well. This is the first time proposing NsdD-mediated GRNs since NsdD was identified in 2001. In terms of the network integrality, the species-specific NsdD networks only offer a partial framework as they were solely done in conidia. To establish the complete NsdD-mediated GRNs in the two *Aspergillus* species, further studies will be needed encompassing diverse developmental stages and different cell types. Nevertheless, this study will provide fundamental information on the regulatory role and mechanism of NsdD for NsdD ortholog studies in other filamentous fungi: *Coprinopsis cinerea* (Liu *et al*., 2022), *Fusarium fujikuroi* (Niehaus *et al*., 2017), *Metarhizium rileyi* (Xin *et al*., 2020), *Neurospora crassa* (Chen *et al*., 2009), *Penicillium oxalicum* (He *et al*., 2018), *Sclerotia sclerotiorum* (Li *et al*., 2018). Furthermore, this study can serve as an experimental template for any research studying regulatory roles of important transcription factors or DNA-binding proteins by providing a well-established network research pipeline.

In conclusion, this study provides a systematic dissection of the regulatory role and mechanism of NsdD in *A. nidulans* and *A. flavus*. In these species, NsdD governs fungal development and metabolism via a species-specific NsdD-mediated gene regulatory network. Within the network, NsdD directly regulates not only crucial upstream regulators of development and metabolism, but also some important genes in fungal biology, which in turn affects the expression of downstream genes, resulting in distinct cellular and metabolic developmental traits in the two distantly related *Aspergillus* species. Moreover, by presenting visualized basic structures of NsdD-mediated GRNs, this study advances the knowledge of the NsdD regulatory mechanism and overall genetic and molecular regulations of *Aspergillus* species.

2.6 References

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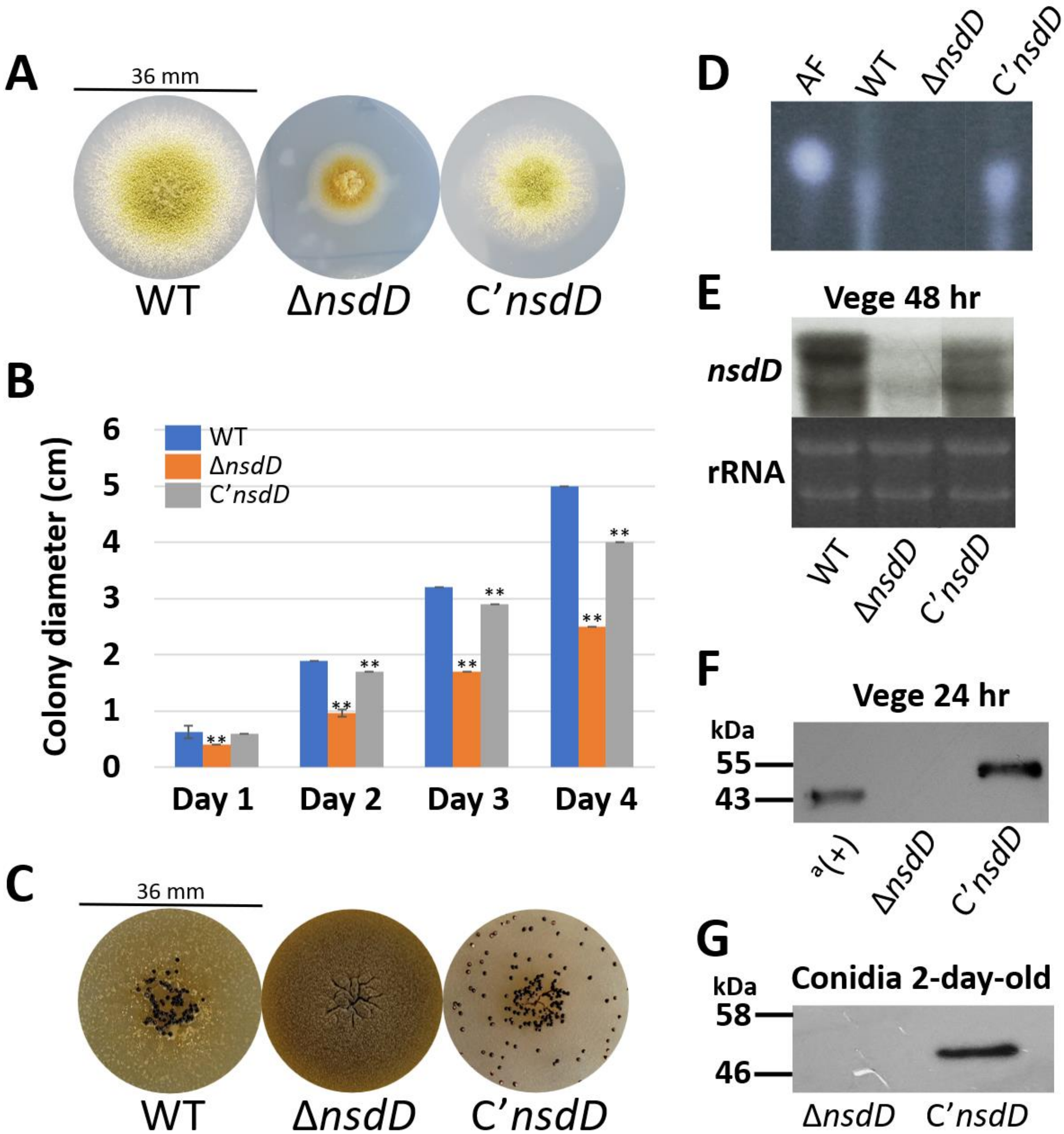


Figure 2-1. Verification of *nsdD* complementation. (A) Photographs of *A. flavus* WT (NRRL3357), Δ*nsdD* (LNJ11), and C'*nsdD* (THM5) strains point inoculated on solid GMM and incubated at 30 °C for 3 days. (B) Colony diameters of WT, Δ*nsdD*, and C'*nsdD* strains were measured on GMM grown at 30 °C for 1-4 days. The colony diameters were counted in triplicates (**$p < 0.01$). (C) Sclerotia formation of WT, Δ*nsdD*, and C'*nsdD* strains point

inoculated on GMM with 0.1% YE, sealed with parafilm, and incubated at 30 °C for 7 days in dark. The plates were gently sprayed with 70% EtOH to reveal sclerotia. (D) Aflatoxin levels of WT, Δ*nsdD*, and C'*nsdD* strains. Strains were stationary cultured in liquid GMM with 0.5% YE at 30 °C for 5 days. AF standard was loaded as a positive control. (E) Northern blot analysis for *nsdD* mRNA levels in WT, Δ*nsdD*, and C'*nsdD* strains. Strains were cultured in liquid GMM at 30 °C for 48 hours. (F) Western blot analysis for NsdD using anti-FLAG antibody in Δ*nsdD* and C'*nsdD* strains. The TMK20 strain expressing McrA::FLAG proteins was used as a positive control. Strains were cultured in liquid GMM at 30 °C for 24 hours. (G) Western blot analysis for NsdD using anti-FLAG antibody in Δ*nsdD* and C'*nsdD* strains. Strains were cultured on solid GMM at 30 °C for 2 days.

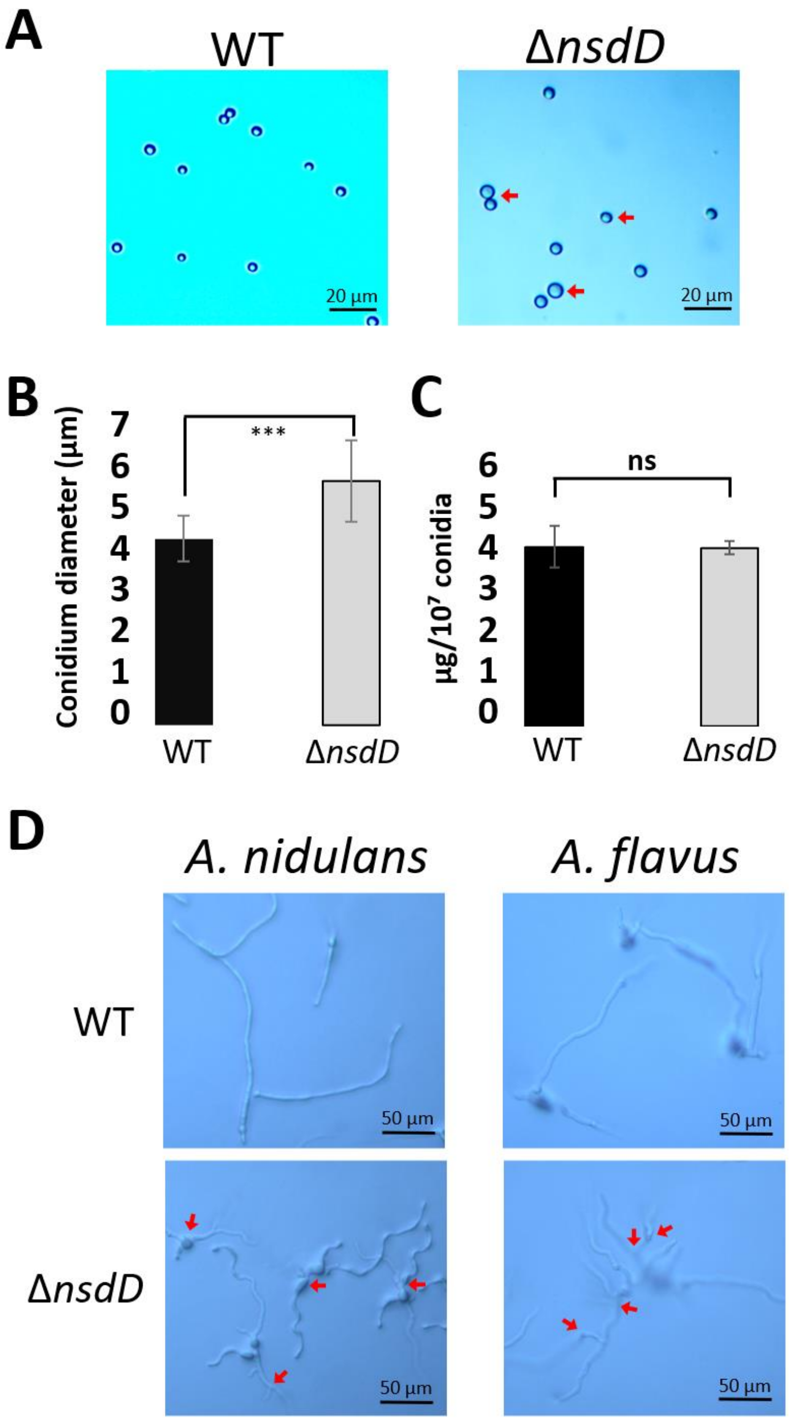
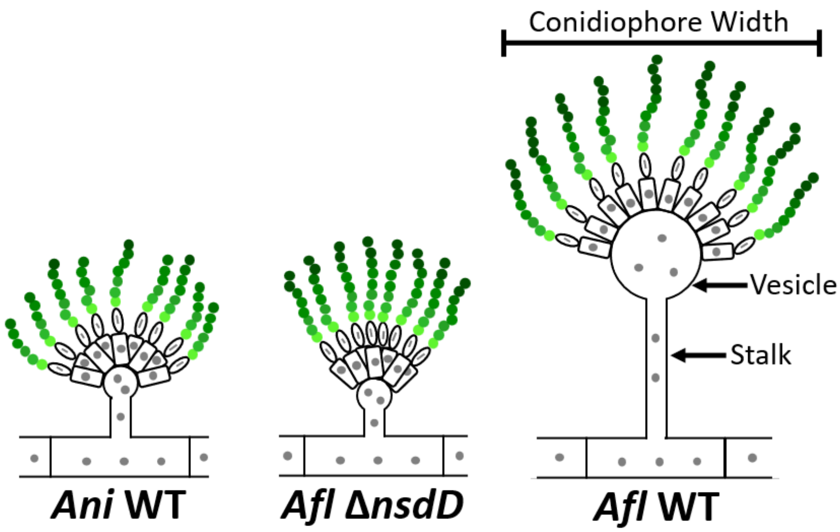


Figure 2-2. Morphological alterations in Δ*nsdD* hyphae and conidia. (A) Photographs of WT and Δ*nsdD* 3-day-old conidia in *A. flavus*. Red arrows indicate conidia showing increased in size. (B) Conidium diameter of WT and Δ*nsdD* 3-day-old conidia in *A. flavus*. The conidium

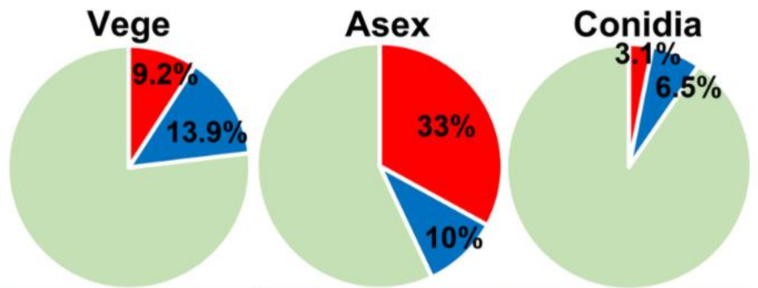
diameters were counted in more than triplicates (***$p < 0.001$). (C) Trehalose amounts (μg) per 10^7 conidia of WT and Δ*nsdD* 3-day-old conidia in *A. flavus*. (D) Photographs of WT and Δ*nsdD* hyphae in *A. nidulans* and *A. flavus*. The 2-day-old conidia of strains were inoculated on solid GMM and incubated at 37 °C and 30 °C for 12 hours, in *A. nidulans* and *A. flavus*, respectively. Red arrows indicate abnormal hyphal branching.



Structure	*Ani* WT	*Afl* Δ*nsdD*	*Afl* WT
Conidiophore width (top)	~79 µm	~23 µm	~92 µm
Vesicle size	~12 µm	~14 µm	~46 µm
Stalk length	~100 µm	~85 µm	~860 µm

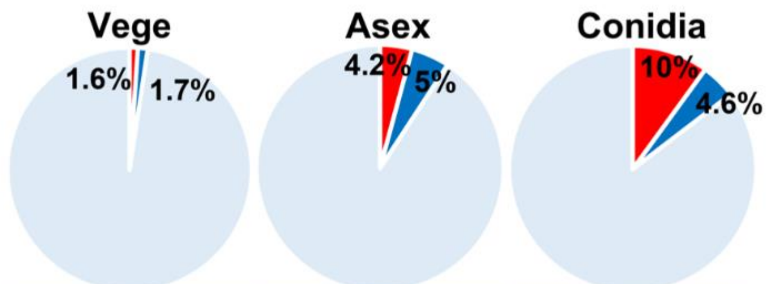
Figure 2-3. Morphological determination of NsdD in asexual developmental structures of *A. flavus*. (A) Diagrammatic representation of conidiophore structures in *A. nidulans* (*Ani*) WT and *A. flavus* (*Afl*) Δ*nsdD* and WT (adopted and modified from Cary *et al*. 2012 and Lee *et al*. 2016). The structural details such as conidiophore width (from top view), vesicle size and stalk length are listed in the table.

A. nidulans



	Vege		Asex		Conidia	
Total gene number	10,988			100%		
DEG number	2,531	**23.1%**	4,724	**43%**	1,052	**9.6%**
DEGs up-regulated in *ΔnsdD*	1,008	9.2%	3,628	33%	341	3.1%
DEGs down-regulated in *ΔnsdD*	1,523	13.9%	1,096	10%	711	6.5%

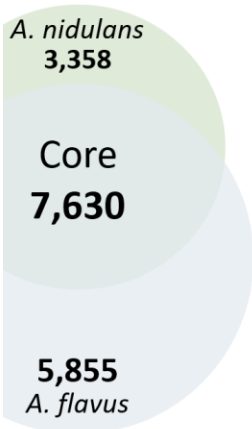
A. flavus



	Vege		Asex		Conidia	
Total gene number	13,485			100%		
DEG number	446	**3.3%**	1,238	**9.2%**	1,964	**14.6%**
DEGs up-regulated in *ΔnsdD*	220	1.6%	569	4.2%	1,339	10%
DEGs down-regulated in *ΔnsdD*	226	1.7%	669	5%	625	4.6%

Up-regulated genes in *ΔnsdD* **Down-regulated genes in *ΔnsdD***

Figure 2-4. The summary of transcriptomic profiling in *A. nidulans* and *A. flavus* Δ*nsdD*. For the RNA-seq analyses, three different cell types were used: vegetatively growing cells (Vege, 36 h), asexually developing cells (Asex, 24 h), and conidia (2 days). DEG is an abbreviation of 'differentially expressed gene'. Numbers in color (red or blue) indicate the number of genes that were up (red)- or down (blue)-regulated in Δ*nsdD* compared to those of WT.



A. nidulans	Vege		Asex		Conidia	
Total gene number	10,988			100%		
DEG number	2,531	**23.1%**	4,724	**43%**	1,052	**9.6%**
Core genes in DEG	1,755	**69.3%**	3,506	**74.2%**	636	**60.4%**
Lineage-specific genes in DEG	776	**30.7%**	1,218	**25.8%**	416	**39.6%**

A. flavus	Vege		Asex		Conidia	
Total gene number	13,485			100%		
DEG number	446	**3.3%**	1,238	**9.2%**	1,964	**14.6%**
Core genes in DEG	195	**43.7%**	529	**42.7%**	1,058	**53.8%**
Lineage-specific genes in DEG	251	**56.3%**	709	**57.3%**	906	**46.2%**

Figure 2-5. The summary of core and lineage-specific genes in *A. nidulans* and *A. flavus* DEGs. Core genes are orthologs commonly found in both *A. nidulans* and *A. flavus* species. Lineage-specific genes are exclusively found in one species (species-specific genes). The numbers in the Venn diagram indicate the number of core genes in the middle, the number of *A. nidulans*-specific genes on the top, and the number of *A. flavus*-specific genes on the bottom. Vegetatively growing cells (Vege, 36 h), asexually developing cells (Asex, 24 h), and conidia (2 days).

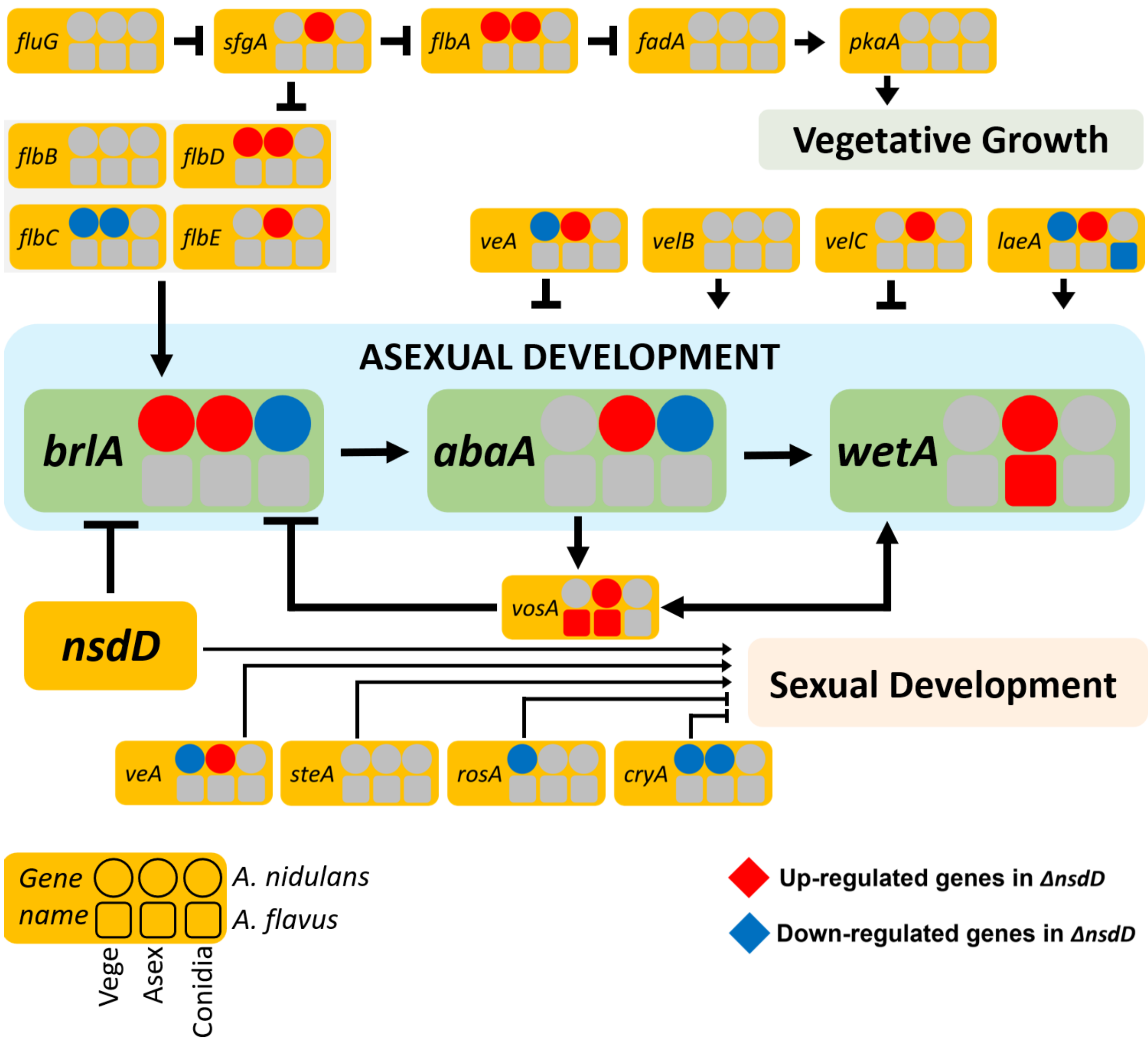


Figure 2-6. NsdD-mediated gene regulation in vegetative growth, asexual development, and sexual development in *A. nidulans* and *A. flavus*. A schematic diagram of the NsdD-mediated regulatory model is shown. Genes with increased, decreased, and unaffected mRNA levels in Δ*nsdD* are labeled with red (up-regulated), blue (down-regulated), and gray (unaffected). Three circles in parallel indicate the expression change of a gene in *A. nidulans* Vege, Asex, and conidia in order. Three rectangles in parallel indicate the expression change of a gene in *A. flavus*

Vege, Asex, and conidia in order. Gene names are present at the left of gene expression changes. The arrow between genes indicates activation; the blunt ended arrow indicates repression.

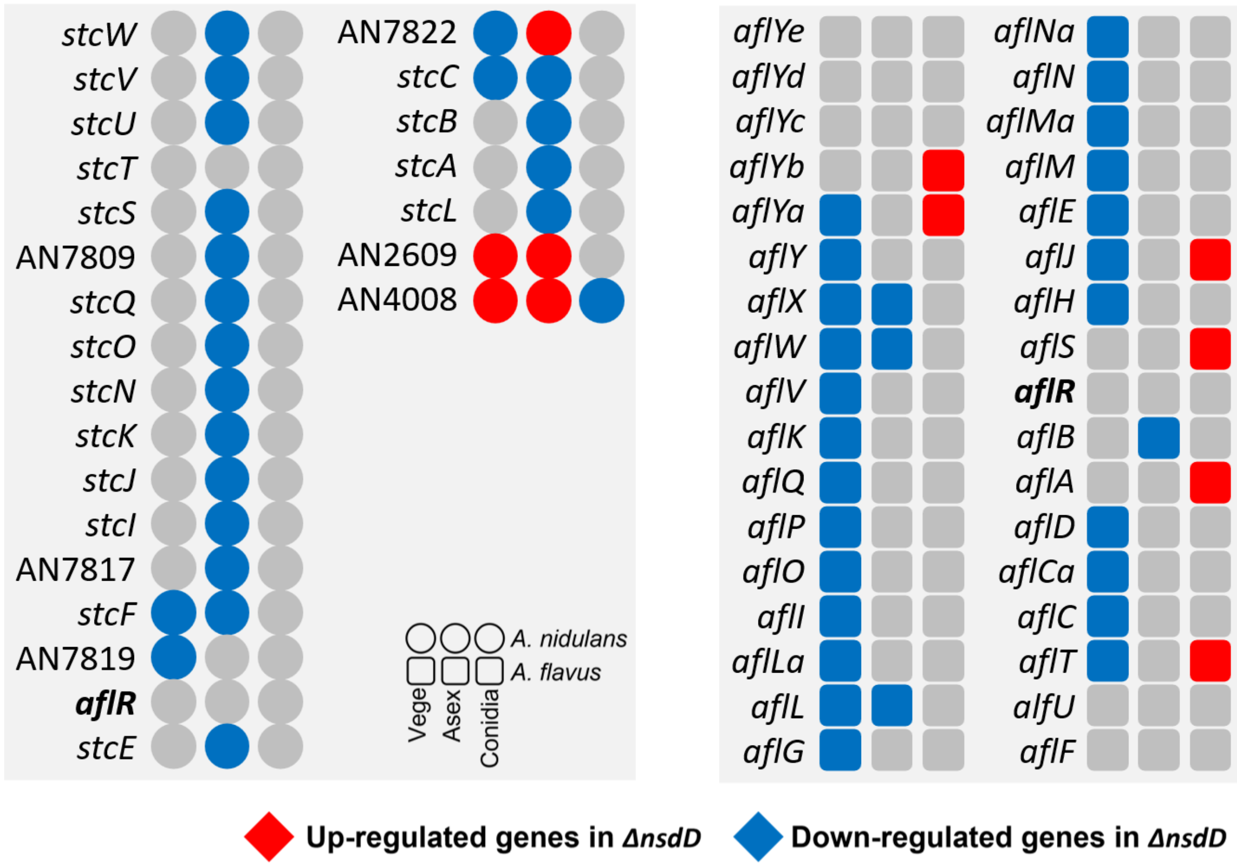
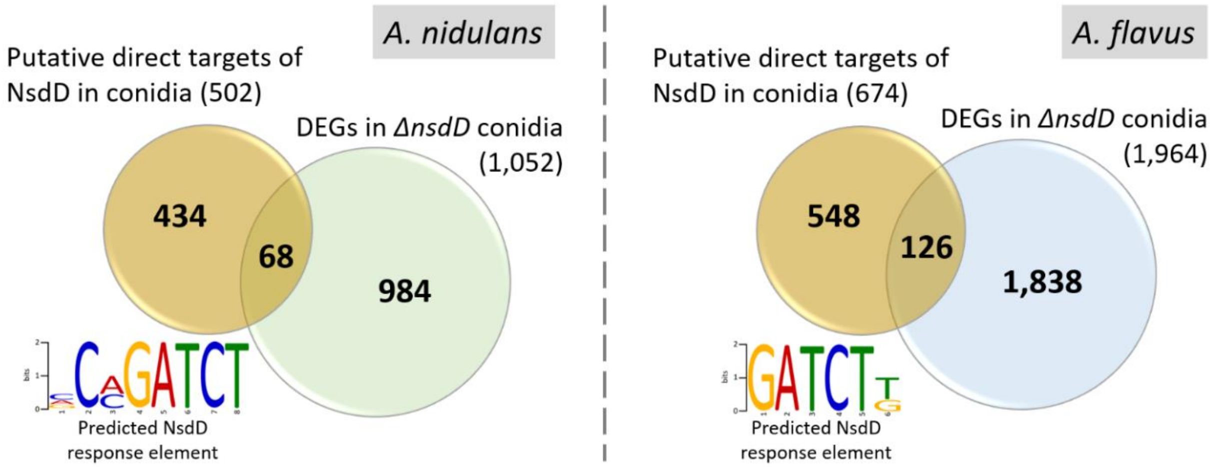


Figure 2-7. Sterigmatocystin/Aflatoxin biosynthetic gene clusters affected in *A. nidulans* and *A. flavus* Δ*nsdD*. Genes consisting of ST/AF biosynthetic gene clusters are shown. Genes with increased, decreased, and unaffected mRNA levels in Δ*nsdD* are labeled with red (up-regulated), blue (down-regulated), and gray (unaffected). Three circles in parallel indicate the expression change of a gene in *A. nidulans* Vege, Asex, and conidia in order. Three rectangles in parallel indicate the expression change of a gene in *A. flavus* Vege, Asex, and conidia in order. Gene names are present at the left of gene expression changes.

A



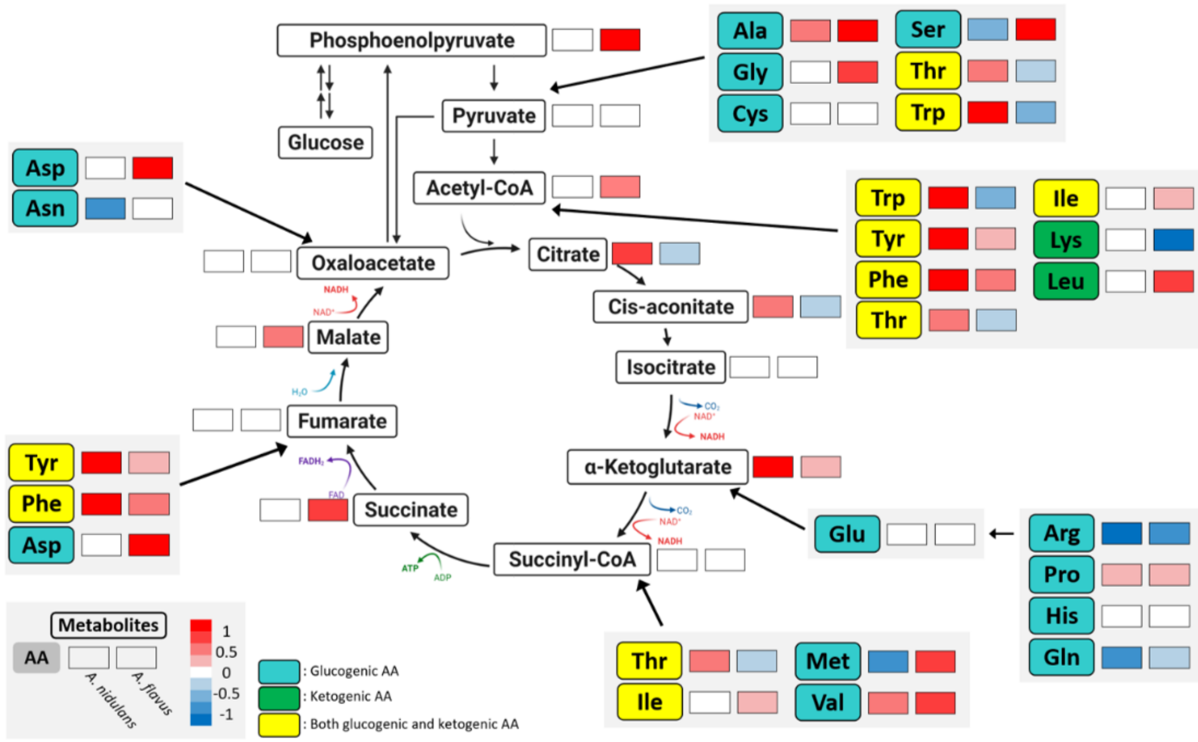
B

Gene ID	log2FC	Gene name	Gene description
Up-regulated genes in *A. nidulans* Δ*nsdD* conidia			
AN2585	-4.46		Substrate-specific transmembrane transporter activity
AN12244	-2.84		Protein of unknown function
AN8390	-2.84		Ethanol- and ethylacetate-induced gene
AN8737	-2.52	*mstA*	High-affinity glucose transporter
AN9295	-2.41		Substrate-specific transmembrane transporter activity
AN5323	-2.16		Substrate-specific transmembrane transporter activity
AN5088	-1.95		Putative potassium-transporting ATPase
AN5943	-1.84		Protein of unknown function
AN0129	-1.70	*ppsA*	Putative dual-specificity protein
AN7828	-1.61	*urhB*	Putative unsaturated rhamnogalacturonan hydrolase
Down-regulated genes in *A. nidulans* Δ*nsdD* conidia			
AN7895	7.00	*cipB*	Putative oxidoreductase
AN9206	4.12		Transcript induced in response to calcium dichloride
AN6703	3.53	*jenB*	Short-chain carboxylic acid transporter
AN8311	3.40		Protein of unknown function
AN3484	2.95		Protein of unknown function
AN6472	2.75	*dfgF*	Putative endo-mannanase GH76 family protein
AN8374	2.74		Oligopeptide transmembrane transporter activity
AN9249	2.44	*ausH*	Protein required for austinol and dehydroaustinol biosynthesis
AN1197	2.43		Predicted carbohydrate binding and catalytic activity
AN0005	2.42		Protein of unknown function

Gene ID	log2FC	Gene name	Gene description
Up-regulated genes in *A. flavus* Δ*nsdD* conidia			
AFLA_104430	-5.22		Putative subtilisin
AFLA_074070	-4.00		Putative siderochrome-iron transporter
AFLA_000930	-3.99		Putative acetolactate synthase
AFLA_122260	-2.88		Putative pantothenate transporter
AFLA_090790	-2.65		Hypothetical protein
AFLA_013400	-2.51		Putative ankyrin repeat-containing protein
AFLA_050150	-2.41		Putative extracellular exo-polygalacturonase
AFLA_078080	-2.27		Hypothetical protein
AFLA_071550	-2.17		Hypothetical protein
AFLA_069460	-2.13		Putative C2H2 transcription factor
Down-regulated genes in *A. flavus* Δ*nsdD* conidia			
AFLA_057180	2.55		Hypothetical protein
AFLA_092300	2.52		Hypothetical protein
AFLA_078820	2.25		Hypothetical protein
AFLA_138060	2.12		c-24(28) sterol reductase
AFLA_113380	2.12		Hypothetical protein
AFLA_116990	2.09		Putative MFS monosaccharide transporter
AFLA_052640	1.99		PH domain protein
AFLA_040260	1.96		Putative HLH DNA binding domain protein
AFLA_031450	1.88		Putative CP2 transcription factor
AFLA_113260	1.87		Allergen Asp F7

Figure 2-8. Identification of NsdD direct targets in *A. nidulans* and *A. flavus* conidia. (A) The Venn diagram display the number of putative direct targets of NsdD and DEGs in Δ*nsdD* conidia. The overlapped part in the Venn diagram indicates the number of direct targets of NsdD. The predicted NsdD response elements (NREs) are shown below the Venn diagram. (B) Summary of potential direct targets of NsdD showing the most changes of the mRNA levels in Δ*nsdD* conidia. The fold change is calculated as WT/Δ*nsdD* so that a negative log2FC value means that a gene is up-regulated in Δ*nsdD* and a positive log2FC value means that a gene is down-regulated in Δ*nsdD.*

A



B

Metabolite	*A. nidulans* Δ*nsdD* conidia		*A. flavus* Δ*nsdD* conidia	
	% change in production	Up or Down	% change in production	Up or Down
AMP	-	-	21.3%	Up
ADP	-	-	149.8%	Up
ATP	-	-	145.8%	Up
GMP	-	-	65.1%	Up
GDP	-	-	357.9%	Up
CMP	-	-	39.4%	Up
UDP	-	-	129.5%	Up
UTP	-	-	144.2%	Up
FAD	-	-	30.4%	Up
NAD+	-20.6%	Down	69.8%	Up
NADH	-	-	52.9%	Up
NADP+	-59.7%	Down	44.9%	Up
NADPH	-	-	28.8%	Up

Figure 2-9. The alteration of primary metabolite production in Δ*nsdD* conidia. (A) A schematic diagram of the NsdD-mediated regulation in primary metabolism is shown. Primary metabolites involved in glycolysis, the citric acid cycle (CAC), and amino acid (AA) biosynthesis are indicated in the diagram. Two rectangles in parallel indicate the alteration in the abundance of a metabolite in *A. nidulans* and in *A.flavus* conidia in order. Red means the

increased abundance of a metabolite and blue means the decreased abundance of a metabolite in Δ*nsdD*. Glucogenic AAs can produce pyruvate or any other glucose precursors during their catabolism, while ketogenic AAs can produce acetyl-CoA during their catabolism. (B) the abundances of energy metabolism-related metabolites such as ATP, NADH, and NADPH in *A. nidulans* and *A. flavus* Δ*nsdD* conidia.

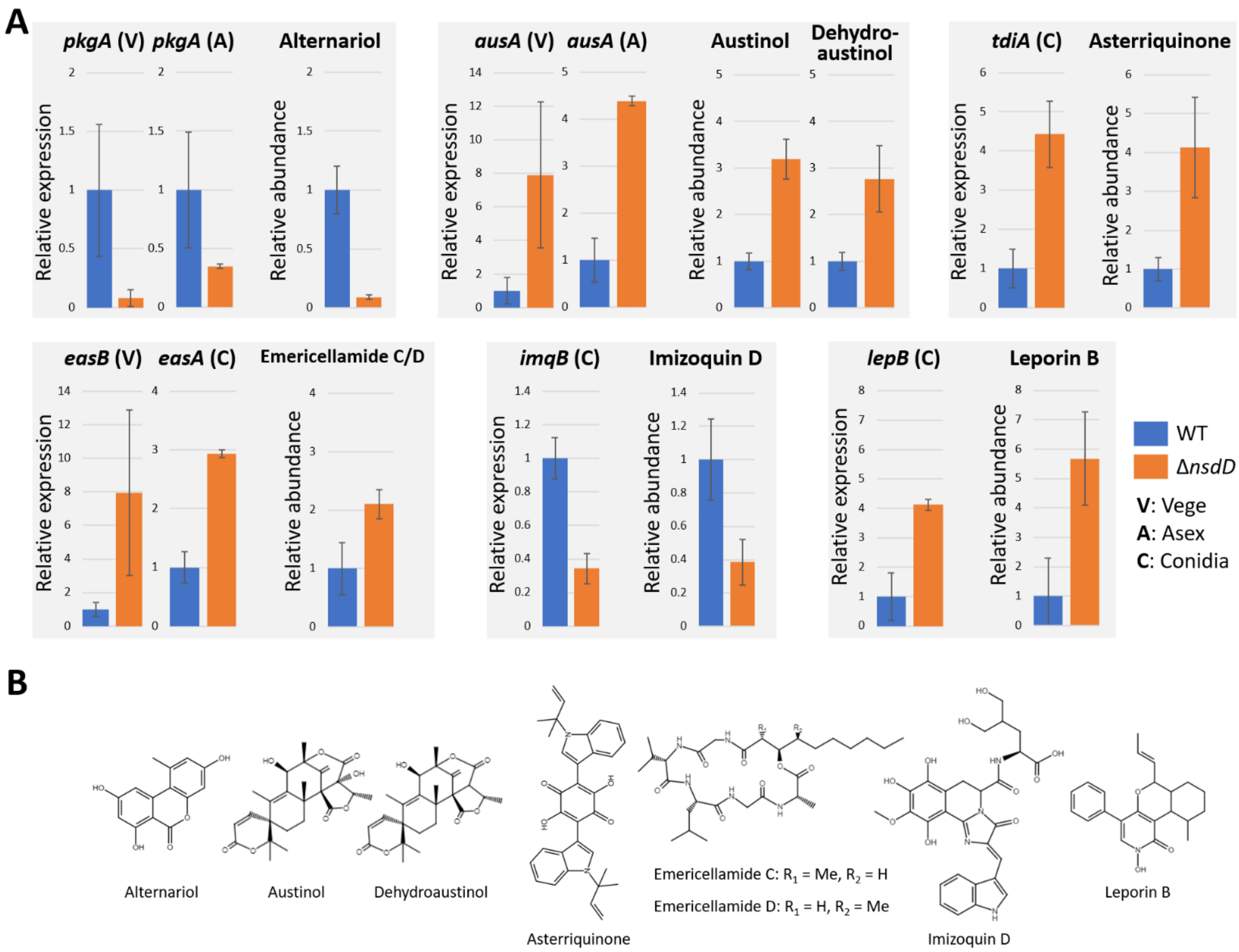


Figure 2-10. The regulatory roles of NsdD in known secondary metabolite production. (A) The relative gene expression level of a backbone gene and the relative abundance of a known secondary metabolite are shown as bar graphs. The gene and metabolite names are displayed on the top of the graphs. All relative differences between WT and Δ*nsdD* are statistically significant ($p < 0.05$). V, A, or C on the right of the gene name indicates the source of the gene expression levels; V: Vege, A: Asex, and C: conidia. (B) Chemical structures of the known secondary metabolites in order. In case of emericellamide C/D, they follow the R1 and R2 combination as shown.

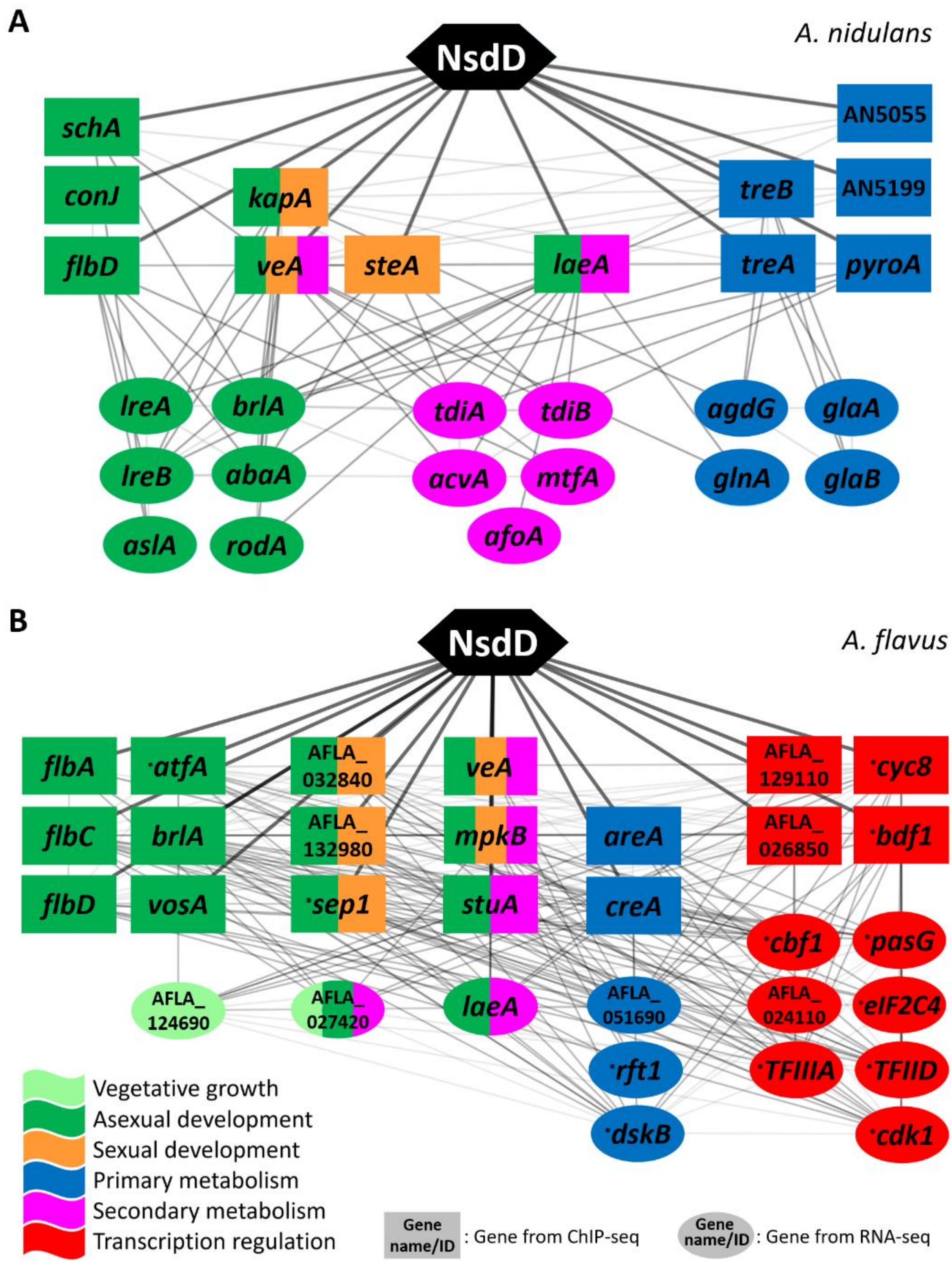

A
A. nidulans
NsdD
schA
conJ
flbD
kapA
veA
steA
laeA
AN5055
AN5199
treB
treA
pyroA
lreA
brlA
lreB
abaA
aslA
rodA
tdiA
tdiB
acvA
mtfA
afoA
agdG
glaA
glnA
glaB
B
A. flavus
NsdD
flbA
flbC
flbD
atfA
brlA
vosA
AFLA_032840
AFLA_132980
sep1
veA
mpkB
stuA
areA
creA
AFLA_129110
AFLA_026850
cyc8
bdf1
cbf1
pasG
AFLA_124690
AFLA_027420
laeA
AFLA_051690
AFLA_024110
eIF2C4
rft1
TFIIIA
TFIID
dskB
cdk1
Vegetative growth
Asexual development
Sexual development
Primary metabolism
Secondary metabolism
Transcription regulation
Gene name/ID : Gene from ChIP-seq
Gene name/ID : Gene from RNA-seq

Figure 2-11. The core networks of NsdD-mediated GRNs in *A. nidulans* and *A. flavus*. Schematic diagrams of the NsdD core networks in *A. nidulans* (A) and *A. flavus* (B) are shown. In the networks, genes are presented in different shapes, which indicate the source of a gene: rectangle for genes from ChIP-seq and ellipse for genes from RNA-seq. Then, these shapes are colored depending on the predicted functional categories: vegetative growth, asexual development, sexual development, primary metabolism, secondary metabolism, and transcription regulation. Each line indicates the interaction between two genes/proteins. The interaction between NsdD and genes from ChIP-seq is highlighted with a thicker line. Gene names marked with an asterisk (*) are predicted, not identified.

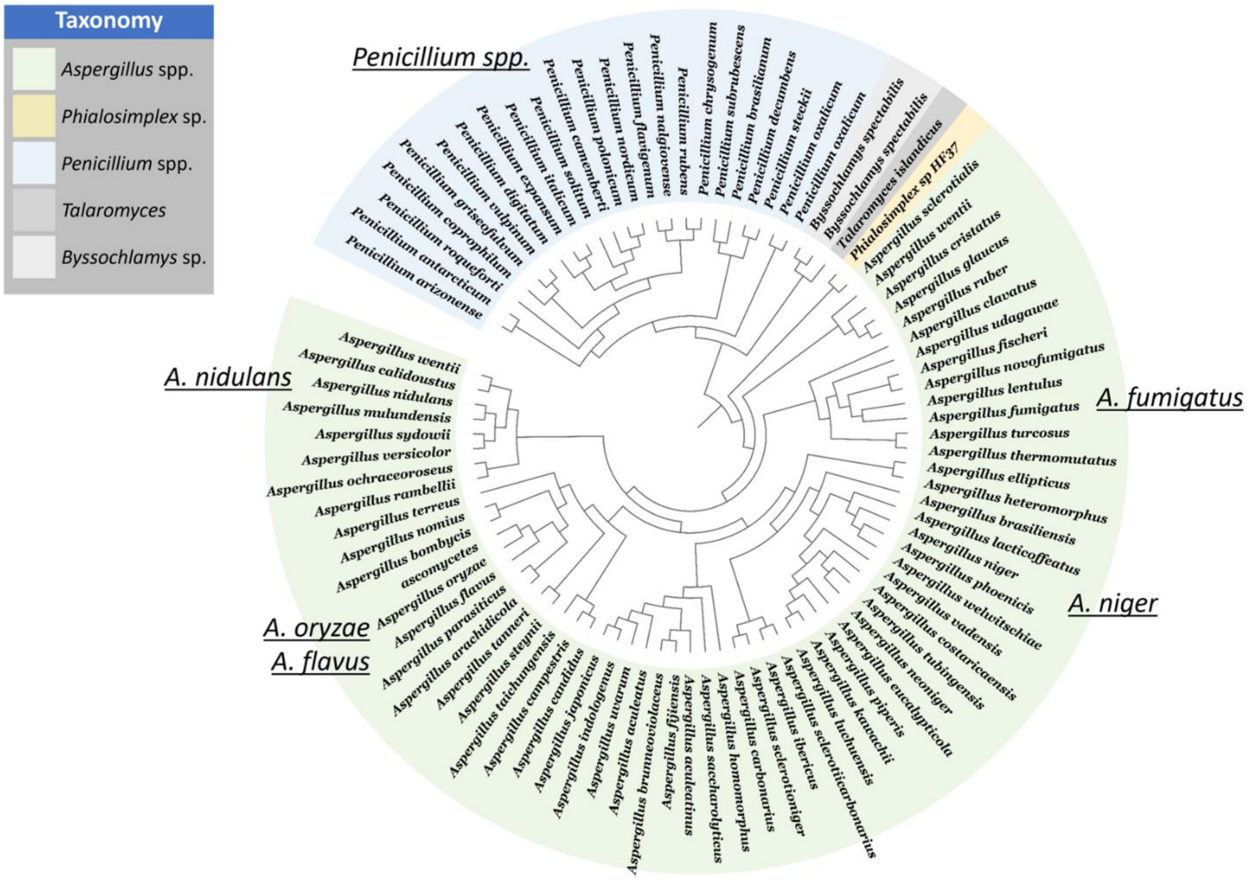


Figure S2-1. The phylogenetic tree of NsdD proteins.

Vege	Asex	Conidia
translation	translation	tyrosine catabolic process
peptide biosynthetic process	peptide biosynthetic process	hexose transmembrane transport
peptide metabolic process	ribonucleoprotein complex biogenesis	aromatic amino acid family catabolic process
amide biosynthetic process	peptide metabolic process	monosaccharide transmembrane transport
cellular amide metabolic process	amide biosynthetic process	carbohydrate transmembrane transport
rRNA transport	ncRNA metabolic process	organic hydroxy compound metabolic process
rRNA export from nucleus	organonitrogen compound biosynthetic process	glucose transmembrane transport
organonitrogen compound biosynthetic process	cellular nitrogen compound metabolic process	carbohydrate transport
monodictyphenone biosynthetic process	ribosome biogenesis	L-phenylalanine catabolic process
monodictyphenone metabolic process	ncRNA processing	erythrose 4-phosphate amino acid catabolic process
carbohydrate metabolic process	secondary metabolite biosynthetic process	austinol biosynthetic process
carbohydrate catabolic process	oxidation-reduction process	austinol metabolic process
polysaccharide metabolic process	secondary metabolic process	dehydroaustinol biosynthetic process
cellular carbohydrate metabolic process	carbohydrate metabolic process	dehydroaustinol metabolic process
cellular polysaccharide metabolic process	organic heteropentacyclic compound biosynthetic process	transmembrane transport
cell wall organization or biogenesis	sterigmatocystin biosynthetic process	secondary metabolite biosynthetic process
polysaccharide catabolic process	cellular carbohydrate metabolic process	fungal-type cell wall beta-glucan metabolic process
DNA strand elongation involved in DNA replication	organic heteropentacyclic compound metabolic process	cell wall beta-glucan metabolic process
transmembrane transport	lipid metabolic process	(1->3)-beta-D-glucan metabolic process
DNA strand elongation	sterigmatocystin metabolic process	inositol phosphoceramide metabolic process

Red : up-regulated in *ΔnsdD* **Blue** : down-regulated in *ΔnsdD*

Figure S2-2. The most enriched GO terms in *A. nidulans* Δ*nsdD* Vege, Asex, and condia.

Vege	Asex	Conidia
oxidation-reduction process	peptide biosynthetic process	carbohydrate transport
peptidoglycan-based cell wall biogenesis	myo-inositol transport	transmembrane transport
glycosaminoglycan biosynthetic process	translation	regulation of cellular biosynthetic process
peptidoglycan biosynthetic process	peptide metabolic process	regulation of biosynthetic process
cellular component macromolecule biosynthetic process	monosaccharide catabolic process	regulation of cellular macromolecule biosynthetic process
cell wall macromolecule biosynthetic process	cellular amide metabolic process	regulation of macromolecule biosynthetic process
obsolete oxygen and reactive oxygen species metabolic process	amide biosynthetic process	regulation of RNA biosynthetic process
hydrogen peroxide catabolic process	D-xylose catabolic process	regulation of transcription, DNA-templated
antibiotic biosynthetic process	D-xylose metabolic process	regulation of nucleic acid-templated transcription
hydrogen peroxide metabolic process	polyol transport	regulation of nucleobase-containing compound metabolic process
organic heteropentacyclic compound biosynthetic process	secondary metabolic process	small molecule catabolic process
aflatoxin metabolic process	oxidation-reduction process	alpha-amino acid catabolic process
aflatoxin biosynthetic process	secondary metabolite biosynthetic process	cellular amino acid catabolic process
organic heteropentacyclic compound metabolic process	obsolete electron transport	oxidation-reduction process
acetate metabolic process	mycotoxin metabolic process	carboxylic acid catabolic process
mycotoxin biosynthetic process	mycotoxin biosynthetic process	organic acid catabolic process
mycotoxin metabolic process	toxin biosynthetic process	aromatic amino acid family catabolic process
toxin metabolic process	toxin metabolic process	tyrosine catabolic process
toxin biosynthetic process	anion transport	obsolete electron transport
secondary metabolite biosynthetic process	fatty acid biosynthetic process	tyrosine metabolic process

Red : up-regulated in *ΔnsdD* **Blue** : down-regulated in *ΔnsdD*

Figure S2-3. The most enriched GO terms in *A. flavus* Δ*nsdD* Vege, Asex, and condia.

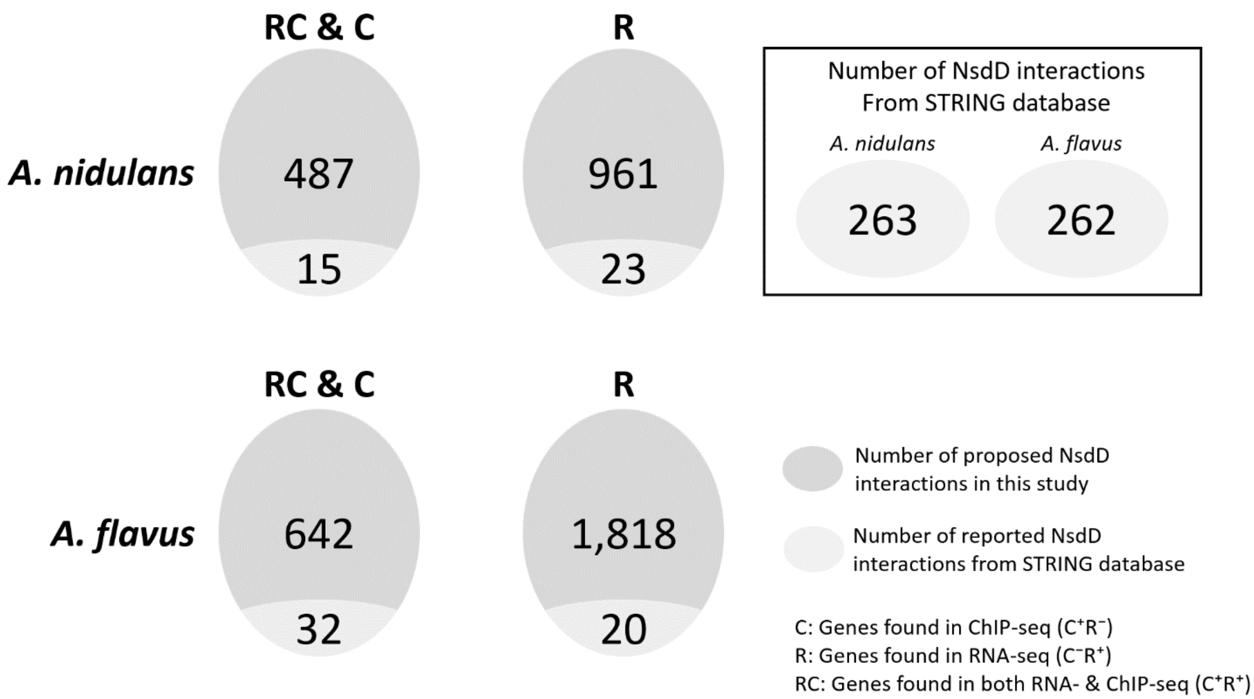


Figure S2-4. The known interactions of NsdD from the STRING database and novel interactions of NsdD proposed in this study.

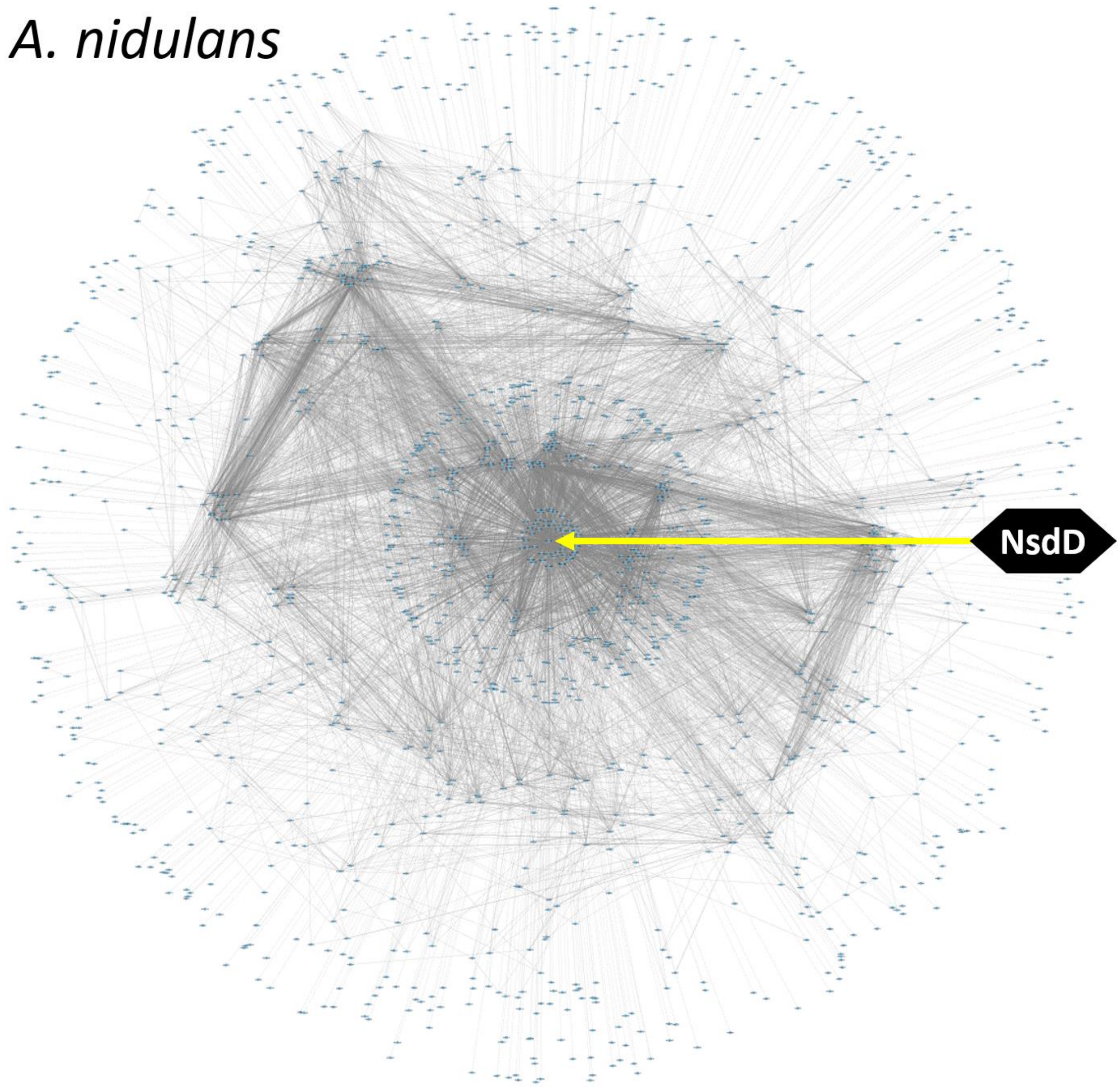


Figure S2-5. The *A. nidulans*-specific NsdD-mediated gene regulatory network.

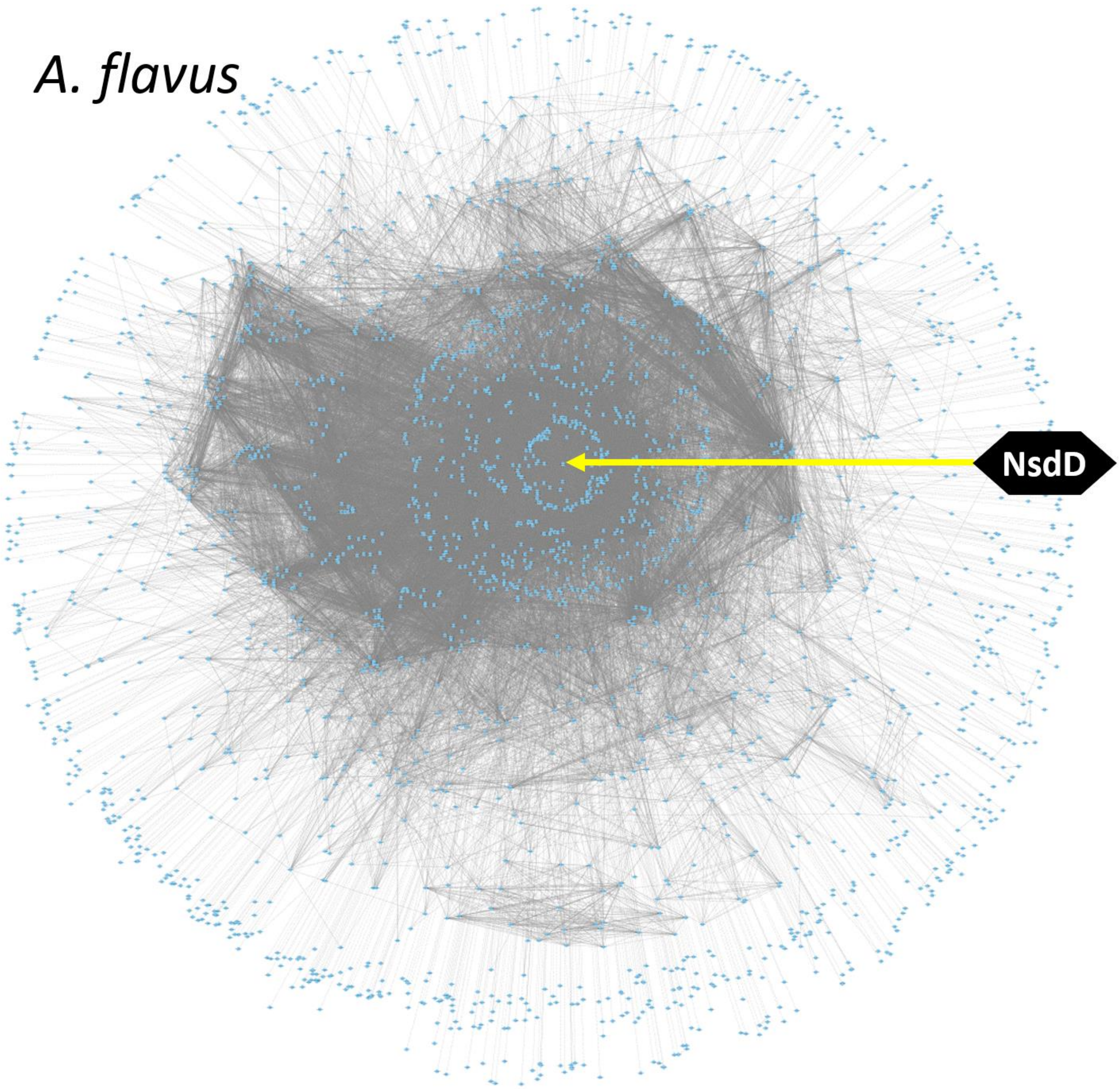


Figure S2-6. The *A. flavus*-specific NsdD-mediated gene regulatory network.

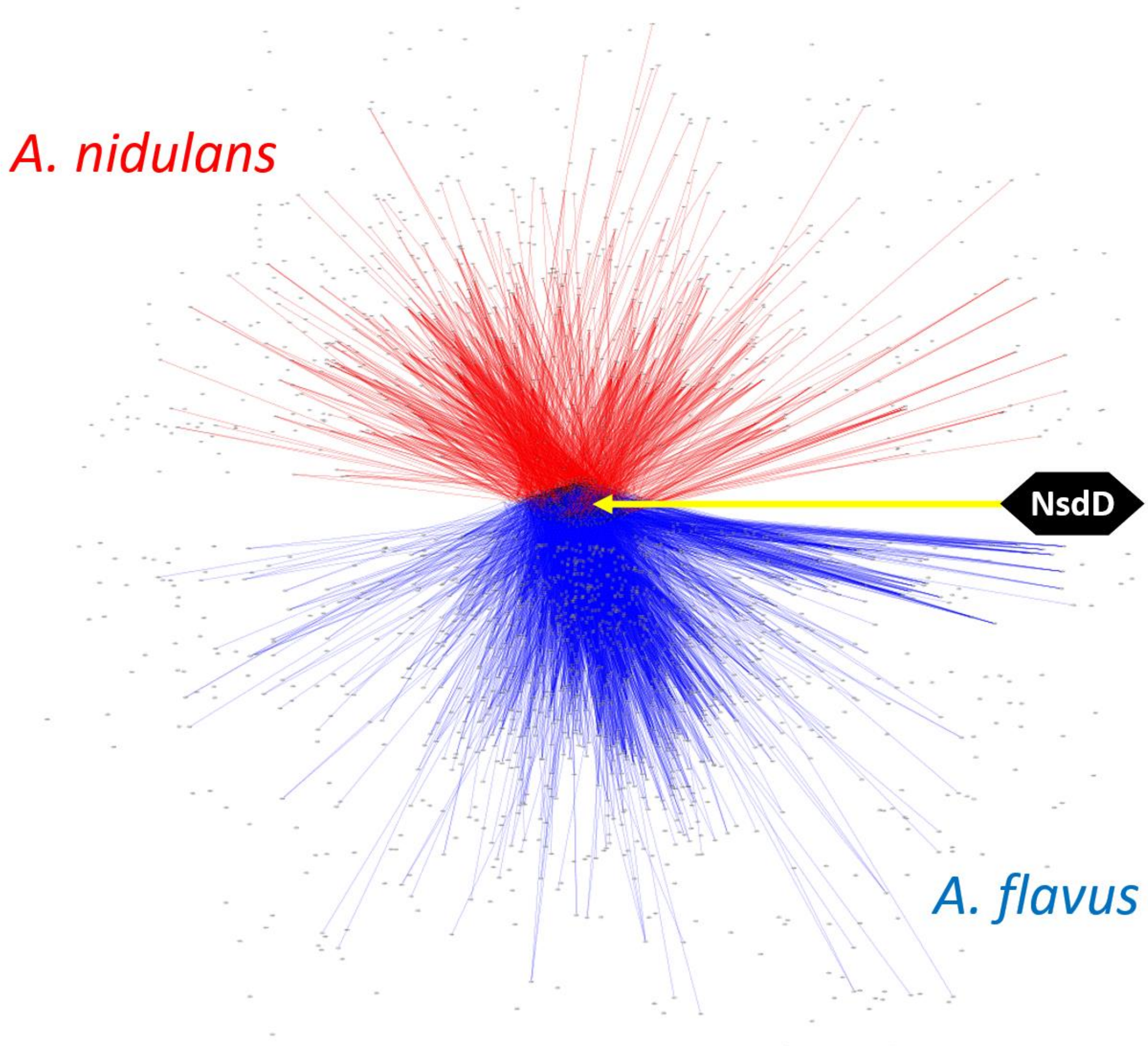


Figure S2-7. The comparative NsdD-mediated gene regulatory network between *A. nidulans* and *A. flavus.*

Table 2-1. *Aspergillus* strains used in this study.

Strain name	Relevant genotype	References
FGSC4	*A. nidulans* wild type	FGSC[a]
TNJ108	*pyrG89*; *pyroA4*; Δ*nsdD*::*AfupyrG*$^{+}$	Lee *et al*. 2014
TMK13	*pyrG89*; $^{3/4}$*pyroA4*::*nsdD*(p)::*nsdD*::FLAG3X::*trpC*(t)::*pyroA*$^{+}$[b]; Δ*nsdD*::*AfupyrG*$^{+}$	Lee *et al*. 2016
NRRL3357	*A. flavus* wild type	FGSC[a]
LNJ11	*AflpyrG*$^{-}$; Δ*nsdD*::*AfupyrG*$^{+}$	Lee *et al*. 2016
THM5	*AflpyrG*$^{-}$; Δ*nsdD*::*AfupyrG*$^{+}$; *nsdD*(p)::*nsdD*::FLAG3X::*trpC*(t)	This study
TMK20	*pyrG89*; Δ*mcrA*::*AfupyrG*$^{+}$; *pyroA4*::*mcrA*(p)::*mcrA*:: FLAG3X::*pyroA*$^{+}$[b]	Lee *et al*. 2020

[a]Fungal Genetics Stock Center (University of Missouri, Kansas City). [b]The $^{3/4}$*pyroA* marker results in the targeted integration at the *pyroA* locus.

Table 2-2. Known secondary metabolites identified from the secondary metabolite analyses.

Species	Metabolite name	Up or Down in Δ*nsdD*	Log_2FC	Molecular Mass
A. nidulans	Asterriquinone	Up	-2.05	507.2276
	Aspernidgulene B1	Up	-1.98	403.2477
	Austinol	Up	-1.67	459.2013
	Aspernidine B	Up	-1.61	386.2324
	Isoaustinone/(5'R)-isoaustinone	Up	-1.55	427.2114
	Dehydroaustinol	Up	-1.47	457.1853
	Protoaustinoid A	Up	-1.46	431.2790
	Emericellin	Up	-1.27	409.2008
	Aspernidgulene B2	Up	-1.27	403.2477
	Aspernidgulene A1	Up	-1.20	419.2427
	Neoaustinone/austinolide	Up	-1.14	443.2062
	Preaspernidgulene A1	Up	-1.07	399.2166
	Emericellamide C/D	Up	-1.07	596.4013
	Austinoneol	Up	-1.03	415.2113
	Terrequinone A	[a]Only detected in Δ*nsdD*	-	491.2325
	Isoversicolorin C	Down	1.69	341.0654
	Nidulol	Down	1.80	195.0649
	2-ω-Dihydroxyemodin	Down	2.13	303.0498
	Citreorosein	Down	2.54	287.0549
	Isosecosterigmatocystin	Down	3.42	363.1072
	Alternariol	Down	3.44	259.0601
	Shamixanthone/epishamixanthone	Down	9.29	407.1851
	Shamixanthone/epishamixanthone	Down	9.46	407.1851
	Chrysophanol	[b]Only detected in WT	-	255.0651
	Norsolorinic acid anthrone	Only detected in WT	-	357.1332
A. flavus	Leporin B	Up	-2.51	352.1905
	Cyclopiazonic acid	Up	-1.70	337.1544
	[c]Circumdatin J	Only detected in Δ*nsdD*	-	378.1445
	(S)-(−)-6,8-di-O-methyl citreoisocoumarin	Down	1.18	307.1174
	Imizoquin D	Down	1.38	581.1888

[a]The metabolite is not detected in WT but is present in Δ*nsdD*. [b]The metabolite is not detected in Δ*nsdD* but is produced in WT. [c]Circumdatin J production is previously reported in *Aspergillus ostianus* but has not been reported in *A. flavus*.

Table 2-3. The genes forming the core section of the NsdD-mediated GRN in *A. nidulans*.

Gene ID	Gene name	Description
AN4238	*schA*	Protein kinase involved in cAMP-dependent signaling during conidial germination; has overlapping functions with PkaA; reduced growth on AVICEL medium; required for CreA derepression and endocellulase production
AN0279	*flbD*	Putative transcription factor involved in regulation of asexual and sexual development and in response to nitrogen starvation; contains a myb-like DNA-binding domain
AN5015	*conJ*	Putative conidiation gene; transcript induced by light in in developmentally competent mycelia; double conF and conJ deletion results in increased cellular glycerol or erythritol leading to delayed germination and desiccation resistance
AN1052	*veA*	Protein involved in light-sensitive control of differentiation and secondary metabolism; localizes to the nucleus in dark and to both nucleus and cytoplasm in the light; induced by light; AspGD sequence represents the veA1 mutant allele
AN2142	*kapA*	Karyopherin (importin) alpha, involved in protein import into nucleus
AN2290	*steA*	STE-like transcription factor with homeobox and zinc finger domains; null mutation blocks sexual cycle but not asexual development, forms Hulle cells but no ascogenous tissue nor cleistothecia
AN0807	*laeA*	Methyltransferase-domain protein; velvet complex component composed of VelB, VeA and LaeA; self-methylates; coordinates asexual development in response to light; regulates secondary metabolism and is required for Hulle cell formation
AN9340	*treA*	Alpha,alpha-trehalase with a role in trehalose hydrolysis; localized to the conidial cell wall; expression upregulated after exposure to farnesol
AN5635	*treB*	Putative alpha, alpha-trehalase with a predicted role in trehalose hydrolysis
AN7725	*pyroA*	Protein required for biosynthesis of pyridoxine; highly conserved throughout fungi, plants and bacteria
AN5199		Ortholog(s) have cytosol, nucleolus localization, methionine aminopeptidase activity
AN5055		Has domain(s) with predicted aminopeptidase activity, metalloexopeptidase activity and role in cellular process, proteolysis
AN0973	*brlA*	C2H2 zinc finger transcription factor, fphA- and lreB-dependent light induced regulator of conidiophore development; locus has 2 overlapping transcriptional units called brlA alpha and brlA beta; see 5' brlA micro-ORF, AN0974
AN0422	*abaA*	TEA/ATTS domain transcriptional activator involved in regulation of conidiation; required for phialide differentiation
AN3435	*lreA*	Putative zinc-finger transcription factor involved in blue-light responsive differentiation; interacts with LreB; similar to N. crassa blue-light-sensing component WC-1

AN3607	*lreB*	Putative zinc-finger transcription factor involved in blue-light responsive differentiation; interacts with VeA, FphA, and LreA; similar to N. crassa blue-light-sensing component WC-2
AN8803	*rodA*	Hydrophobin; protein involved in conidium development; required for the formation of outer hydrophobic layer (rodlet layer) of the conidium wall; transcriptionally regulated by BrlA; predicted glycosylphosphatidylinositol (GPI)-anchor
AN5583	*aslA*	Putative C2H2 zinc finger transcription factor, involved in vacuolar sequestration of potassium and vacuolar biogenesis
AN1029	*afoA*	Protein with homology to CtnR, citrinin biosynthesis transcriptional activator; contains a Zn(2)Cys(6) domain; involved in asperfuranone biosynthesis; overexpression induces expression of the asperfuranone biosynthesis gene cluster
AN8741	*mtfA*	Putative C2H2 transcription factor involved in regulation of secondary metabolism and morphogenesis
AN8513	*tdiA*	Putative single-module nonribosomal peptide synthetase (NRPS); member of the tdi (terrequinone A biosynthesis) gene cluster; transcriptionally regulated by LaeA
AN8514	*tdiB*	Asterriquinone prenyltransferase; member of the tdi gene cluster; required for terrequinone A production; catalyzes the reverse prenylation event during terrequinone A biosynthesis; lacks canonical prenyl diphosphate binding motif (D/N)DXXD
AN2621	*acvA*	Delta-(L-alpha-aminoadipyl)-L-cysteinyl-D-valine synthetase, nonribosomal peptide synthase, the first enzyme of the penicillin biosynthesis pathway
AN11143	*glaA*	Putative glucoamylase with a predicted role in starch metabolism
AN7402	*glaB*	Putative glucoamylase with a predicted role in starch metabolism
AN4159	*glnA*	Putative glutamate-ammonia ligase with a predicted role in glutamate and glutamine metabolism; intracellular; transcript upregulated by nitrate limitation; protein abundance decreased by menadione stress and induced by farnesol
AN4843	*agdG*	Putative alpha-glucosidase with a predicted role in maltose metabolism

Table 2-4. The genes forming the core section of the NsdD-mediated GRN in *A. flavus*.

Gene ID	Gene name	Description
AFLA_134030	*flbA*	developmental regulator FlbA
AFLA_137320	*flbC*	C2H2 conidiation transcription factor FlbC
AFLA_080170	*flbD*	MYB family conidiophore development protein FlbD, putative
AFLA_031340	*atfA*	bZIP transcription factor (AtfA), putative
AFLA_082850	*brlA*	C2H2 type conidiation transcription factor BrlA
AFLA_026900	*vosA*	developmental regulator VosA
AFLA_046990	*stuA*	APSES transcription factor StuA
AFLA_066460	*veA*	developmental regulator AflYf / VeA
AFLA_034170	*mpkB*	MAP kinase FUS3/KSS1
AFLA_049870	*areA*	GATA transcriptional activator AreA
AFLA_134680	*creA*	C2H2 transcription factor (Crea), putative
AFLA_032840		forkhead domain protein
AFLA_048110	*sep1*	forkhead transcription factor (Sep1), putative
AFLA_132980		forkhead domain protein
AFLA_129110		sensory transduction histidine kinase, putative
AFLA_134730	*cyc8*	transcriptional corepressor Cyc8, putative
AFLA_026610	*bdf1*	transcription regulator BDF1, putative
AFLA_026850		HMG box protein, putative
AFLA_051690		cutinase gene palindrome-binding protein, putative
AFLA_124690		vegetative incompatibility WD repeat protein, putative
AFLA_027420		F-box and WD domain protein
AFLA_033290	*laeA*	regulator of secondary metabolism LaeA
AFLA_074470	*rft1*	nuclear division Rft1 protein, putative
AFLA_061790	*cbf1*	centromere-binding factor 1, cbf1, putative
AFLA_033520	*dskB*	ubiquitin-like protein DskB, putative
AFLA_018440	*pasG*	SNF2 family helicase/ATPase PasG, putative
AFLA_024110		set and mynd domain containing protein, putative
AFLA_030080	*TFIIIA*	C2H2 transcription factor (TFIIIA), putative
AFLA_031650	*eIF2C4*	eukaryotic translation initiation factor eIF-2C4, putative
AFLA_032740	*TFIID*	transcription factor TFIID
AFLA_035750	*cdk1*	Cdk1, putative

Table 2-5. The most enriched GO terms from the comparative network.

The Orthologs (253)	***Genes only regulated in A. nidulans*** **(1,233)**	***Genes only regulated in A. flavus*** **(2,259)**
transmembrane transport	austinol metabolic process	regulation of RNA biosynthetic process
tyrosine catabolic process	austinol biosynthetic process	regulation of nucleic acid-templated transcription
organic hydroxy compound metabolic process	regulation of phospholipid translocation	regulation of transcription, DNA-templated
aromatic amino acid family catabolic process	regulation of phospholipid transport	regulation of nucleobase-containing compound metabolic process
L-phenylalanine catabolic process	regulation of lipid transport	nicotinamide mononucleotide transport
melanin biosynthetic process	regulation of lipid localization	regulation of RNA metabolic process
erythrose 4-phosphate/phosphoenolpyruvate family amino acid catabolic process	transmembrane transport	regulation of cellular biosynthetic process
positive regulation of developmental process	dehydroaustinol biosynthetic process	regulation of biosynthetic process
positive regulation of sporulation	dehydroaustinol metabolic process	regulation of macromolecule biosynthetic process
positive regulation of cell differentiation	fungal-type cell wall (1→3)-alpha-glucan metabolic process	regulation of cellular macromolecule biosynthetic process
positive regulation of sexual sporulation resulting in formation of a cellular spore	alpha-glucan metabolic process	regulation of cellular metabolic process

Table S2-1. Oligonucleotides used in this study

Name	Sequence (5' → 3')	Purpose
OMK718	GGTT**GAATTC**CTAGCTCTTTCGCTGGACCCT	5' *AflnsdD* with *Eco*RI
OMK719	GGAA**CATATG**ACGGCCAGTTGGAGATGC	3' *AflnsdD* with *Nde*I
OHM39	AAATCACTAGAAGGCACTCTTTGC	3' pHM1 sequencing
OHM40	TGACGATTTGGTACCCTCTCCCAC	5' pHM1 sequencing
OHM42	GCAGAAGCATCTCGGGCGAGTTTG	5' pHM1 sequencing
OHM43	ATGTATGCCGGCCAGCCATTACC	5' *AflnsdD* probe
OHM44	TTAACGGCCAGTTGGAGATGCGGA	3' *AflnsdD* probe
OHM97	CGAGGCAGTTGTCTCCTAGTCT	5' *AflnsdD* ChIP PCR (-1,378)
OHM98	GGATGACAAGAGATATGAGTGCG	3' *AflnsdD* ChIP PCR (-1,145)
OHM99	CTTGGCTTCCTCTTACGTCC	5' *AflnsdD* ChIP PCR (-743)
OHM100	TTGCCGTCCTTCAAGTGCG	3' *AflnsdD* ChIP PCR (-510)
OHM101	GGTGGCCTGATAATTTCTATCT	5' *AflnsdD* ChIP PCR (-632)
OHM102	AATAAGACAATAGAAGTGTGGGC	3' *AflnsdD* ChIP PCR (-391)
ONK1037	TCCCTCGAGGCAGTTGTCTCCTAG	5' flanking of *AflnsdD*
ONK1038	GCTTTGGCCTGTATCATGACTTCATCCGGTTATATCCTTGCGATGTCG	3' *AflnsdD* with *AfupyrG* tail
ONK1039	ATCGACCGAACCTAGGTAGGGTAAACGAAGAAAGGCTTTGGGATTAG	5' *AflnsdD* with *AfupyrG* tail
ONK1040	AGAGCCTCAATTCTTGGGAATGAC	3' flanking of *AflnsdD*
ONK1041	TGTGGTCTTCCTCTATCATCTATC	5' nest of *AflnsdD*
ONK1042	TCAGTATCTAGTTAGAACCACTGG	3' nest of *AflnsdD*

Table S2-2. The most up-regulated 50 DEGs in *A. nidulans* Δ*nsdD* Vege.

Gene ID	Gene Name	Log2 FC	Description
AN9219		-9.33	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN9220		-8.68	Protein of unknown function
AN4809	*gtaA*	-8.20	Putative glutaminase A with a predicted role in glutamate and glutamine metabolism
AN7116		-7.66	Protein of unknown function
AN9313	*CYP58 D1*	-7.29	Putative cytochrome P450; secondary metabolism gene cluster member with AN9314
AN8775		-7.17	Protein of unknown function
AN8360		-7.09	Has domain(s) with predicted oxidoreductase activity
AN6401		-7.06	Putative hydrophobin
AN1941		-7.01	Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN11999		-6.90	Protein of unknown function
AN10325	*srpkD*	-6.71	Has domain(s) with predicted ATP binding, protein tyrosine kinase activity and role in protein phosphorylation
AN11049		-6.27	NmrA-like domain containing protein; predicted secondary metabolism gene cluster member
AN10573	*ivoC*	-6.24	Putative cytochrome P450; transcrip/tionally coregulated with ivoA/AN10576
AN5444		-6.16	Putative tryptophan synthase with a predicted role in aromatic amino acid biosynthesis
AN7539		-6.12	Putative hydrophobin; transcript is induced by nitrate
AN3931	*pilB*	-6.11	Putative conserved eisosome protein
AN10049	*mdpB*	-5.99	Protein with homology to scytalone dehydratase; member of the monodictyphenone (mdp) secondary metabolite biosynthesis gene cluster; transcript is induced by nitrate
AN7414		-5.80	Protein of unknown function
AN7217		-5.77	Protein of unknown function
AN8910		-5.67	Putative polyketide synthase (PKS)
AN2755	*MAT1*	-5.67	Alpha-domain mating-type protein; regulator of sexual development; acts with Mat2 HMG domain protein; null mutant cleistothecia are sterile; gene expression is induced during sexual development
AN0638		-5.60	Protein of unknown function
AN12226		-5.58	Protein of unknown function
AN2912		-5.56	Protein of unknown function
AN0146	*mdpC*	-5.45	Protein with homology to versicolorin ketoreductase; member of the (mdp) monodictyphenone secondary metabolite biosynthesis gene cluster; required for monodictyphenone biosynthesis
AN2398		-5.38	Has domain(s) with predicted O-methyltransferase activity
AN11670		-5.31	Protein of unknown function
AN8958		-5.25	Protein of unknown function
AN9348		-5.19	Putative aryl-alcohol oxidase-related protein

AN5942		-5.12	Protein of unknown function
AN6552		-5.07	Putative F-box protein
AN8198		-5.04	Protein of unknown function
AN9314		-4.98	Protein with homology to entkaurene synthases; prediction backbone enzyme of a secondary metabolite biosynthesis gene cluster
AN8909		-4.95	Protein of unknown function
AN0231	*ivoB*	-4.90	Conidiophore-specific phenol oxidase; mutant conidiophores, metulae and phialides lack pigmentation; ivoB mutants accumulate the substrate N-acetyl-6-hydroxytryptophan (AHT); repressed by light in developmentally competent mycelia
AN2888		-4.89	Protein of unknown function
AN9174		-4.87	Has domain(s) with predicted role in transmembrane transport and membrane localization
AN0230		-4.82	Protein of unknown function
AN2924		-4.75	Putative nonribosomal peptide synthetase (NRPS)-like enzyme
AN7907		-4.71	Putative glyoxylate-bleomycin resistance protein; member of the F9775 secondary metabolite gene cluster
AN11882		-4.70	Protein of unknown function
AN2750		-4.69	Protein of unknown function
AN4691		-4.65	Ortholog(s) have L-arabinitol 2-dehydrogenase activity and role in D-arabinose catabolic process, D-arabitol catabolic process to xylulose 5-phosphate
AN10887	*CYP5095B1*	-4.57	Putative cytochrome P450; predicted secondary metabolism gene cluster member
AN4148	*xtrE*	-4.55	Putative xylose transporter; transcriptionally induced by growth on xylose
AN12225		-4.53	Protein of unknown function
AN11624		-4.52	Protein of unknown function; transcript repressed by nitrate
AN2228		-4.52	Protein of unknown function
AN8612		-4.48	Protein of unknown function
AN0973	*brlA*	-4.44	C2H2 zinc finger transcription factor, fphA- and lreB-dependent light induced regulator of conidiophore development; locus has 2 overlapping transcriptional units called brlA alpha and brlA beta; see 5' brlA micro-ORF, AN0974

Table S2-3. The most down-regulated 50 DEGs in *A. nidulans* Δ*nsdD* Vege.

Gene ID	Gene Name	Log2 FC	Description
AN20018	*ndhC*	12.39	Mitochondrially encoded subunit 3 of NADH dehydrogenase
AN6407		8.84	Ortholog(s) have oxidoreductase activity and role in steroid metabolic process
AN20013		7.55	Putative mitochondrial ribosomal protein S5, encoded within the intron of the large mitochondrial ribosomal rRNA gene (L-rRNA); similar to open reading frames in the introns of N. crassa and Penicil
AN20019	*oxiB*	7.28	Subunit II of cytochrome c oxidase, which is the terminal member of the mitochondrial inner membrane electron transport chain; one of three mitochondrially-encoded subunits
AN3567		7.11	Protein of unknown function
AN3388	*amyF*	6.98	Putative alpha-amylase with a predicted role in starch metabolism; transcriptionally induced by isomaltose in an amyR-dependent manner
AN7932	*CYP682A1*	6.96	Putative cytochrome P450
AN7933	*llmC*	6.89	Putative LaeA-like methyltransferase
AN10372		6.73	Protein of unknown function
AN6456		6.69	Protein of unknown function
AN12458		6.39	Has domain(s) with predicted catalytic activity and role in metabolic process
AN9038		6.21	Has domain(s) with predicted role in Mo-molybdopterin cofactor biosynthetic process, molybdopterin cofactor biosynthetic process
AN8526		6.08	Ortholog(s) have role in ergot alkaloid biosynthetic process
AN7396	*bglM*	6.07	Putative beta-glucosidase
AN3780		5.94	Putative alpha-L-rhamnosidase
AN11673		5.94	Protein of unknown function
AN7773	*CYP573A3*	5.93	Putative cytochrome P450
AN2392		5.80	Has domain(s) with predicted protein dimerization activity
AN5267	*faeC*	5.78	with ferulic acid esterase activity, involved in degradation of xylans
AN8666		5.60	Has domain(s) with predicted RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN8985		5.48	Has domain(s) with predicted oxidoreductase activity, oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor activity and role in oxidation-reduction process
AN9310		5.44	Transcript induced by light in in developmentally competent mycelia
AN4629		5.41	Putative unsaturated glucuronyl hydrolase

AN9036		5.35	Protein of unknown function
AN3615		5.35	Has domain(s) with predicted ATP binding, nucleoside-triphosphatase activity
AN4643	*CYP675A1*	5.28	Putative cytochrome P450
AN3561		5.25	Protein of unknown function
AN7089		5.20	Protein of unknown function
AN8637	*catA*	5.18	Conidia-specific catalase; predicted role in gluconic acid and gluconate metabolism
AN5069		5.17	Protein of unknown function
AN7884		5.17	Putative nonribosomal peptide synthase (NRPS) similar to ferrichrome peptide synthetases involved in siderophore biosynthesis; predicted backbone enzyme of a secondary metabolism gene cluster member
AN5068		5.14	Has domain(s) with predicted arylformamidase activity and role in tryptophan catabolic process to kynurenine
AN5324	*dlpA*	5.13	Dehydrin-like protein; protein induced by farnesol
AN9037		5.13	Has domain(s) with predicted molybdenum ion binding, oxidoreductase activity and role in oxidation-reduction process
AN4123		5.13	Has domain(s) with predicted RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN8134		5.12	Has domain(s) with predicted transferase activity, transferring acyl groups other than amino-acyl groups activity
AN3248		5.08	Has domain(s) with predicted role in oxidation-reduction process
AN0016		5.05	Putative nonribosomal peptide synthase
AN9045	*pgxD*	5.04	Protein with polygalacturonase activity, involved in degradation of pectin
AN7881	*CYP548C1*	5.02	Putative cytochrome P450; predicted secondary metabolism gene cluster member
AN7584		5.02	Has domain(s) with predicted DNA binding, zinc ion binding activity, role in transcription, DNA-templated and nucleus localization
AN7152	*aglD*	5.00	Protein with alpha-galactosidase activity, involved in degradation of mannans, predicted role in galactose and galactitol metabolism
AN2649		4.96	Protein of unknown function
AN3205		4.93	Putative aldehyde dehydrogenase
AN3504		4.88	Putative alpha-1,4-glucosidase; transcript is induced by nitrate
AN8550		4.86	Protein of unknown function
AN3614		4.81	Protein of unknown function

AN8465	*rhaF*	4.81	Putative alpha-L-rhamnosidase; expression upregulated after exposure to farnesol
AN5289		4.80	Protein of unknown function
AN5938		4.76	Ortholog(s) have cytosol, nucleus localization

Table S2-4. The most up-regulated 50 DEGs in *A. nidulans* Δ*nsdD* Asex.

Gene ID	Gene Name	Log2 FC	Description
AN9042	*agnC*	-13.08	Putative alpha-1,3-glucanase
AN7349	*mutA*	-12.49	Protein with alpha-1,3-glucanase (mutanase) activity, involved in carbohydrate catabolism; highly expressed during sexual development, specifically expressed in Hulle cells
AN0195		-9.45	Protein of unknown function
AN9128		-8.54	Has domain(s) with predicted catalytic activity and role in metabolic process
AN7963		-8.24	Protein of unknown function
AN4620		-8.18	Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN2610	*CYP566C1*	-8.08	Putative cytochrome P450
AN7116		-7.94	Protein of unknown function
AN3568		-7.81	Protein of unknown function
AN2607	*CYP661A1*	-7.69	Putative cytochrome P450
AN7345	*agdC*	-7.65	Protein with glucosidase activity, involved in degradation of glucans; predicted role in maltose metabolism
AN4871	*chiB*	-7.62	Class V chitinase; glycoside hydrolase family 18 (GH18) protein with a role in the age-dependent autolysis
AN7344		-7.45	Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN10325	*srpkD*	-7.36	Has domain(s) with predicted ATP binding, protein tyrosine kinase activity and role in protein phosphorylation
AN2608		-7.31	Ortholog(s) have intracellular localization
AN2912		-7.28	Protein of unknown function
AN10385		-7.01	Protein of unknown function
AN3783		-6.99	Protein of unknown function
AN6798		-6.98	Has domain(s) with predicted catalytic activity and role in metabolic process
AN11024		-6.97	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN9129		-6.96	Ortholog(s) have role in pathogenesis
AN0012		-6.85	Protein of unknown function
AN11049		-6.84	NmrA-like domain containing protein; predicted secondary metabolism gene cluster member
AN8510	*CYP686A1P*	-6.81	Putative cytochrome P450; possibly a pseudogene
AN0245		-6.76	Putative glucanase with a predicted role beta-glucan hydrolysis

AN4825		-6.74	Putative glucan 1,3-beta-glucosidase with a predicted role in glucan metabolism
AN6382		-6.61	Has domain(s) with predicted phosphoric diester hydrolase activity and role in lipid metabolic process
AN1723		-6.61	Ortholog(s) have cell septum, hyphal tip localization
AN0638		-6.56	Protein of unknown function
AN1088		-6.55	Protein of unknown function
AN11897		-6.50	Has domain(s) with predicted RNA binding, ribonuclease T2 activity
AN7343		-6.43	Putative Zn(II)2Cys6-domain containing transcription factor; transcript is induced by nitrate
AN8433		-6.42	Has domain(s) with predicted catalytic activity and role in metabolic process
AN1604	*agnE*	-6.35	Putative alpha-1,3-glucanase; predicted glycosyl phosphatidylinositol (GPI)-anchor
AN3915		-6.32	Has domain(s) with predicted transporter activity, role in transport and membrane localization
AN5444		-6.27	Putative tryptophan synthase with a predicted role in aromatic amino acid biosynthesis
AN4394		-6.22	Ortholog(s) have role in asexual sporulation resulting in formation of a cellular spore, positive regulation of asexual sporulation resulting in formation of a cellular spore, regulation of transcription, DNA-templated
AN3966		-6.20	Protein of unknown function
AN12225		-6.18	Protein of unknown function
AN1419		-6.14	Protein of unknown function
AN5355		-6.09	Has domain(s) with predicted oxidoreductase activity, transferase activity, transferring acyl groups other than amino-acyl groups, zinc ion binding activity and role in oxidation-reduction process
AN2398		-6.05	Has domain(s) with predicted O-methyltransferase activity
AN7414		-6.04	Protein of unknown function
AN4809	*gtaA*	-6.04	Putative glutaminase A with a predicted role in glutamate and glutamine metabolism
AN5309		-6.04	Putative cutinase with a predicted role in the hydrolysis of cutin
AN1307		-6.00	Protein of unknown function
AN8432		-5.98	ain(s) with predicted catalytic activity
AN7702		-5.96	Ortholog(s) have tripeptide transmembrane transporter activity and role in glutathione transmembrane transport
AN2471		-5.95	Protein of unknown function
AN0972		-5.93	Protein of unknown function

Table S2-5. The most down-regulated 50 DEGs in *A. nidulans* Δ*nsdD* Asex.

Gene ID	Gene Name	Log2 FC	Description
AN20013		8.22	Putative mitochondrial ribosomal protein S5, encoded within the intron of the large mitochondrial ribosomal rRNA gene (L-rRNA); similar to open reading frames in the introns of N. crassa and Penicil
AN5324	*dlpA*	7.50	Dehydrin-like protein; protein induced by farnesol
AN7933	*llmC*	7.09	Putative LaeA-like methyltransferase
AN7932	*CYP682A1*	6.61	Putative cytochrome P450
AN9310		6.03	Transcript induced by light in in developmentally competent mycelia
AN3390	*pmeA*	5.92	Protein with pectinesterase activity, involved in degradation of pectin
AN3495	*inpA*	5.66	Putative nonribosomal peptide synthetase with a role in asperfuranone biosynthesis; inp secondary metabolite gene cluster member; expression controlled by scpR; transcriptionally silent under standard laboratory conditions
AN6407		5.56	Ortholog(s) have oxidoreductase activity and role in steroid metabolic process
AN20019	*oxiB*	5.47	Subunit II of cytochrome c oxidase, which is the terminal member of the mitochondrial inner membrane electron transport chain; one of three mitochondrially-encoded subunits
AN7903	*pkeA*	5.33	Polyketide synthase; involved in orsellinic acid and violaceol production; member of the dba gene cluster; dba cluster expression is coregulated
AN8329		5.32	Putative glucose oxidase-related protein
AN11313		5.14	Protein of unknown function
AN7139		5.09	Protein of unknown function
AN10977		5.07	Ortholog(s) have extracellular region localization
AN0468		5.02	Protein of unknown function
AN8077		5.00	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN0528		4.98	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN8138	*aglC*	4.97	Alpha-galactosidase, involved in degradation of mannans; predicted role in galactose and galactitol metabolism; glycoside hydrolase family 36 (GH36); transcriptionally induced by growth on xylose
AN5624		4.96	Ortholog(s) have cytosol, endoplasmic reticulum, mitotic spindle pole body, nucleus localization
AN12413		4.93	Protein of unknown function
AN2792		4.79	Protein of unknown function
AN3872		4.78	Transcript induced by light in in developmentally competent mycelia

AN6456		4.73	Protein of unknown function
AN9266		4.71	Protein of unknown function
AN3388	*amyF*	4.67	Putative alpha-amylase with a predicted role in starch metabolism; transcriptionally induced by isomaltose in an amyR-dependent manner
AN7402	*glaB*	4.62	Putative glucoamylase with a predicted role in starch metabolism
AN20006	*I-AniI*	4.49	Group I intron maturase encoded by the first exon and part of the intron of the mitochondrial cobA gene; facilitates the splicing of the cobA group I intron; also has DNA endonuclease activity
AN8666		4.43	Has domain(s) with predicted RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN7341		4.43	Protein of unknown function
AN6633		4.39	Protein of unknown function; transcript is induced by nitrate
AN6445	*cicC*	4.36	Putative aryl-alcohol oxidase-related protein; coregulated with the NRPS/AN6444; encoded within the cichorine gene cluster
AN2817		4.35	Protein of unknown function
AN3201	*lacD*	4.33	Putative beta-galactosidase with a predicted role in lactose metabolism
AN0026		4.31	Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-template
AN6455		4.30	Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN7153		4.29	Has domain(s) with predicted UDP-N-acetylmuramate dehydrogenase activity, flavin adenine dinucleotide binding, oxidoreductase activity and role in oxidation-reduction process
AN5338		4.29	Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN5090		4.28	Predicted ADP ribosylation factor (Arf) GTPase
AN20008	*ndhD*	4.26	Mitochondrially encoded subunit 4 of NADH dehydrogenase; sequence in AspGD is truncated at the 3' end
AN6820	*hk-8-3*	4.26	Putative histidine-containing phosphotransfer protein
AN0567		4.25	Putative alcohol oxidase with a predicted role in glycerol metabolism
AN0294		4.25	Ortholog(s) have cytoplasm localization
AN1792		4.23	Has domain(s) with predicted hydrolase activity, acting on ester bonds activity and role in lipid metabolic process
AN8195		4.23	Protein of unknown function
AN4113	*hk-8-2*	4.23	Histidine kinase, histidine-containing phosphotransfer protein; expression upregulated after exposure to farnesol; palA-dependent expression independent of pH
AN12379		4.23	Protein of unknown function

AN7618		4.23	Has domain(s) with predicted transporter activity, role in transport and membrane localization
AN0467		4.21	Protein of unknown function
AN7902	*dbaH*	4.19	FAD-binding monooxygenase with a role in secondary metabolism; member of the dba gene cluster; dba cluster expression is coregulated
AN0314		4.18	Putative aspartyl-tRNA synthetase with a predicted role in tRNA aminoacylation; expression upregulated after exposure to farnesol

Table S2-6. The most up-regulated 50 DEGs in *A. nidulans* Δ*nsdD* conidia.

Gene ID	Gene Name	Log2 FC	Description
AN8656		-6.98	Has domain(s) with predicted 2 iron, 2 sulfur cluster binding, iron ion binding, oxidoreductase activity, oxidoreductase activity and acting on paired donors, more
AN6798		-5.04	Has domain(s) with predicted catalytic activity and role in metabolic process
AN8657		-4.69	Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN6382		-4.56	Has domain(s) with predicted phosphoric diester hydrolase activity and role in lipid metabolic process
AN1029	*afoA*	-4.53	Protein with homology to CtnR, citrinin biosynthesis transcriptional activator; contains a Zn(2)Cys(6) domain; involved in asperfuranone biosynthesis; overexpression induces expression of the asperfuranone biosynthesis gene cluster
AN2585		-4.46	Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN3574		-4.37	Protein of unknown function
AN1033	*afoD*	-4.11	Putative salicylate hydroxylase; required for asperfuranone biosynthesis; transcriptionally induced by scrP overexpression
AN1032	*afoC*	-4.10	Putative oxidoreductase; required for asperfuranone biosynthesis
AN7663		-3.83	Has domain(s) with predicted NAD binding, oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor activity and role in oxidation-reduction process
AN7863		-3.68	Protein of unknown function
AN3210		-3.60	Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN8516	*tdiD*	-3.33	Putative aminotransferase; member of the tdi (terrequinone A biosynthesis) gene cluster; transcriptionally regulated by LaeA
AN4605		-3.25	Has domain(s) with predicted metal ion binding activity
AN1899	*hpdA*	-3.22	Putative 4-hydroxyphenylpyruvate dioxygenase with a predicted role in aromatic amino acid biosynthesis; expression induced by phenylalanine and repressed by glucose; mutants unable to use phenylalanine as a sole carbon source
AN6747		-2.95	Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN8654		-2.88	Putative aminomethyltransferase with a predicted role in glycine, serine, and threonine metabolism

AN12082		-2.87	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN12244		-2.85	Protein of unknown function
AN8390		-2.84	GPR1/FUN34/YaaH family member; ethanol- and ethylacetate-induced gene
AN0638		-2.83	Protein of unknown function
AN1897	*hmgA*	-2.79	Homogentisate 1,2-dioxygenase, enzyme in phenylalanine catabolism; required for growth on phenylalanine or phenylacetate as the sole carbon source; mutation in human ortholog results in alkaptonuria
AN11575		-2.76	Protein of unknown function
AN7061		-2.73	Putative transcription factor; predicted role in secondary metabolite production
AN7349	*mutA*	-2.72	Protein with alpha-1,3-glucanase (mutanase) activity, involved in carbohydrate catabolism; highly expressed during sexual development, specifically expressed in Hulle cells
AN10668		-2.70	Has domain(s) with predicted NAD binding, oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor activity and role in oxidation-reduction process
AN6153		-2.67	Protein of unknown function
AN8043		-2.66	Protein of unknown function
AN8953	*agdB*	-2.65	Putative alpha-glucosidase with a predicted role in maltose metabolism; transcriptionally induced by isomaltose; induced by rapamycin-induced autophagy
AN7402	*glaB*	-2.64	Putative glucoamylase with a predicted role in starch metabolism
AN3041		-2.63	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN1028		-2.58	Has domain(s) with predicted RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN4871	*chiB*	-2.57	Class V chitinase; glycoside hydrolase family 18 (GH18) protein with a role in the age-dependent autolysis
AN8737	*mstA*	-2.52	High-affinity glucose transporter active in hyphae under glucose-limiting conditions
AN8154		-2.43	Protein of unknown function
AN9295		-2.42	Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN8655		-2.41	Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN11389		-2.40	Protein of unknown function
AN9042	*agnC*	-2.40	Putative alpha-1,3-glucanase

AN7865	*bglJ*	-2.39	Putative beta-glucosidase
AN9164		-2.37	Has domain(s) with predicted oxidoreductase activity
AN5993		-2.35	Has domain(s) with predicted calcium ion binding activity
AN4825		-2.33	Putative glucan 1,3-beta-glucosidase with a predicted role in glucan metabolism
AN8143		-2.29	Has domain(s) with predicted role in isoprenoid biosynthetic process
AN0195		-2.25	Protein of unknown function
AN7066	*CYP670A1*	-2.21	Putative cytochrome P450
AN4131		-2.20	Has domain(s) with predicted solute:proton antiporter activity, role in cation transport, transmembrane transport and integral component of membrane localization
AN7181		-2.19	Protein of unknown function
AN9336		-2.18	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN5323		-2.17	Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization

Table S2-7. The most down-regulated 50 DEGs in *A. nidulans* Δ*nsdD* conidia.

Gene ID	Gene Name	Log2 FC	Description
AN7895	*cipB*	7.01	Putative oxidoreductase; contains Zn-dependent alcohol dehydrogenase domain; protein expressed at increased levels during osmoadaptation
AN10431		6.43	Protein of unknown function; transcript is induced by nitrate; predicted NirA binding site in promoter
AN0528		5.68	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN5540		5.67	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN9280		5.54	Has domain(s) with predicted role in transmembrane transport and membrane localization
AN11491		5.19	Protein of unknown function
AN12418		5.10	Protein of unknown function
AN7875		5.03	Protein of unknown function; predicted secondary metabolism gene cluster member
AN6948	*crhE*	5.00	Putative transglycosidase with a predicted role in glucan processing
AN8366		4.90	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN7893		4.88	Has domain(s) with predicted oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors
AN12446		4.82	Protein of unknown function
AN5421		4.65	Protein of unknown function
AN7157		4.64	Protein of unknown function
AN8308		4.62	Protein of unknown function
AN11946		4.62	Protein of unknown function
AN11945		4.60	Protein of unknown function
AN7899	*dbaE*	4.56	Putative esterase/lipase with a role in secondary metabolism; member of the dba gene cluster
AN11215		4.52	Has domain(s) with predicted metal ion transmembrane transporter activity, role in metal ion transport, transmembrane transport and membrane localization
AN8381	*ausC*	4.47	Protein of unknown function; required for austinol and dehydroaustinol biosynthesis, aus secondary metabolism gene cluster member
AN11210		4.39	Protein of unknown function; transcript repressed by nitrate
AN11206	*ausM*	4.30	Predicted monooxygenase; required for austinol and dehydroaustinol biosynthesis; aus secondary metabolism gene cluster member
AN11871		4.28	Protein of unknown function
AN0587		4.23	Putative Hsp70 family chaperone; transcript is induced by nitrate

AN6482		4.23	Protein of unknown function
AN5281		4.15	Putative pyranose oxidase
AN9206		4.13	Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN11281		4.09	Protein of unknown function
AN3996		4.09	Has domain(s) with predicted methyltransferase activity and role in metabolic process
AN3205		4.08	Putative aldehyde dehydrogenase
AN6447	*cicE*	4.06	ed O-methyltransferase; coregulated with the PKS pkbA/AN6448; encoded within the cichorine gene cluster
AN9279		4.05	Putative delta-1-pyrroline-5-carboxylate dehydrogenase with a predicted role in glutamate and glutamine metabolism
AN7937	*cipC*	4.05	Protein responsive to Concanamycin A
AN8432		4.03	ain(s) with predicted catalytic activity
AN8384	*ausD*	4.03	Protein of unknown function; required for austinol and dehydroaustinol biosynthesis; aus secondary metabolism gene cluster member
AN6404		4.03	Has domain(s) with predicted zinc ion binding activity
AN5304		4.02	Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN8310		3.93	Protein of unknown function
AN7906		3.93	Protein of unknown function
AN5312		3.91	Protein of unknown function
AN2370		3.89	Protein of unknown function
AN9272		3.88	Protein of unknown function
AN5501		3.85	Ortholog(s) have lipase activity
AN8439		3.70	Protein of unknown function; transcript is induced by nitrate; predicted NirA binding site
AN3519		3.69	Protein of unknown function
AN9186		3.69	Putative acid phosphatase with a predicted role in gluconic acid and gluconate metabolism; expression repressed by the addition of inorganic phosphate to the growth medium
AN8624		3.67	Protein of unknown function
AN2116		3.66	Has domain(s) with predicted catalytic activity, coenzyme binding activity and role in cellular metabolic process
AN1837		3.64	Putative hydrophobin; predicted glycosyl phosphatidylinositol (GPI)-anchor
AN2037		3.61	Predicted NAD binding oxidoreductase; predicted secondary metabolism gene cluster member

Table S2-8. The most up-regulated 50 DEGs in *A. flavus* Δ*nsdD* Vege.

Gene ID	Gene Name	Log2 FC	Description
AFLA_053560		-4.68	conserved hypothetical protein
AFLA_020230		-4.58	hypothetical protein
AFLA_106080		-4.55	hypothetical protein
AFLA_020220		-4.39	aminotransferase class III, putative
AFLA_102420		-4.21	hypothetical protein
AFLA_049520		-4.06	conserved hypothetical protein
AFLA_092300		-3.99	hypothetical protein
AFLA_096130		-3.96	conserved hypothetical protein
AFLA_105240		-3.86	hypothetical protein
AFLA_102780		-3.83	WD-repeat protein, putative
AFLA_044800		-3.67	conidiation protein Con-6, putative
AFLA_097540		-3.61	hypothetical protein
AFLA_106070		-3.53	conserved hypothetical protein
AFLA_035280		-3.43	cell wall biogenesis protein, putative
AFLA_009320		-3.39	conserved hypothetical protein
AFLA_100330		-3.34	FAD dependent oxidoreductase, putative
AFLA_101960		-3.31	conserved hypothetical protein
AFLA_096210		-3.30	catalase, putative
AFLA_006730		-3.22	alcohol dehydrogenase, putative
AFLA_092090		-3.20	oxidoreductase, short-chain dehydrogenase/reductase family
AFLA_082110		-3.19	wd40 protein, putative
AFLA_005350		-3.16	hypothetical protein
AFLA_085640		-3.13	hypothetical protein
AFLA_042540		-3.11	conserved hypothetical protein
AFLA_108370		-2.97	tyrosinase, putative
AFLA_101950		-2.97	conserved hypothetical protein
AFLA_028420		-2.95	conserved hypothetical protein
AFLA_024610		-2.95	NADP-dependent alcohol dehydrogenase
AFLA_009790		-2.95	conserved hypothetical protein
AFLA_016580		-2.94	conserved hypothetical protein
AFLA_097270		-2.92	conserved hypothetical protein
AFLA_050590		-2.81	conserved hypothetical protein
AFLA_062050		-2.79	putative allantoate permease of the major facilitator superfamily
AFLA_048440		-2.79	conserved hypothetical protein
AFLA_002020		-2.77	spherulin 4-like cell surface protein, putative
AFLA_041370		-2.77	isoflavone reductase family protein
AFLA_005440		-2.73	nonribosomal peptide synthase, putative
AFLA_096220		-2.72	HHE domain protein
AFLA_107790		-2.72	glucan 1,3-beta-glucosidase precursor, putative
AFLA_060180		-2.71	conserved hypothetical protein
AFLA_081530		-2.71	hypothetical protein
AFLA_033450		-2.66	gamma-glutamylputrescine oxidoreductase, putative

AFLA_051180		-2.61	erythromycin esterase, putative
AFLA_044790		-2.61	conidiation-specific family protein
AFLA_110190		-2.60	anticoagulant protein C, putative
AFLA_108940		-2.57	esterase/lipase, putative
AFLA_083110	*conJ*	-2.57	conidiation-specific protein (Con-10), putative
AFLA_058620		-2.55	ferric-chelate reductase FREA
AFLA_013520		-2.53	cytochrome P450, putative
AFLA_100250		-2.50	catalase Cat

Table S2-9. The most down-regulated 50 DEGs in *A. flavus* Δ*nsdD* Vege.

Gene ID	Gene Name	Log2 FC	Description
AFLA_082960		6.43	conserved hypothetical protein
AFLA_007810		6.35	gibberellin 20-oxidase family protein
AFLA_025720		5.73	C6 transcription factor NosA
AFLA_064330		5.66	conserved hypothetical protein
AFLA_039180		5.40	MFS gliotoxin efflux transporter GliA
AFLA_064340		5.11	conserved hypothetical protein
AFLA_119190		4.95	hypothetical protein
AFLA_007830		4.91	conserved hypothetical protein
AFLA_024100		4.85	conserved hypothetical protein
AFLA_094120		4.83	conserved hypothetical protein
AFLA_065460		4.83	conserved hypothetical protein
AFLA_007430		4.70	conserved hypothetical protein
AFLA_126530		4.65	conserved hypothetical protein
AFLA_064470		4.56	cytochrome P450, putative
AFLA_086470		4.56	DUF124 domain protein
AFLA_028830		4.52	FG-GAP repeat protein, putative
AFLA_007870		4.31	phosphatidylserine decarboxylase, putative
AFLA_127130		4.24	conserved hypothetical protein
AFLA_075100		4.20	conserved hypothetical protein
AFLA_139170	*aflW*	4.18	aflW/ moxY/ monooxygenase
AFLA_139230	*aflI*	4.15	aflI/ avfA/ cytochrome P450 monooxygenase
AFLA_139180	*aflV*	4.13	aflV/ cypX/ cytochrome P450 monooxygenase
AFLA_131310		4.10	conserved hypothetical protein
AFLA_139410	*aflC*	4.10	aflC / pksA / pksL1 / polyketide synthase
AFLA_139210	*aflP*	4.06	aflP/ omtA/ omt-1/ O-methyltransferase A
AFLA_139250	*aflL*	4.06	aflL/ verB/ desaturase/ P450 monooxygenase
AFLA_119200		4.04	conserved hypothetical protein
AFLA_057710		4.01	carboxyphosphonoenolpyruvate phosphonomutase, putative
AFLA_040800		3.97	conserved hypothetical protein
AFLA_111290		3.95	conserved hypothetical protein
AFLA_021920		3.93	cellular morphogenesis protein Boi1-like, putative
AFLA_139190	*aflK*	3.92	aflK/ vbs/ VERB synthase
AFLA_139310	*aflE*	3.92	aflE/ norA/ aad/ adh-2/ NOR reductase/ dehydrogenase
AFLA_139220	*aflO*	3.90	aflO/ omtB/ dmtA/ O-methyltransferase B
AFLA_139300	*aflM*	3.89	aflM/ ver-1/ dehydrogenase/ ketoreductase
AFLA_139240	*aflLa*	3.87	aflLa/ hypB/ hypothetical protein
AFLA_139140	*aflYa*	3.83	aflYa/ nadA/ NADH oxidase
AFLA_139260	*aflG*	3.82	aflG/ avnA/ ord-1/ cytochrome P450 monooxygenase
AFLA_139200	*aflQ*	3.81	aflQ/ ordA/ ord-1/ oxidoreductase/ cytochrome P450 monooxigenase
AFLA_139400	*aflCa*	3.79	aflCa / hypC / hypothetical protein
AFLA_139330	*aflH*	3.77	aflH/ adhA/ short chain alcohol dehydrogenase
AFLA_038870		3.75	integral membrane protein

AFLA_064290		3.71	O-methyltransferase, putative
AFLA_064270		3.65	gibberellin 2-oxidase, putative
AFLA_101640		3.64	conserved hypothetical protein
AFLA_139280	*aflN*	3.64	aflN/ verA/ monooxygenase
AFLA_011300		3.63	conserved hypothetical protein
AFLA_139290	*aflMa*	3.60	aflMa/ hypE/ hypothetical protein
AFLA_124930		3.60	monooxygenase, putative
AFLA_108510		3.58	conserved hypothetical protein

Table S2-10. The most up-regulated 50 DEGs in *A. flavus* Δ*nsdD* Asex.

Gene ID	Gene Name	Log2 FC	Description
AFLA_138610		-7.05	hypothetical protein
AFLA_108020		-6.74	conserved hypothetical protein
AFLA_009330		-6.54	conserved hypothetical protein
AFLA_097270		-6.18	conserved hypothetical protein
AFLA_069600		-5.57	conserved hypothetical protein
AFLA_121970		-5.36	pectate lyase, putative
AFLA_020220		-5.27	aminotransferase class III, putative
AFLA_138620		-5.08	conserved hypothetical protein
AFLA_108000		-4.79	conserved hypothetical protein
AFLA_065050		-4.74	Defensin domain protein
AFLA_013880		-4.51	lipase 2 precursor, putative
AFLA_002020		-4.46	spherulin 4-like cell surface protein, putative
AFLA_000980		-4.26	conserved hypothetical protein
AFLA_074740		-4.17	conserved hypothetical protein
AFLA_122950		-4.15	conserved hypothetical protein
AFLA_121840		-4.08	NAD dependent epimerase/dehydratase family protein
AFLA_061400		-4.02	aminotransferase, putative
AFLA_004410		-4.01	conserved hypothetical protein
AFLA_009320		-3.97	conserved hypothetical protein
AFLA_121160		-3.84	oxalate decarboxylase oxdC, putative
AFLA_038920		-3.84	conserved hypothetical protein
AFLA_138340		-3.71	conserved hypothetical protein
AFLA_009740		-3.71	hypothetical protein
AFLA_036400		-3.68	conserved hypothetical protein
AFLA_124110		-3.65	galactose-proton symport, putative
AFLA_065110		-3.64	neutral amino acid permease
AFLA_012930		-3.63	conserved hypothetical protein
AFLA_124660		-3.62	pectin lyase precursor, putative
AFLA_001800		-3.62	hypothetical protein
AFLA_124790		-3.57	GNAT family acetyltransferase, putative
AFLA_059940		-3.56	conserved hypothetical protein
AFLA_097290		-3.54	conserved hypothetical protein
AFLA_034690		-3.54	conserved hypothetical protein
AFLA_104640		-3.52	Asp hemolysin-like protein
AFLA_126170		-3.52	conserved hypothetical protein
AFLA_117120		-3.45	hypothetical protein
AFLA_127520		-3.45	glucose-methanol-choline (gmc) oxidoreductase, putative
AFLA_020240		-3.43	hypothetical protein
AFLA_076470		-3.41	conserved hypothetical protein
AFLA_104490		-3.38	conserved hypothetical protein
AFLA_062550		-3.36	phosphatidylserine decarboxylase, putative
AFLA_015330		-3.33	conserved hypothetical protein
AFLA_124560		-3.31	conserved hypothetical protein

AFLA_077840	-3.29	endoglucanase, putative
AFLA_120800	-3.21	ATP-binding cassette transporter, putative
AFLA_036280	-3.21	conserved hypothetical protein
AFLA_063400	-3.20	hypothetical protein
AFLA_116190	-3.19	conserved hypothetical protein
AFLA_107790	-3.18	glucan 1,3-beta-glucosidase precursor, putative
AFLA_008640	-3.13	ferric-chelate reductase, putative

Table S2-11. The most down-regulated 50 DEGs in *A. flavus* Δ*nsdD* Asex.

Gene ID	Gene Name	Log2 FC	Description
AFLA_089950		9.31	conserved hypothetical protein
AFLA_064380		8.42	conserved hypothetical protein
AFLA_074940		8.30	viral-enhancing factor, putative
AFLA_105630		8.16	cytochrome P450 monooxygenase, putative
AFLA_042330		8.02	conserved hypothetical protein
AFLA_064400		8.01	benzoate 4-monooxygenase cytochrome P450, putative
AFLA_089960		7.91	conserved hypothetical protein
AFLA_023020		7.73	NRPS-like enzyme, putative
AFLA_004300		7.71	prenyltransferase, putative
AFLA_038260		7.66	conserved hypothetical protein
AFLA_076160		7.60	conserved hypothetical protein
AFLA_126710		7.45	polyketide synthase, putative
AFLA_038430		7.35	pyridoxal-dependent decarboxylase domain protein
AFLA_105460		7.33	conserved hypothetical protein
AFLA_065160		7.27	hypothetical protein
AFLA_042200		7.17	conserved hypothetical protein
AFLA_057450		7.12	hypothetical protein
AFLA_023010		6.84	GA4 desaturase family protein
AFLA_065280		6.83	tyrosinase domain protein
AFLA_071790		6.72	galactose oxidase, putative
AFLA_065960		6.66	fucose-specific lectin FleA
AFLA_023030		6.62	cytochrome P450 oxidoreductase GliC-like, putative
AFLA_064390		6.61	cytochrome P450 oxidoreductase GliF
AFLA_042210		6.58	conserved hypothetical protein
AFLA_101370		6.40	conserved hypothetical protein
AFLA_056480		6.40	glycosyl transferase, putative
AFLA_023000		6.40	Ankyrin domain protein
AFLA_124500		6.36	nitric oxide synthase, putative
AFLA_064440		6.34	transport protein, putative
AFLA_126530		6.29	conserved hypothetical protein
AFLA_042230		6.22	ATP-dependent helicase, putative
AFLA_049600		6.20	conserved hypothetical protein
AFLA_042340		6.04	conserved hypothetical protein
AFLA_014450		6.00	conserved hypothetical protein
AFLA_042220		5.87	conserved hypothetical protein
AFLA_065450		5.86	penicillolysin/deuterolysin metalloprotease, putative
AFLA_077910		5.83	alpha-1,3-glucanase, putative
AFLA_062630		5.78	conserved hypothetical protein
AFLA_135210		5.73	conserved hypothetical protein
AFLA_038710		5.70	conserved hypothetical protein
AFLA_125280		5.63	hypothetical protein
AFLA_016150		5.60	lipase, putative
AFLA_072030		5.59	conserved hypothetical protein

AFLA_104990	5.53	conserved hypothetical protein
AFLA_035570	5.51	conserved hypothetical protein
AFLA_042320	5.47	conserved hypothetical protein
AFLA_128070	5.32	conserved hypothetical protein
AFLA_040690	5.24	hypothetical protein
AFLA_065170	5.18	conserved hypothetical protein
AFLA_042300	5.18	conserved hypothetical protein

Table S2-12. The most up-regulated 50 DEGs in *A. flavus* Δ*nsdD* conidia.

Gene ID	Gene Name	Log2 FC	Description
AFLA_118600		-6.27	conserved hypothetical protein
AFLA_011400		-5.48	conserved hypothetical protein
AFLA_065410		-5.29	conserved hypothetical protein
AFLA_104430		-5.23	subtilisin, putative
AFLA_074740		-4.95	conserved hypothetical protein
AFLA_054330		-4.94	conserved hypothetical protein
AFLA_076470		-4.74	conserved hypothetical protein
AFLA_023000		-4.63	Ankyrin domain protein
AFLA_010590		-4.57	siderophore biosynthesis lipase/esterase, putative
AFLA_017060		-4.49	conserved hypothetical protein
AFLA_063080		-4.46	conserved hypothetical protein
AFLA_009510		-4.46	sodium/chloride dependent neurotransmitter transporter, putative
AFLA_019160		-4.37	conserved hypothetical protein
AFLA_118910		-4.29	conserved hypothetical protein
AFLA_036690		-4.20	hypothetical protein
AFLA_136190		-4.12	conserved hypothetical protein
AFLA_059940		-4.08	conserved hypothetical protein
AFLA_004160		-4.06	ankyrin repeat-containing protein, putative
AFLA_054060		-4.06	ATP/GTP-binding protein, putative
AFLA_005190		-4.03	hypothetical protein
AFLA_060780		-4.01	hydrophobin family protein
AFLA_074070		-4.00	siderochrome-iron transporter, putative
AFLA_066440		-4.00	conserved hypothetical protein
AFLA_000930		-3.99	acetolactate synthase, large subunit, putative
AFLA_123590		-3.97	glutathione S transferase, putative
AFLA_023080		-3.95	integral membrane protein TmpA
AFLA_056600		-3.85	conserved hypothetical protein
AFLA_039840		-3.84	alpha/beta hydrolase, putative
AFLA_097270		-3.82	conserved hypothetical protein
AFLA_005030		-3.81	conserved hypothetical protein
AFLA_138550		-3.81	O-methyltransferase, putative
AFLA_060090		-3.80	conserved hypothetical protein
AFLA_075810		-3.78	efflux pump antibiotic resistance protein, putative
AFLA_009180		-3.74	conserved hypothetical protein
AFLA_013500		-3.73	conserved hypothetical protein
AFLA_007740		-3.73	heat shock protein Hsp20/Hsp26, putative
AFLA_019940		-3.70	sugar transporter, putative
AFLA_099170		-3.69	conserved hypothetical protein
AFLA_041140		-3.67	hypothetical protein
AFLA_116260		-3.64	N-hydroxyarylamine O-acetyltransferase, putative
AFLA_108510		-3.63	conserved hypothetical protein
AFLA_009320		-3.63	conserved hypothetical protein

AFLA_097640	-3.57	conserved hypothetical protein
AFLA_089720	-3.52	isopropylmalate synthase, putative
AFLA_041850	-3.52	phthalate transporter, putative
AFLA_124470	-3.45	conserved hypothetical protein
AFLA_058300	-3.42	conserved hypothetical protein
AFLA_059830	-3.41	glucose dehydrogenase, putative
AFLA_128330	-3.40	UMTA methyltransferase family protein
AFLA_070880	-3.40	acyl-coenzyme A:Isopenicillin N acyltransferase PenDE

Table S2-13. The most down-regulated 50 DEGs in *A. flavus* Δ*nsdD* conidia.

Gene ID	Gene Name	Log2 FC	Description
AFLA_064400		7.28	benzoate 4-monooxygenase cytochrome P450, putative
AFLA_070490		7.04	conserved hypothetical protein
AFLA_064380		6.74	conserved hypothetical protein
AFLA_077600		6.27	conserved hypothetical protein
AFLA_064410		6.23	conserved hypothetical protein
AFLA_037820		6.03	heat shock protein Hsp30-like, putative
AFLA_071800		5.97	branched-chain amino acid aminotransferase, putative
AFLA_118200		5.81	C-14 sterol reductase, putative
AFLA_064450		5.68	aminotransferase GliI-like, putative
AFLA_126690		5.57	conserved hypothetical protein
AFLA_071790		5.56	galactose oxidase, putative
AFLA_126670		5.46	hypothetical protein
AFLA_064440		5.43	transport protein, putative
AFLA_126660		5.41	ankyrin repeat protein
AFLA_004140		5.27	hypothetical protein
AFLA_126680		5.11	WD repeat-containing protein, putative
AFLA_041060		4.86	cell wall associated protein, putative
AFLA_116430		4.78	UDP-glucose dehydrogenase Ugd1, putative
AFLA_040330		4.69	chitin binding domain protein Peritrophin-A, putative
AFLA_072030		4.61	conserved hypothetical protein
AFLA_071780		4.55	Dyp-type peroxidase family protein
AFLA_135200		4.54	conserved hypothetical protein
AFLA_016410		4.45	hypothetical protein
AFLA_045600		4.40	cyclohexanone monooxygenase, putative
AFLA_126810		4.37	conserved hypothetical protein
AFLA_126730		4.36	conserved hypothetical protein
AFLA_086090		4.33	conserved hypothetical protein
AFLA_064470		4.32	cytochrome P450, putative
AFLA_126710		4.28	polyketide synthase, putative
AFLA_071920		4.28	class III aminotransferase, putative
AFLA_062570		4.16	phosphatidylserine decarboxylase, putative
AFLA_064460		4.04	conserved hypothetical protein
AFLA_064600		4.01	conserved hypothetical protein
AFLA_077610		3.98	MFS drug efflux transporter, putative
AFLA_097750		3.98	conserved hypothetical protein
AFLA_042180		3.89	conserved hypothetical protein
AFLA_064550		3.89	membrane dipeptidase GliJ-like, putative
AFLA_009450		3.78	hydroxymethylglutaryl-coenzyme A reductase family protein
AFLA_071180		3.78	indoleamine 2,3-dioxygenase family protein
AFLA_040860		3.70	hypothetical protein
AFLA_120710		3.68	cytochrome P450 monooxygenase, putative
AFLA_041080		3.68	hypothetical protein

AFLA_112850	3.62	O-methyltransferase, putative
AFLA_135420	3.61	NmrA-like family protein
AFLA_057520	3.57	conserved hypothetical protein
AFLA_121520	3.54	NRPS-like enzyme, putative
AFLA_124930	3.53	monooxygenase, putative
AFLA_077660	3.48	hypothetical protein
AFLA_121480	3.48	phytanoyl-CoA dioxygenase family protein
AFLA_124050	3.47	conserved hypothetical protein

Table S2-14. DEGs related to asexual development in *A. nidulans*.

Gene ID	Gene Name	Log2 FC Vege	Log2 FC Asex	Log2 FC conidia
AN0422	*abaA*	-	-2.06	1.70
AN2623	*acyA*	-	-3.95	-
AN2936	*ams1*	-	1.56	-
AN8667	*areA*	1.20	1.64	-
AN4409	*argB*	-	-1.69	-
AN2911	*atfA*	-	1.67	-
AN6849	*atfB*	3.69	2.33	-
AN5131	*atgH*	-1.87	-1.98	-
AN0973	*brlA*	-4.44	-3.11	1.70
AN1168	*cch1*	1.92	-	-
AN4566	*chsA*	1.26	-	-
AN2047	*cmdA*	-	-1.15	-
AN8820	*cnaA*	1.04	-2.51	-
AN6566	*cnaB*	-1.06	-2.25	-
AN8006	*dewA*	-1.65	-4.96	2.57
AN9121	*esdC*	1.92	1.05	-
AN2505	*fbx15*	1.42	-	-
AN5893	*flbA*	-1.87	-2.30	-
AN2421	*flbC*	1.31	2.68	-
AN0279	*flbD*	-3.24	-4.98	-
AN0721	*flbE*	-	-1.39	-
AN9008	*fphA*	1.28	-	-
AN3765	*gprC*	3.35	-	-
AN3387	*gprD*	-	-4.18	-
AN8269	*hsp90*	-	-1.29	-
AN6243	*ime2*	1.23	1.16	-
AN0807	*laeA*	2.26	-1.83	-
AN8945	*llmB/vipC*	-	-1.06	-
AN3435	*lreA*	-	-	-1.13
AN3607	*lreB*	-	-	-1.09
AN6230	*medA*	1.11	-	-
AN6288	*mob1*	-	-3.10	-
AN3719	*mpkB*	-	-1.53	-
AN0787	*msdS*	2.77	-	-
AN1652	*msnA*	1.08	-	-
AN8741	*mtfA*	-	-1.40	1.27
AN8863	*napA*	-	-2.16	-
AN7683	*nce102*	-2.27	-	-
AN0420	*nudG*	-	-2.05	-
AN1037	*odeA*	-1.40	-1.79	1.28
AN3441	*orlA*	1.49	-	-
AN3074	*pac2/osaB*	2.28	-	-
AN0453	*pcl1*	2.00	-1.94	-
AN8209	*pksP*	-	-3.71	-

AN1967	*ppoA*	2.17	-	-
AN6320	*ppoB*	-	-2.43	-
AN5028	*ppoC*	-	-2.07	-
AN3129	*prpA*	-	-	1.18
AN0182	*rasA*	-	-1.24	-
AN5832	*rasB*	1.39	-	-
AN8868	*rhbA*	-1.04	-1.12	-
AN1661	*ricA*	-	-2.46	-
AN8803	*rodA*	-2.59	-2.76	3.11
AN0081	*sfaD*	1.23	-	-
AN8129	*sfgA*	-	-1.35	-
AN8751	*sidB*	-	-1.44	-
AN9467	*ssc1*	-	-1.07	-
AN5836	*stuA*	-	-1.86	-
AN6037	*swoM*	-1.57	-	-
AN0641	*tcpA*	-	-1.82	-
AN0055	*tmpA*	-	-3.74	-
AN5523	*tpsA*	1.21	-	-
AN10533	*tpsC*	-	1.78	-
AN1052	*veA*	1.70	-1.82	-
AN2059	*velC*	-	-3.23	-
AN1959	*vosA*	-	-3.41	-
AN1937	*wetA*	-	-3.20	-
AN4674	*wsc1*	-	-	1.87

Table S2-15. DEGs related to asexual development in *A. flavus*.

Gene ID	Gene Name	Log2 FC Vege	Log2 FC Asex	Log2 FC conidia
AFLA_070880	*acyA*	-1.67	-	-3.40
AFLA_114720	*bem1*	-	-	-1.46
AFLA_060590	*chsG*	-	-	-1.07
AFLA_098750	*cnaA*	-	-	-1.02
AFLA_042780	*chsA*	-	-	1.02
AFLA_114760	*chsB*	-	-	1.26
AFLA_060780	*dewA*	1.57	-	-4.01
AFLA_019100	*fbx15*	-	-	-1.00
AFLA_033290	*laeA*	-	-	2.15
AFLA_121330	*llmB/vipC*	-	2.14	-
AFLA_051690	*lreB*	-	-	-1.21
AFLA_051240	*mkkB*	-	-	-1.05
AFLA_066330	*odeA*	-	-	-1.05
AFLA_055650	*osaA*	-	-	-1.01
AFLA_008970	*llmF*	-	-	1.85
AFLA_110650	*msnA*	-	-	-2.08
AFLA_006170	*pksP*	-	-2.61	-
AFLA_120760	*ppoB*	-	-2.76	-
AFLA_021100	*ppoD*	-	-	-1.19
AFLA_086590	*rlmA*	-	-1.21	-1.11
AFLA_098380	*rodA*	-	-	3.16
AFLA_014260	*rodB*	-	2.81	-
AFLA_093580	*tmpA*	-	-	-2.01
AFLA_087630	*tpsB*	-	-	1.51
AFLA_093580	*tmpA*	-	-	-3.43
AFLA_026900	*vosA*	-1.04	-1.26	-
AFLA_074470	*vosB*	-	-	1.21
AFLA_052030	*wetA*	-	-1.28	-

Table S2-16. DEGs related to G protein signaling pathway in *A. nidulans*.

Gene ID	Gene Name	Log2 FC Vege	Log2 FC Asex	Log2 FC conidia
AN5893	*flbA*	-1.87	-2.30	-
AN3090	*ganA*	-	-1.59	-
AN2520	*gprA*	-	-2.58	-
AN7743	*gprB*	-2.28	-2.61	-1.42
AN3765	*gprC*	3.35	-	-
AN3387	*gprD*	-	-4.18	-
AN9199	*gprE*	-	-3.24	-
AN12206	*gprF*	-	1.34	-
AN10166	*gprG*	-2.87	-1.52	-1.55
AN8262	*gprH*	-1.08	-	-1.47
AN6680	*gprM*	1.80	-	-
AN5508	*gprN*	3.54	1.99	-
AN4932	*gprO*	-	-1.74	-
AN5151	*gprP*	-	-1.60	-
AN3361	*nopA*	-	2.97	-
AN2740	*pdeB*	1.63	1.93	-
AN0081	*sfaD*	1.23	-	-

Table S2-17. DEGs related to G protein signaling pathway in *A. flavus*.

Gene ID	Gene Name	Log2 FC Vege	Log2 FC Asex	Log2 FC conidia
AFLA_079780	*ganA*	-	-	-1.14
AFLA_124830	*gaoC*	-	3.58	-
AFLA_006920	*gprH*	-	-1.03	-1.84
AFLA_009790	*gprK*	-2.95	-	-
AFLA_088190	*gprP*	-	-	-1.26
AFLA_023070	*gprR*	2.81	1.64	-

Table S2-18. DEGs predicted to encode transcription factors in *A. nidulans*.

Gene ID	Gene Name	Log2 FC Vege	Log2 FC Asex	Log2 FC conidia
AN8129	*sfgA*	-	-1.35	-
AN0148	*mdpE*	-1.60	-3.09	-
AN0273	*rsrA*	1.12	-	-
AN5806		-	-2.23	-
AN11962		1.15	-	-
AN0585		-	-2.14	-
AN0671		1.04	-	-
AN1251	*zfpB*	-	-	2.58
AN5929		2.06	-	-
AN1298		-	-1.81	-
AN0973	*brlA*	-4.44	-3.11	1.70
AN10059		-	-2.21	-
AN10083		1.84	1.47	-
AN5170	*rosA*	2.41	-	-
AN10120		1.16	-	-1.36
AN10384		3.27	2.64	-
AN4680	*rfeG*	-	-1.09	1.33
AN3667		-2.33	-3.12	-
AN10543	*galX*	-	-2.86	-
AN10548		2.09	-	-
AN10600		-	-2.16	-1.53
AN11222		2.72	-	-
AN1134	*qutA*	-	-1.73	-
AN1937	*wetA*	-	-3.20	-
AN1217		-	-1.28	-
AN3761		-	-	1.07
AN3063		-	1.34	-
AN7661	*srbA*	-	-	1.43
AN1265	*zapA*	-	-1.63	-
AN0568		1.47	-	-
AN2009	*rfeB*	1.54	-	-
AN1652	*msnA*	1.08	-	-
AN1736		2.89	2.38	-
AN1812	*jlbA*	-2.17	-1.81	-
AN8596		3.10	-	-
AN2025		-	3.60	-
AN1212		1.04	-	-
AN2421	*flbC*	1.31	2.68	-
AN2855	*pacC*	-	-2.00	-
AN7170		-	1.76	-
AN2996	*mbf1*	-	-1.58	-
AN0422	*abaA*	-	-2.06	1.70
AN3075	*oefC*	1.44	-	-
AN0026		4.04	4.31	-

AN0096		1.09	-	-
AN3435	*lreA*	-	-	-1.13
AN3607	*lreB*	-	-	-1.09
AN7610		1.17	-	-
AN0279	*flbD*	-3.24	-4.98	-
AN1959	*vosA*	-	-3.41	-
AN3769		-	-2.35	-
AN0709	*rpn4*	-1.85	1.61	-
AN4001		1.19	-	-
AN1028	*sln1*	1.00	-	-2.58
AN4324		-	-	2.11
AN10334		1.28	-	-
AN4773		-1.05	-3.23	-
AN4821		1.18	-	-
AN11073		-	-	3.00
AN11793		1.65	1.40	-
AN4878		-	2.52	-
AN12485	*btf3*	1.38	1.48	-
AN1500	*zfpA*	-	-3.42	2.41
AN1906		-	-1.73	-
AN5583	*aslA*	-	-	1.66
AN2020	*htfA*	-	-1.06	-
AN5775		1.80	-	-
AN5836	*stuA*	-	-1.86	-
AN5870		1.18	-	-
AN2854	*fkh1/2*	1.06	-	-1.01
AN6221	*areB*	-	-1.96	-
AN3024		-	2.78	-
AN3154		1.74	-	-
AN6715		1.59	-	-
AN4013		1.54	-	-
AN4035	*amdR*	-	-2.11	-
AN7061		2.70	-	-2.73
AN4873	*ace2*	-	1.12	-
AN7073		2.39	-	-
AN7343		-2.26	-6.43	1.42
AN7734	*anbH1*	-2.07	-	-
AN7896	*dbaA*	3.57	-	-
AN7919		3.13	-	-
AN5924		-	-1.90	-
AN8111		1.73	-	-
AN6396		-	2.47	-
AN6503	*azf1*	-1.74	-	-
AN6747		-	-	-2.95
AN8271	*palcA*	1.72	-	-
AN8355		2.36	-	-

AN8414	*apdR*	1.02	-	-
AN8535		-	-1.95	-
AN6846		-	-	-1.49
AN8655		2.08	1.31	-2.41
AN8676	*mcmA*	1.32	-	-
AN8741	*mtfA*	-	-1.40	1.27
AN7592		1.05	1.39	-
AN8918		-	-2.09	-
AN8949		-	2.65	-
AN8978	*alcR*	-	-1.89	-1.45
AN9013		4.30	2.57	-
AN8894		-	2.72	1.51
AN9492	*amdX*	1.02	-	-
AN7468	*acuK*	-	-1.54	-
AN1962		-	-3.35	-
AN1848	*nosA*	1.61	-	-
AN10906		-	1.01	-
AN10550		1.30	-	-

Table S2-19. DEGs predicted to encode transcription factors in *A. flavus*.

Gene ID	Gene Name	Log2 FC Vege	Log2 FC Asex	Log2 FC conidia
AFLA_013090		-	-	-1.31
AFLA_017290		-	-	1.23
AFLA_069460	*zfpB*	-	-	-2.14
AFLA_083820		-	-	-2.31
AFLA_082910		-	-	-1.22
AFLA_108220		-	-1.20	-
AFLA_099460	*rfeG*	-	-	-1.43
AFLA_113510		-	-	-1.51
AFLA_000770		-	-	1.10
AFLA_124220		-1.72	-	-
AFLA_052030	*wetA*	-	-1.28	-
AFLA_120780		-	3.90	-
AFLA_068970		-	-	-1.23
AFLA_040260		-	-	1.97
AFLA_086590	*rlmA*	-	-1.21	-1.11
AFLA_032740	*tbp*	-	-	-1.01
AFLA_085170	*oefC*	-	-	-1.10
AFLA_026130	*prtT*	-	1.20	-
AFLA_103640		-	-	1.46
AFLA_123840		-	-	-1.16
AFLA_051690	*lreB*	-	-	-1.21
AFLA_026900	*vosA*	-1.04	-1.26	-
AFLA_074200		-	-	-1.11
AFLA_017640		-	-	1.04
AFLA_093110		-2.17	-	-2.03
AFLA_101420		-	-2.00	-
AFLA_139560		-	-1.47	-
AFLA_040300		-	-	-1.16
AFLA_113790		-	-	1.17
AFLA_100950		-	-	-1.19
AFLA_009490		-	-	-1.90
AFLA_031450	*btf3*	-	-	1.88
AFLA_088390	*metZ*	-	-	1.05
AFLA_078920	*zfpA*	-	-	1.49
AFLA_026100	*htfA*	-2.12	-	-
AFLA_037760		-	3.07	-
AFLA_017040		-	-	-1.05
AFLA_020130		-	-	-1.26
AFLA_076560		-	-	-1.55
AFLA_061790	*anbH1*	-	-	-1.27
AFLA_024040		-	-	-1.08
AFLA_005740		-	-	-1.10
AFLA_015850		-	1.85	-
AFLA_048110	*mcnB*	-	-	-1.27

AFLA_089270	*hacA*	-	-1.06	-
AFLA_071050		-	-	-1.79
AFLA_002290	*amdX*	1.20	-	-
AFLA_074180		-	-1.66	-2.64
AFLA_038900		-	-	-1.53
AFLA_124010		-	-	-1.54
AFLA_105530		1.33	-	-
AFLA_034610		-	-	-1.29
AFLA_086110		-	-	-1.49
AFLA_134920		-	-	1.15
AFLA_049410		-	-2.16	-
AFLA_026850		-	-	1.42
AFLA_064330	*fmpR*	5.66	-	-
AFLA_025720	*nosA*	5.73	-	1.14
AFLA_070980		-	-	-1.64
AFLA_012100	*xprG*	-	-	-1.30
AFLA_012010	*farB2*	-	-	-1.16

Table S2-20. DEGs predicted to encode kinases in *A. nidulans*.

Gene ID	Gene Name	Log2 FC Vege	Log2 FC Asex	Log2 FC conidia
AN0034	*dakA*	-	1.74	-
AN0038	*atmA*	-	-1.51	-
AN0124	*rio2*	-	-2.01	-
AN0156		-	-1.87	-
AN0235	*ireA*	-	-1.05	-
AN0259		-	-2.68	-
AN0699		-	-2.32	-
AN0708	*aromA*	-	1.85	-
AN0931	*pbsA*	-	2.37	-
AN10082	*srpkF*	-	-1.47	-
AN10156		-	-1.39	-
AN10188	*null*	-	-2.30	-
AN10462	*srpkG*	-1.10	-	-
AN10485	*stk21*	-	-1.00	-
AN10646		-	1.55	-
AN10682		-	1.25	-
AN10731		-	-1.97	-
AN10937	*srpkE*	1.84	-1.95	-
AN11101	*gin4*	-	-2.56	-
AN11814		-2.58	-2.13	-1.18
AN11932		-	-1.49	-
AN1194	*sD*	-1.20	-2.84	-
AN1246	*pgkA*	-2.25	-	-
AN1315	*srpkC*	-1.40	-2.72	-
AN1315		-1.40	-2.72	-
AN1370		-	-2.58	-
AN1800	*sln1*	1.44	-	-
AN1854		-1.28	-1.82	-
AN1867	*phoB*	-	-2.42	-
AN2272		-1.66	-2.82	-
AN2373	*ffkC*	-2.51	-2.62	-
AN2489	*ssn3*	-	-2.08	-
AN2513		-	-3.24	-
AN2766		-	1.02	-
AN3001	*isr1*	2.06	-	-
AN3065		-	-1.76	-
AN3110		1.13	1.42	-
AN3450	*cdc7*	-	-1.99	-
AN3648		-	-1.20	-
AN3719	*mpkB*	-	-1.53	-
AN3869	*erg12*	1.41	-	-
AN4258	*ura6*	-	-1.79	-
AN4278	*stt4*	1.10	1.07	-
AN4310	*phoC*	1.77	-	-

AN4382		-	-2.90	-
AN4483		1.32	3.84	-
AN4536	*psk1*	-	1.18	-
AN4914		-	1.83	-
AN4957		1.16	-2.00	-
AN4984		-	1.20	-
AN5122		-	-1.42	-
AN5167		1.08	-	-
AN5210	*pkiA*	-	-2.06	-
AN5296	*fos-1*	1.31	-	-
AN5529		-	-1.69	-
AN5666	*mpkA*	-	-2.49	-
AN5674	*mst1*	1.24	-	-
AN5719		-	-2.25	-
AN5728	*stk22*	2.65	-	-
AN5757		1.13	-	-
AN5817		-	-2.04	-
AN6053		1.19	-	-
AN6192	*ffkG*	-	-	1.75
AN6243	*imeB*	1.23	1.16	-
AN6288	*mob1*	-	-3.10	-
AN6347		1.05	-	-
AN6363	*sudD*	-	-2.48	-
AN6367		-	2.98	-
AN6508	*gsk3*	-1.62	-	-
AN6758	*ffkJ*	-2.82	-3.19	-
AN6943		3.55	2.72	-
AN6975	*uvsb*	-	-1.07	-
AN7469		-2.12	-	-
AN7502		-	-2.01	-
AN7537	*ppk33*	1.25	-	-
AN7737		-1.05	-2.68	-
AN7787		2.04	-	-
AN7945		1.70	1.66	-
AN7986	*ffkA*	3.47	-	-
AN7995		1.29	-2.54	-
AN8213		-	-2.19	-
AN8216	*swoH*	-2.17	-3.11	-
AN8751		-	-1.44	-
AN8827	*cmkC*	1.00	-	-
AN8843		-	-2.27	-
AN8859		-	-3.02	-
AN8865	*ptkA*	-	-1.38	-
AN9022		-2.09	-2.87	-
AN9024		-	-	1.55
AN9302	*ffkK*	-	-2.14	-

AN9446	*panK*	-	-2.04	-
AN9500		-	1.06	-
AN8261	*phoA*	-	-1.96	-
AN9008	*fphA*	1.28	-	-
AN6985		-1.22	-	-
AN1568		-	-	1.18
AN1017		-	2.03	-
AN5589		-	1.31	-
AN3916		-	-2.79	-

Table S2-21. DEGs predicted to encode kinases in *A. flavus*.

Gene ID	Gene Name	Log2 FC Vege	Log2 FC Asex	Log2 FC conidia
AFLA_009240	*srpkE*	-	-	-1.43
AFLA_067680		1.48	-	-
AFLA_026880		-	-1.21	-
AFLA_119130		-	-1.34	-
AFLA_025890		-	-	-1.03
AFLA_056720		-	1.03	-
AFLA_019840	*ssn3*	-	-	-1.88
AFLA_021030		-	-	-1.04
AFLA_020620		-	-	-1.55
AFLA_024540		-	-1.77	-
AFLA_103840	*cdc7*	-	-	-1.20
AFLA_112040	*erg12*	-	-	-1.11
AFLA_113230		-	-	1.01
AFLA_032170	*ran1*	-	-	-1.33
AFLA_106830	*fos-1*	-	-	-1.23
AFLA_027910		-	-	-1.06
AFLA_073630	*mst1*	-	-	-1.14
AFLA_045240		-	-	-1.05
AFLA_138010	*sudD*	-	-	-1.37
AFLA_114720	*bem1*	-	-	-1.46
AFLA_129090	*ppk33*	-	-	-1.27
AFLA_061710		-	-	-1.46
AFLA_006300	*swoH*	-	-1.27	-
AFLA_098240	*iki3*	-1.84	-	-
AFLA_049940		-	-	-1.17
AFLA_073810		-1.60	-	-
AFLA_009250		-	3.91	-2.17
AFLA_043510		-	3.60	-
AFLA_095440		-	1.58	-
AFLA_101910		-1.02	-	-
AFLA_097830		-	-	1.68
AFLA_119810		-	-	-1.59
AFLA_097830		-	-	1.68
AFLA_068090		-	-	-1.41
AFLA_128100		-	-	-1.01

Table S2-22. The most enriched 100 putative direct target genes of NsdD in *A. nidulans* conidia.

Gene ID	**Gene Name**	**Description**
AN11321		Protein of unknown function
AN8279		Ortholog of S. cerevisiae Can1p which has arginine transmembrane transporter activity; basic amino acid transporter; expression reduced after exposure to farnesol
AN3036	*figA*	Putative low affinity Ca2+ channel family protein
AN5225		Protein of unknown function
AN6278		Protein of unknown function
AN6538		Has domain(s) with predicted phospholipid binding activity
AN2177		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN4034	*hapC*	Component of AnCP/AnCF CCAAT-binding complex; can act as both a positive and negative regulator of transcription
AN1199		Ortholog(s) have role in cell-abiotic substrate adhesion
AN6104		Protein of unknown function
AN6783		Has domain(s) with predicted nucleobase transmembrane transporter activity, transporter activity, role in nucleobase transport, transmembrane transport and integral component of membrane, membrane localization
AN0379		Protein of unknown function
AN0709		Putative zinc-finger protein; expression upregulated after exposure to farnesol
AN5489		Protein of unknown function
AN2488		Protein of unknown function
AN0859		Protein of unknown function
AN5055		Has domain(s) with predicted aminopeptidase activity, metalloexopeptidase activity and role in cellular process, proteolysis
AN5445		Protein of unknown function
AN2300	*atrD*	Putative ATP-binding cassette (ABC) transporter of the P-glycoprotein cluster; has a role in protection against cytotoxic agents, in antibiotic secretion and in the efflux of the azole-related fungicide fenarimol; upregulated by farnesol
AN8387		Protein of unknown function
AN7795	*gprK*	Putative heterotrimeric G-protein coupled receptor component; contains both a 7-transmembrane domain and an RGS signaling domain
AN5354		Has domain(s) with predicted oxidoreductase activity, transferase activity, transferring acyl groups other than amino-acyl groups activity and role in oxidation-reduction process
AN0468		Protein of unknown function
AN0391		Protein of unknown function
AN6403		Protein of unknown function
AN3881		Has domain(s) with predicted ADP binding, catalytic activity and role in nucleoside metabolic process
AN5357		Predicted glycosylphosphatidylinositol (GPI)-anchored protein

AN0732		Putative transporter of the major facilitator superfamily (MFS); expression upregulated after exposure to farnesol
AN4482		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane localization
AN5492		Has domain(s) with predicted deaminase activity and role in purine ribonucleoside monophosphate biosynthetic process
AN2001		Has domain(s) with predicted RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN3216		Has domain(s) with predicted sequence-specific DNA binding, transcription factor activity, sequence-specific DNA binding activity and role in regulation of transcription, DNA-templated
AN0129	*ppsA*	Putative dual-specificity protein tyrosine/serine/threonine phosphatase
AN7725	*pyroA*	Protein required for biosynthesis of pyridoxine; highly conserved throughout fungi, plants and bacteria
AN2500		Putative nicotinamide N-methyltransferase
AN6472	*dfgF*	Putative endo-mannanase GH76 family protein
AN4758		Ortholog(s) have role in apoptotic process and cytoplasm, nucleus localization
AN5283		Protein of unknown function
AN3203		Putative F-box protein
AN1430		Ortholog(s) have betaine-aldehyde dehydrogenase activity
AN20051		Protein of unknown function
AN6831		Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN11920		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN3152	*nsdD*	Predicted GATA-type zinc-finger transcription factor required for sexual development; transcript induced by nitrositive stress
AN11262		Protein of unknown function
AN1509		Protein of unknown function
AN8534	*azgA*	Purine transporter with high affinity for hypoxanthine and adenine; takes up purines for nucleotide salvage and as nitrogen sources; induced by uric acid; regulated by UaY and AreA
AN1715		Putative mannose-6-phosphate isomerase with a predicted role in mannose/mannitol, fructose, and sorbose/sorbitol metabolism
AN10040		Protein of unknown function
AN5015	*conJ*	Putative conidiation gene; transcript induced by light in in developmentally competent mycelia; double conF and conJ deletion results in increased cellular glycerol or erythritol leading to delayed germination and desiccation resistance
AN12458		Has domain(s) with predicted catalytic activity and role in metabolic process

AN8605	*cyp1*	Putative peptidyl-prolyl cis-trans isomerase (PPIase); cyclophilin
AN1894		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN8751	*sidB*	Septation initiation network (SIN) component; required for septation and conidiation; interacts with MobA; localizes to spindle pole bodies and sites of septation; S. pombe Mob1p is required for the activity of the sidB ortholog, Sid2p
AN11724		Protein of unknown function
AN7662	*freA*	Putative metalloreductase with a predicted role in iron homeostasis; regulated by iron independently of SreA
AN5752		Ortholog(s) have RNA polymerase II transcription factor activity, TBP-class protein binding, involved in preinitiation complex assembly and transcription factor activity, more
AN1800	*tcsB*	Transmembrane histidine kinase, part of a two-component signal transducer involved in the HOG signaling pathway that regulates osmotic stress response' transcript upregulated by growth in glycerol
AN1052	*veA*	Protein involved in light-sensitive control of differentiation and secondary metabolism; localizes to the nucleus in dark and to both nucleus and cytoplasm in the light; induced by light; AspGD sequence represents the veA1 mutant allele
AN6881		Protein of unknown function
AN3931	*pilB*	Putative conserved eisosome protein
AN5573		Protein of unknown function
AN0279	*flbD*	Putative transcription factor involved in regulation of asexual and sexual development and in response to nitrogen starvation; contains a myb-like DNA-binding domain
AN5563	*gldB*	Putative dehydrogenase with a predicted role in carbohydrate metabolism; NADP+-dependent glycerol dehydrogenase; required for osmotolerance; transcript downregulated by growth in glycerol; expression upregulated after exposure to farnesol
AN8004	*CYP54 1B1*	Putative cytochrome P450
AN8603		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN6713		Ortholog(s) have cytosol, nucleus localization
AN6823		Protein of unknown function
AN11981		Has domain(s) with predicted UDP-N-acetylmuramate dehydrogenase activity, flavin adenine dinucleotide binding activity and role in oxidation-reduction process
AN1731	*prnD*	Putative proline dehydrogenase with a predicted role in proline metabolism; expression is regulated by carbon and nitrogen repression; negatively regulated by CreA
AN8945	*llmB*	Component of the plasma membrane-associated VapA-VipC-VapB methyltransferase complex that controls differentiation; TAM domain methyltransferase; ortholog of A. fumigatus Afu8g01930

AN6749	*llmF*	Putative LaeA-like methyltransferase; negative regulator of sterigmatocystin production and sexual development
AN7158		Has domain(s) with predicted hydrolase activity, hydrolase activity, acting on ester bonds activity and role in lipid metabolic process
AN0176	*sreA*	Predicted GATA transcription factor involved in iron uptake, acts as a repressor of siderophore biosynthesis under high-iron conditions; mutants accumulate increased amounts of iron
AN11100		Protein of unknown function
AN8628		Has domain(s) with predicted oxidoreductase activity, transferase activity, transferring acyl groups other than amino-acyl groups, zinc ion binding activity and role in oxidation-reduction process
AN0771		Putative ABC multidrug transporter; confers resistance to azole antifungal drugs
AN5660	*wscA*	Putative plasma membrane sensor-transducer; N- and O- glycosylated and localized in the cell wall and membrane; mutants display a high frequency of swollen hyphae under hypo-osmotic conditions; required for conidiation
AN2366		Putative trypsin-like protease with a role in the proteolytic cleavage of NmrA
AN4199		Protein of unknown function
AN6604		Ortholog(s) have ATPase activity, tRNA binding activity, role in tRNA modification and Elongator holoenzyme complex, cytosol, nucleus localization
AN10307		Has domain(s) with predicted zinc ion binding activity
AN9025		Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, nucleic acid binding, zinc ion binding activity
AN0807	*laeA*	Methyltransferase-domain protein; velvet complex component composed of VelB, VeA and LaeA; self-methylates; coordinates asexual development in response to light; regulates secondary metabolism and is required for Hulle cell formation
AN7744		Protein of unknown function
AN0412		Protein of unknown function; this locus is reported to contain an upstream open reading frame (uORF)
AN1094	*ndiF*	Putative mitochondrial NADH dehydrogenase (ubiquinone) with a predicted role in energy metabolism
AN2011	*dnfC*	Putative flippase, type 4 P-type ATPase involved in distribution lipids
AN6487		Putative aspartyl protease; ortholog of S. cerevisiae BAR1; expression reduced after exposure to farnesol
AN1174		Has domain(s) with predicted ATP binding, ATPase activity, ATPase activity, coupled to transmembrane movement of substances, nucleoside-triphosphatase activity, nucleotide binding activity and role in transport
AN1415		Protein of unknown function
AN1797		Ortholog(s) have fructose transmembrane transporter activity, glucose transmembrane transporter activity, mannose transmembrane transporter activity

AN12229	Ortholog(s) have cytosol, nucleus localization
AN1082	Putative nucleoside phosphatase; ortholog of S. cerevisiae Gda1p; expression reduced after exposure to farnesol; this locus is reported to contain an upstream open reading frame (uORF)
AN11477	Protein of unknown function
AN4622	Protein of unknown function
AN7587	Protein of unknown function
AN7018	Protein of unknown function
AN1219	Protein of unknown function
AN4353	Ortholog(s) have glyoxysome localization

Table S2-23. The most enriched 100 putative direct target genes of NsdD in *A. flavus* conidia.

Gene ID	Gene Name	Description
AFLA_006980		HLH transcription factor (PalcA), putative
AFLA_019710		conserved hypothetical protein
AFLA_088800		conserved hypothetical protein
AFLA_105190		NRPS-like enzyme, putative
AFLA_114230		hypothetical protein
AFLA_114510		hypothetical protein
AFLA_130040		ammonium transporter MeaA
AFLA_048290		homoserine kinase
AFLA_131900		conserved hypothetical protein
AFLA_000930		acetolactate synthase, large subunit, putative
AFLA_121240		phosphate-repressible phosphate permease, putative
AFLA_052640		PH domain protein
AFLA_114220		hypothetical protein
AFLA_013400		ankyrin repeat-containing protein, putative
AFLA_019870		phosphatidylserine decarboxylase Psd2, putative
AFLA_113390		hypothetical protein
AFLA_130300		uracil DNA N-glycosylase Ung1, putative
AFLA_111260		mannose-6-phosphate isomerase, class I
AFLA_033720		conserved hypothetical protein
AFLA_068250		conserved hypothetical protein
AFLA_037590		conserved hypothetical protein
AFLA_005920		Ulp1 protease family protein
AFLA_009650		hypothetical protein
AFLA_104910		conserved hypothetical protein
AFLA_001480		hypothetical protein
AFLA_079660		conserved hypothetical protein
AFLA_093580		membrane oxidoreductase TmpA, putative
AFLA_054460		hypothetical protein
AFLA_027120		conserved hypothetical protein
AFLA_070090		conserved hypothetical protein
AFLA_011480		amino acid permease (Can1), putative
AFLA_011950		suppressor protein SRP40, putative
AFLA_088660		DUF431 domain protein
AFLA_084800		hypothetical protein
AFLA_100340		nonribosomal peptide synthase, putative
AFLA_078310		hypothetical protein
AFLA_039630		conserved hypothetical protein
AFLA_135520		conserved hypothetical protein
AFLA_041930		conserved hypothetical protein
AFLA_099400		hypothetical protein
AFLA_136390		hypothetical protein
AFLA_034450		WD repeat protein
AFLA_139510		conserved hypothetical protein

AFLA_008520		alpha-amylase, putative
AFLA_083090		tRNA processing endoribonuclease Trz1, putative
AFLA_041810		hypothetical protein
AFLA_086600		morphogenesis protein (Msb1), putative
AFLA_128470		efflux pump antibiotic resistance protein, putative
AFLA_046680		hypothetical protein
AFLA_102850		Rho GTPase activator (Rgd1), putative
AFLA_048630		conserved hypothetical protein
AFLA_043140		CUE domain protein, putative
AFLA_119840		hypothetical protein
AFLA_015790		C6 transcription factor (Leu3), putative
AFLA_037540		hypothetical protein
AFLA_127440		cAMP-dependent protein kinase-like, putative
AFLA_076780		ribonucleoprotein, putative
AFLA_005180		AAA family ATPase, putative
AFLA_010630		ABC multidrug transporter SidT
AFLA_096080		hypothetical protein
AFLA_042590		hypothetical protein
AFLA_101910		sensor histidine kinase/response regulator, putative
AFLA_052490		C2H2 finger domain protein, putative
AFLA_110610		high affinity methionine permease
AFLA_110560		protein kinase, putative
AFLA_094310		conserved hypothetical protein
AFLA_025950		conserved hypothetical protein
AFLA_126790		threonine synthase Thr4, putative
AFLA_002790		vacuolar sorting protein SNF7 family protein, putative
AFLA_031330		conserved hypothetical protein
AFLA_135320		MFS multidrug transporter, putative
AFLA_104980		hypothetical protein
AFLA_092240		calcium/calmodulin-dependent protein kinase, putative
AFLA_134030	*flbA*	developmental regulator FlbA
AFLA_134730		transcriptional corepressor Cyc8, putative
AFLA_105450		polyketide synthase, putative
AFLA_123470		hypothetical protein
AFLA_067680		mucin, putative
AFLA_082230		pantothenate transporter, putative
AFLA_034030		trehalose-phosphate synthase/phosphatase complex subunit Tps1
AFLA_105490		phosphoribulokinase/uridine kinase family protein
AFLA_071370		meiotic recombination protein (Dmc1), putative
AFLA_029130		proline utilization protein PrnX-like, putative
AFLA_131520		arsenite resistance protein Ars2, putative
AFLA_046900		CorA family metal ion transporter, putative
AFLA_022340		glutamate synthase Glt1, putative
AFLA_033540		arrestin (or S-antigen), N-terminal domain protein
AFLA_045350		conserved hypothetical protein
AFLA_101220		annexin ANXC4

AFLA_000860		tripeptidyl peptidase A
AFLA_003490		pseudouridine synthase
AFLA_116990		MFS monosaccharide transporter, putative
AFLA_069090		GPI anchored protein, putative
AFLA_082850	*brlA*	C2H2 type conidiation transcription factor BrlA
AFLA_020660		conserved hypothetical protein
AFLA_096700		NRPS-like enzyme, putative
AFLA_046890		phospholipid metabolism enzyme regulator, putative
AFLA_058550		conserved hypothetical protein
AFLA_043510		protein kinase domain-containing protein
AFLA_094020		hypothetical protein

Table S2-24. Putative direct targets of NsdD predicted to encode transcription factors in *A. nidulans* conidia.

Gene ID	Gene Name	Description
AN4034	*hapC*	Component of AnCP/AnCF CCAAT-binding complex; can act as both a positive and negative regulator of transcription
AN2001		Has domain(s) with predicted RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN3216		Has domain(s) with predicted sequence-specific DNA binding, transcription factor activity, sequence-specific DNA binding activity and role in regulation of transcription, DNA-templated
AN3152	*nsdD*	Predicted GATA-type zinc-finger transcription factor required for sexual development; transcript induced by nitrositive stress
AN5752		Ortholog(s) have RNA polymerase II transcription factor activity, TBP-class protein binding, involved in preinitiation complex assembly and transcription factor activity, more
AN0279	*flbD*	Putative transcription factor involved in regulation of asexual and sexual development and in response to nitrogen starvation; contains a myb-like DNA-binding domain
AN0176	*sreA*	Predicted GATA transcription factor involved in iron uptake, acts as a repressor of siderophore biosynthesis under high-iron conditions; mutants accumulate increased amounts of iron
AN9025		Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, nucleic acid binding, zinc ion binding activity
AN4485		Has domain(s) with predicted RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN7872		Predicted transcription factor; predicted secondary metabolism gene cluster member
AN8125		Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN8636		Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN0742		Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN5170	*rosA*	Putative Zn(II)2Cys6 transcription factor; negative regulator of sexual development

AN8978	*alcR*	Transcription factor involved in positive regulation of the ethanol regulon; contains Zn(II)2Cys6 DNA-binding domain
AN10378	*zipC*	Putative bZIP transcription factor
AN4558		Ortholog(s) have role in positive regulation of transcription from RNA polymerase II promoter involved in cellular response to chemical stimulus and cytosol, nucleus localization
AN0162		Has domain(s) with predicted DNA binding, transcription factor activity, sequence-specific DNA binding activity and role in regulation of transcription, DNA-templated
AN8506		Putative transcription factor; predicted role in secondary metabolite production
AN2290	*steA*	STE-like transcription factor with homeobox and zinc finger domains; null mutation blocks sexual cycle but not asexual development, forms Hulle cells but no ascogenous tissue nor cleistothecia
AN5955		Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN8298		Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN0533		Putative transcription factor; predicted role in secondary metabolite production
AN11131		Ortholog(s) have RNA polymerase II core binding activity, role in chromatin-mediated maintenance of transcription, transcription elongation from RNA polymerase II promoter and transcription elongation factor complex localization

Table S2-25. Putative direct targets of NsdD predicted to encode transcription factors in *A. flavus* conidia.

Gene ID	Gene Name	Description
AFLA_006980	*palcA*	HLH transcription factor (PalcA), putative
AFLA_015790	*leu3*	C6 transcription factor (Leu3), putative
AFLA_134730	*cyc8*	transcriptional corepressor Cyc8, putative
AFLA_082850	*brlA*	C2H2 type conidiation transcription factor BrlA
AFLA_134680	*creA*	C2H2 transcription factor (Crea), putative
AFLA_048110	*sep1*	forkhead transcription factor (Sep1), putative
AFLA_069460	*egr2*	C2H2 transcription factor (Egr2), putative
AFLA_088390	*btf3*	transcription factor btf3, putative
AFLA_043760	*con7*	C2H2 transcription factor (Con7), putative
AFLA_083100	*lziP*	bZIP transcription factor (LziP), putative
AFLA_005520		C6 transcription factor, putative
AFLA_103640	*fcr1*	C6 transcription factor (Fcr1), putative
AFLA_067020		jumonji family transcription factor, putative
AFLA_083460		C6 transcription factor RosA-like, putative
AFLA_084720		C6 transcription factor, putative
AFLA_078500		bZIP transcription factor, putative
AFLA_031450		CP2 transcription factor, putative
AFLA_049870	*areA*	GATA transcriptional activator AreA
AFLA_091090		C6 transcription factor, putative
AFLA_087350	*ace1*	C2H2 transcription factor (Ace1), putative
AFLA_026250	*rfeB*	homeobox transcription factor (RfeB), putative
AFLA_103270	*TFIID*	transcription initiation factor TFIID subunit 12, putative
AFLA_040300		C6 transcription factor, putative
AFLA_137320	*flbC*	C2H2 conidiation transcription factor FlbC
AFLA_031340	*atfA*	bZIP transcription factor (AtfA), putative
AFLA_020210	*nsdD*	sexual development transcription factor NsdD
AFLA_089270	*hacA*	bZIP transcription factor HacA
AFLA_054800	*azf1*	C2H2 transcription factor (Azf1), putative
AFLA_110650	*seb1*	C2H2 transcription factor (Seb1), putative
AFLA_046990	*stuA*	APSES transcription factor StuA
AFLA_026100		homeobox transcription factor, putative
AFLA_071390	*csx1*	mRNA binding post-transcriptional regulator (Csx1), putative
AFLA_086590	*smp1*	transcription factor smp1, putative
AFLA_025430		C6 transcription factor, putative
AFLA_017640	*rpn4*	C2H2 transcription factor (Rpn4), putative
AFLA_069100		homeobox transcription factor, putative
AFLA_044060	*rfeC*	C2H2 transcription factor (RfeC), putative
AFLA_054970		C6 transcription factor, putative
AFLA_084410		C6 transcription factor, putative
AFLA_031790	*meaB*	bZIP transcription factor (MeaB), putative
AFLA_026610	*bdf1*	transcription regulator BDF1, putative

Table S2-26. Potential direct targets of NsdD in *A. nidulans* conidia.

Gene ID	Log2 FC	Gene Name	Description
AN2585	-4.46		Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN12244	-2.85		Protein of unknown function
AN8390	-2.84		GPR1/FUN34/YaaH family member; ethanol- and ethylacetate-induced gene
AN8737	-2.52	*mstA*	High-affinity glucose transporter active in hyphae under glucose-limiting conditions
AN9295	-2.42		Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN5323	-2.17		Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN5088	-1.95		Putative potassium-transporting ATPase with a predicted role in energy metabolism
AN5943	-1.85		Protein of unknown function
AN0129	-1.70	*ppsA*	Putative dual-specificity protein tyrosine/serine/threonine phosphatase
AN7828	-1.62	*urhB*	Putative unsaturated rhamnogalacturonan hydrolase
AN6783	-1.60		Has domain(s) with predicted nucleobase transmembrane transporter activity, transporter activity, role in nucleobase transport, transmembrane transport and integral component of membrane, membrane localization
AN8812	-1.46		Has domain(s) with predicted ATP binding, ATPase activity, nucleoside-triphosphatase activity, nucleotide binding activity
AN8978	-1.45	*alcR*	Transcription factor involved in positive regulation of the ethanol regulon; contains Zn(II)2Cys6 DNA-binding domain
AN3776	-1.44		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN7131	-1.31	*CYP52 H1*	Putative cytochrome P450
AN2629	-1.26		Protein of unknown function
AN1276	-1.24		Has domain(s) with predicted substrate-specific transmembrane transporter activity, transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN10287	-1.11		Has domain(s) with predicted FAD binding, oleate hydratase activity and role in fatty acid metabolic process

AN8017	-1.04		Ortholog(s) have cytosol, mitotic spindle pole body, nucleus localization
AN8421	1.02	*dfgB*	Putative endo-mannanase GH76 family protein; role in polysaccharide degradation
AN1174	1.07		Has domain(s) with predicted ATP binding, ATPase activity, ATPase activity, coupled to transmembrane movement of substances, nucleoside-triphosphatase activity, nucleotide binding activity and role in transport
AN1509	1.08		Protein of unknown function
AN11321	1.10		Protein of unknown function
AN6538	1.10		Has domain(s) with predicted phospholipid binding activity
AN1958	1.12		Protein of unknown function
AN3114	1.12		Protein of unknown function
AN9340	1.13	*treA*	Alpha,alpha-trehalase with a role in trehalose hydrolysis; localized to the conidial cell wall; expression upregulated after exposure to farnesol
AN7607	1.17		Protein of unknown function
AN12458	1.17		Has domain(s) with predicted catalytic activity and role in metabolic process
AN8892	1.19		Has domain(s) with predicted ATP binding, ATPase activity, ATPase activity, coupled to transmembrane movement of substances, nucleoside-triphosphatase activity, nucleotide binding activity and role in transport
AN0701	1.26		Has domain(s) with predicted NAD binding, oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor activity and role in oxidation-reduction process
AN1415	1.28		Protein of unknown function
AN5492	1.34		Has domain(s) with predicted deaminase activity and role in purine ribonucleoside monophosphate biosynthetic process
AN6835	1.39	*CYP505A8*	Putative cytochrome P450; expression upregulated after exposure to farnesol
AN2669	1.40		Has domain(s) with predicted role in response to stress and integral component of membrane localization
AN1465	1.43		Protein of unknown function
AN1058	1.44		Protein of unknown function
AN2730	1.51	*sB*	Putative transporter with a predicted role in small molecule transport; transcript negatively regulated by sulfate and methionine
AN2134	1.51		Protein of unknown function; transcript is induced by nitrate
AN4482	1.52		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane localization
AN5175	1.54		Ortholog(s) have mitochondrial outer membrane localization
AN7754	1.64		Protein of unknown function
AN1614	1.73		Has domain(s) with predicted methyltransferase activity and role in metabolic process

AN2649	1.82		Protein of unknown function
AN6962	1.83		Ortholog of the non-ribosomal peptide synthetase (NRPS) of A. fumigatus, nrps14/Afu8g00540; predicted secondary metabolism gene cluster member; coregulated with AN6961
AN9073	1.83		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN7662	1.92	*freA*	Putative metalloreductase with a predicted role in iron homeostasis; regulated by iron independently of SreA
AN11920	1.93		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN1199	1.97		Ortholog(s) have role in cell-abiotic substrate adhesion
AN3152	1.98	*nsdD*	Predicted GATA-type zinc-finger transcription factor required for sexual development; transcript induced by nitrositive stress
AN5283	2.07		Protein of unknown function
AN0019	2.16		Protein of unknown function
AN9347	2.18		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN5474	2.20		Protein of unknown function
AN2366	2.26		Putative trypsin-like protease with a role in the proteolytic cleavage of NmrA
AN3247	2.33		Has domain(s) with predicted ATP binding, ATPase activity, ATPase activity, coupled to transmembrane movement of substances, nucleoside-triphosphatase activity, nucleotide binding activity and role in transport
AN5033	2.37		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN1894	2.41		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN0005	2.43		Protein of unknown function
AN1197	2.43		Has domain(s) with predicted carbohydrate binding, catalytic activity and role in carbohydrate metabolic process
AN9249	2.45	*ausH*	Protein required for austinol and dehydroaustinol biosynthesis
AN8374	2.74		Ortholog(s) have oligopeptide transmembrane transporter activity
AN6472	2.75	*dfgF*	Putative endo-mannanase GH76 family protein
AN3484	2.96		Protein of unknown function
AN8311	3.41		Protein of unknown function
AN6703	3.54	*jenB*	Short-chain carboxylic acid transporter involved in uptake of lactate, succinate, pyruvate and malate
AN9206	4.13		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN7895	7.01	*cipB*	Putative oxidoreductase; contains Zn-dependent alcohol dehydrogenase domain; protein expressed at increased levels during osmoadaptation

Table S2-27. Potential direct targets of NsdD in *A. flavus* conidia.

Gene ID	Log2 FC	Gene Name	Description
AFLA_104430	-5.23		subtilisin, putative
AFLA_074070	-4.00		siderochrome-iron transporter, putative
AFLA_000930	-3.99		acetolactate synthase, large subunit, putative
AFLA_122260	-2.89		pantothenate transporter, putative
AFLA_090790	-2.66		conserved hypothetical protein
AFLA_013400	-2.52		ankyrin repeat-containing protein, putative
AFLA_050150	-2.41		extracellular exo-polygalacturonase, putative
AFLA_078080	-2.27		conserved hypothetical protein
AFLA_071550	-2.17		conserved hypothetical protein
AFLA_069460	-2.14	*erg2*	C2H2 transcription factor (Egr2), putative
AFLA_022190	-2.08		hypothetical protein
AFLA_130040	-2.03	*meaA*	ammonium transporter MeaA
AFLA_093580	-2.01	*tmpA*	membrane oxidoreductase TmpA, putative
AFLA_047190	-1.94	*sidA*	L-ornithine N5-oxygenase SidA
AFLA_113410	-1.88	*mlh3*	DNA mismatch repair protein (Mlh3), putative
AFLA_139510	-1.82		conserved hypothetical protein
AFLA_004410	-1.75		conserved hypothetical protein
AFLA_000360	-1.75		hypothetical protein
AFLA_121240	-1.73		phosphate-repressible phosphate permease, putative
AFLA_122580	-1.68		protein phosphatase type 1 complex subunit Hex2/Reg1, putative
AFLA_001480	-1.65		hypothetical protein
AFLA_083460	-1.64	*rosA*	C6 transcription factor RosA-like, putative
AFLA_068250	-1.58		conserved hypothetical protein
AFLA_122370	-1.58		MFS peptide transporter, putative
AFLA_132940	-1.58	*hmt1*	vacuolar ABC heavy metal transporter (Hmt1), putative
AFLA_130010	-1.53		hypothetical protein
AFLA_026320	-1.52		conserved hypothetical protein
AFLA_088350	-1.52		hydrolase, alpha/beta fold family protein
AFLA_066200	-1.47		C6 finger domain protein, putative
AFLA_114720	-1.46	*bem1*	protein kinase activator Bem1, putative
AFLA_075120	-1.46		mitochondrial carrier protein, putative
AFLA_045030	-1.45		hypothetical protein
AFLA_098320	-1.45		conserved fungal protein
AFLA_081140	-1.43		conserved hypothetical protein
AFLA_127210	-1.42	*cap20*	pathogenesis associated protein Cap20, putative
AFLA_051930	-1.41		glutamine synthetase
AFLA_037440	-1.39		conserved hypothetical protein
AFLA_044630	-1.38		conserved hypothetical protein
AFLA_081540	-1.37		integral membrane protein
AFLA_054120	-1.36		DUF1212 domain membrane protein
AFLA_136040	-1.34		chitin synthase, putative
AFLA_032170	-1.33	*ran1*	protein serine/threonine kinase (Ran1), putative

AFLA_004060	-1.32		conserved hypothetical protein
AFLA_099240	-1.31	*arbD*	D-arabinitol dehydrogenase ArbD, putative
AFLA_012100	-1.30	*pcaG*	NDT80_PhoG domain protein PcaG
AFLA_113350	-1.28		conserved hypothetical protein
AFLA_087220	-1.27		UDP-glucose 4-epimerase, putative
AFLA_084410	-1.27		C6 transcription factor, putative
AFLA_048110	-1.27	*sep1*	forkhead transcription factor (Sep1), putative
AFLA_112560	-1.26	*sok1*	cAMP-mediated signaling protein Sok1, putative
AFLA_110610	-1.24		high affinity methionine permease
AFLA_132510	-1.23		MHYT domain signaling protein, putative
AFLA_071440	-1.23		conserved hypothetical protein
AFLA_113490	-1.21		dDENN domain protein
AFLA_012000	-1.19		hypothetical protein
AFLA_033630	-1.18		conserved hypothetical protein
AFLA_128870	-1.18		feruloyl esterase, putative
AFLA_061460	-1.17		conserved hypothetical protein
AFLA_135560	-1.16	*sat1*	intracellular protein transport protein (Sat1), putative
AFLA_040300	-1.16		C6 transcription factor, putative
AFLA_092010	-1.16		HMG-CoA reductase
AFLA_029680	-1.16		conserved hypothetical protein
AFLA_108250	-1.11		pantothenate transporter, putative
AFLA_086590	-1.11		transcription factor smp1, putative
AFLA_094020	-1.10		hypothetical protein
AFLA_088340	-1.10		conserved hypothetical protein
AFLA_083090	-1.09	*trz1*	tRNA processing endoribonuclease Trz1, putative
AFLA_084720	-1.09		C6 transcription factor, putative
AFLA_013830	-1.08		amino acid transporter, putative
AFLA_105490	-1.07		phosphoribulokinase/uridine kinase family protein
AFLA_058660	-1.07		P-type ATPase, putative
AFLA_019870	-1.06	*psd2*	phosphatidylserine decarboxylase Psd2, putative
AFLA_033540	-1.05		arrestin (or S-antigen), N-terminal domain protein
AFLA_051240	-1.05	*mkk2*	MAP kinase kinase (Mkk2), putative
AFLA_018980	-1.05		hypothetical protein
AFLA_079850	-1.05		phosphatidyl synthase
AFLA_003490	-1.05		pseudouridine synthase
AFLA_132660	-1.05		conserved hypothetical protein
AFLA_021030	-1.04		serine/threonine protein kinase, putative
AFLA_029130	-1.04		proline utilization protein PrnX-like, putative
AFLA_028790	-1.03	*sac2*	GARP complex subunit (Sac2), putative
AFLA_135840	-1.01		mitochondrial AAA ATPase, putative
AFLA_055650	-1.01		camp independent regulatory protein
AFLA_009070	-1.01		conserved hypothetical protein
AFLA_032710	-1.01		conserved hypothetical protein
AFLA_094890	-1.00		FHA domain protein
AFLA_037660	-1.00		conserved hypothetical protein
AFLA_015600	1.03		MFS transporter family protein, putative

AFLA_017640	1.04	*rpn4*	C2H2 transcription factor (Rpn4), putative
AFLA_088390	1.05	*btf3*	transcription factor btf3, putative
AFLA_015680	1.05		conserved hypothetical protein
AFLA_135610	1.06		hypothetical protein
AFLA_027070	1.10		acetate--CoA ligase
AFLA_039470	1.12		membrane bound cation transporter, putative
AFLA_105450	1.16		polyketide synthase, putative
AFLA_095240	1.20		hypothetical protein
AFLA_036560	1.21		conserved threonine rich protein
AFLA_119840	1.22		hypothetical protein
AFLA_058550	1.32		conserved hypothetical protein
AFLA_093590	1.36		arrestin (or S-antigen), N-terminal domain protein
AFLA_127500	1.38		cytochrome P450, putative
AFLA_026850	1.42		HMG box protein, putative
AFLA_087820	1.43		cell wall integrity signaling protein Lsp1/Pil1, putative
AFLA_103640	1.46	*fcr1*	C6 transcription factor (Fcr1), putative
AFLA_117370	1.47		conserved hypothetical protein
AFLA_002350	1.47		hypothetical protein
AFLA_037580	1.49		MFS transporter, putative
AFLA_078920	1.49		C2H2 finger domain protein, putative
AFLA_135620	1.51		hypothetical protein
AFLA_023270	1.52		MFS multidrug transporter, putative
AFLA_084870	1.53		hypothetical protein
AFLA_017230	1.59		conserved hypothetical protein
AFLA_104190	1.73		hypothetical protein
AFLA_090870	1.75		hypothetical protein
AFLA_125810	1.80		quinone oxidoreductase, putative
AFLA_113260	1.88		allergen Asp F7
AFLA_031450	1.88		CP2 transcription factor, putative
AFLA_040260	1.97		HLH DNA binding domain protein, putative
AFLA_052640	2.00		PH domain protein
AFLA_116990	2.09		MFS monosaccharide transporter, putative
AFLA_113380	2.12		hypothetical protein
AFLA_138060	2.13		c-24(28) sterol reductase
AFLA_078820	2.26		conserved hypothetical protein
AFLA_092300	2.52		hypothetical protein
AFLA_057180	2.55		conserved hypothetical protein
AFLA_020210	7.56	*nsdD*	sexual development transcription factor NsdD

Table S2-28. Primary metabolites affected in the abundance in *A. nidulans* Δ*nsdD* conidia.

Metabolite ID	Metabolite Name	Log2FC	Median m/z ([M-H]-)	Median retention time	Maximum intensity
M159T15_4		-5.40	159.1276	15.35	1482956
M217T2_7		-5.22	217.0350	1.73	4130693
M137T10_4		-3.98	137.0609	10.19	3262043
M199T10_6		-3.74	199.0613	10.19	12045441
M89T16_1		-3.66	88.9772	16.12	1944879
M157T1_8		-3.36	157.0620	1.10	1124917
M311T1_2		-2.93	311.1100	1.24	3031808
M223T1_1		-2.89	222.9558	0.73	1461687
M131T0		-2.86	130.9443	0.23	1116606
M156T12_1		-2.84	156.0668	12.01	1331684
M457T14_1		-2.47	457.1762	14.04	1080622
M292T1_5		-2.04	292.1517	1.25	1236784
M343T1_3		-1.99	343.1360	1.28	1411611
M111T2_2		-1.92	111.0200	1.56	12711126
M460T1_2		-1.85	460.1676	1.34	3367672
M180T2_2	Tyrosine	-1.74	180.0669	1.84	10433505
M203T7	Tryptophan	-1.72	203.0827	6.63	1262994
M155T7_4		-1.72	155.0100	7.12	1748069
M312T2		-1.72	312.1667	1.88	2188114
M388T1_3		-1.66	388.1493	1.26	3578758
M247T12_3		-1.66	247.0251	12.41	1514445
M257T12_2		-1.63	257.0095	11.91	1620022
M301T12		-1.62	300.9992	11.91	1099405
M517T14_2		-1.57	517.2082	13.92	1734484
M249T12_2		-1.55	249.0043	12.25	1243297
M457T14_2		-1.53	457.1872	13.92	13741594
M493T14_2		-1.41	493.1637	13.92	1843547
M258T1_4		-1.40	258.0751	1.46	1580262
M450T1		-1.36	450.1291	1.32	1400941
M249T1_1		-1.33	249.0208	1.46	1174568
M244T1_2		-1.31	244.1305	1.45	1244872
M335T6_1		-1.26	335.0752	5.78	5331751
M317T12_1		-1.25	317.0305	11.63	1147402
M473T1		-1.24	473.1628	1.31	16106876
M325T1_2		-1.22	325.1254	1.25	1861860
M247T15_3		-1.17	247.0251	14.53	3260872
M267T6_2		-1.16	267.0723	6.05	2082203
M239T1_4		-1.14	239.0775	1.24	16314747
M271T12_4		-1.14	271.0250	12.43	6561150
M911T14		-1.13	911.3503	14.01	1084717
M471T1_3		-1.13	471.1516	1.26	1356862
M215T1_3		-1.13	215.0330	1.23	2169069

M458T1_2		-1.09	458.1882	1.47	2708157
M188T15_1		-1.09	188.1116	14.71	2966901
M431T1_2		-1.07	431.1605	1.32	1100568
M245T6_1		-1.06	245.0434	6.03	9604744
M456T1_3		-1.02	456.1727	1.37	3416658
M145T10_4	alpha-ketoglutarate	-1.01	145.0143	10.46	26010028
M205T12_4		-1.01	205.0354	11.70	4897869
M745T1		-1.00	745.2518	1.36	1150735
M164T4	Phenylalanine	-1.00	164.0718	4.07	2411061
M202T1_3		-1.00	202.1086	1.41	1238580
M371T1_2		1.01	371.1675	1.39	9256569
M133T7_7		1.03	133.0508	6.79	4933666
M244T2_1		1.03	244.0658	1.80	1105844
M201T10_1		1.04	200.8590	9.64	2415378
M243T2_1	Uridine	1.05	243.0625	1.80	10362362
M217T10_1		1.05	216.8539	9.64	1832552
M370T1_1		1.06	370.1407	1.36	1134879
M200T1_1		1.06	199.8051	0.58	1206349
M103T10		1.07	103.0036	10.17	13704847
M137T9		1.07	136.8917	9.33	1547949
M135T11_1		1.09	134.8949	11.43	2145061
M371T1_1		1.09	371.1370	1.36	4032246
M283T1_5		1.10	283.1150	1.29	1184741
M372T1_2		1.10	372.1711	1.39	1765426
M157T11_3		1.11	157.0508	11.42	1331716
M175T11_9		1.13	175.0613	11.24	9822554
M116T1_1		1.15	115.9208	0.98	1828358
M131T1_1		1.15	130.9443	0.99	7313031
M263T1_2		1.15	263.0975	1.09	2129529
M360T1_2		1.16	360.1515	1.28	2274756
M231T10_1		1.18	230.8696	9.63	3272512
M152T1_2		1.18	152.0387	1.41	1303932
M200T7_1		1.22	199.8051	6.73	1864555
M229T9_3	Ribulose 5-phosphate	1.23	229.0120	9.49	1394239
M410T1		1.23	410.1606	1.43	1472570
M242T14_3		1.25	242.1400	14.40	2246504
M201T9_2		1.26	200.8590	9.32	2029648
M271T9_1		1.26	270.9318	9.04	1633215
M159T2_1		1.31	158.9392	1.80	3150369
M137T2_2		1.33	136.8918	2.21	2634476
M173T1_2	Arginine	1.39	173.1045	1.09	1866839
M327T1_1		1.44	327.1253	1.29	1355427
M269T1_2		1.55	269.0881	1.40	2076802
M135T7_3		1.58	134.8949	7.38	5028898

M246T6_1	1.69	245.9713	5.55	2999049
M135T6_2	1.94	134.8949	5.88	4624119
M211T1_2	1.96	211.0825	1.33	36638364
M198T7_1	1.99	197.8080	6.72	4042754
M268T1_2	1.99	268.1404	1.47	2845494
M135T2_6	2.08	135.0315	1.73	3196945
M340T1_3	2.14	340.1255	1.37	1074089
M240T1_4	2.18	240.1092	1.32	4127259
M184T6_2	2.21	183.9708	5.55	1187706
M301T1_1	2.28	301.1079	1.32	1968971
M151T1	2.34	151.0613	1.33	166410528
M266T1_3	2.54	266.1248	1.37	1105201
M297T1_2	2.55	297.1306	1.31	5186613
M187T1_2	2.76	187.0380	1.41	3618250
M189T1_2	2.79	189.0350	1.47	1567255
M156T10	3.53	156.0748	10.19	1884515
M341T1_2	3.61	341.1263	1.37	3883235
M163T1_5	3.69	163.0806	1.25	4710551
M267T2_1	3.90	267.0736	2.44	1200455
M121T1	4.51	121.0507	1.35	6606994
M269T1_1	4.64	269.0549	1.46	1799206
M145T13_5	5.05	145.0620	13.25	1834397
M283T15_2	5.74	283.0250	14.65	2193527
M281T1_4	7.93	281.1052	1.38	3051543
M251T1_4	9.04	251.0947	1.38	1787654

Table S2-29. Primary metabolites affected in the abundance in *A. flavus* Δ*nsdD* conidia.

Metabolite ID	Metabolite Name	Log2FC	Median m/z ([M-H]-)	Median retention time	Maximum intensity
M117T13_4		-7.50	116.9726	13.18	31478208
M117T15_1		-6.62	116.9726	14.74	12747400
M117T7_1		-5.56	116.8935	6.66	1179636
M377T1_3		-5.42	377.0496	1.48	3348842
M218T6_3		-4.79	218.0670	6.11	1444480
M185T6_1		-4.66	185.0221	6.26	12221452
M101T11_2		-4.34	100.9855	11.27	2502151
M159T15_3		-4.32	159.1274	15.34	1530688
M337T15_2		-3.73	337.1560	15.12	16854034
M556T1		-3.47	556.1649	1.32	3079285
M316T1_2		-2.60	316.1167	1.25	3497818
M229T1_5		-2.56	229.0679	1.46	1021328
M229T10_2		-2.53	229.0119	10.08	1184026
M402T1_2		-2.49	402.1648	1.26	3516573
M157T6_5		-2.27	157.0264	6.22	2039585
M214T1_3		-2.25	214.0487	1.28	23244762
M388T1_3		-2.25	388.1492	1.26	1500508
M396T1_2		-2.23	396.1278	1.29	15016115
M142T7_2		-2.21	142.0510	7.39	1923821
M187T10_2		-2.16	187.0248	10.47	5728511
M458T1_2		-2.16	458.1880	1.49	3828426
M683T1_2		-2.14	683.2257	1.36	4463985
M205T12_3		-2.09	205.0353	11.67	22408474
M430T1		-2.08	430.1569	1.33	7382913
M245T6_2		-2.05	245.0434	5.86	3783559
M743T1		-2.04	743.2460	1.37	4798504
M275T6_2		-1.97	275.0539	5.75	1630472
M269T12		-1.96	269.0932	12.19	1396225
M829T1_2		-1.95	829.2938	1.33	1192372
M203T5		-1.93	203.0674	5.49	1191336
M199T13_1		-1.88	198.9086	12.95	20700264
M319T13_2		-1.81	318.8581	12.95	1643102
M371T1_4		-1.80	371.2302	1.49	17948644
M435T1_1		-1.78	434.9482	1.07	1931764
M303T10_1		-1.71	302.5337	10.11	1277408
M155T7_3		-1.68	155.0099	7.06	5671048
M239T13_1		-1.66	238.8911	12.95	1357209
M174T1_2	Citrulline	-1.66	174.0886	1.34	1067254
M678T12		-1.65	678.0957	11.67	2476731
M456T1_2		-1.64	456.1725	1.38	3481190
M517T1_1		-1.60	516.9510	1.07	2252264
M399T13_2		-1.60	398.8247	12.95	51577464

M401T13_1		-1.59	400.8288	12.95	1548160
M531T1_2		-1.59	531.2043	1.39	12613301
M188T10_2		-1.48	188.0565	10.35	1908226
M309T9_2		-1.48	309.1126	9.07	1490156
M158T15_2		-1.47	158.1187	15.07	1996566
M305T6_2		-1.46	305.0646	5.56	2197812
M287T11		-1.45	287.0199	11.20	9612406
M379T1_4		-1.44	379.0828	1.39	1367483
M236T10_2		-1.42	236.0236	10.05	1004752
M231T12_1		-1.41	230.5298	12.15	1570956
M599T1_1		-1.41	598.9541	1.07	2399198
M918T10_1		-1.36	917.7223	9.81	1330364
M231T10_3		-1.35	231.0147	10.37	2254854
M423T5		-1.34	423.0911	4.80	1038011
M462T12		-1.34	462.0670	12.15	6048583
M918T10_2		-1.34	918.2219	9.81	1055104
M917T10		-1.33	917.2210	9.81	1723501
M426T11	ADP	-1.32	426.0223	11.31	1890625
M242T2_2		-1.31	242.0800	2.49	3050736
M255T1_1		-1.30	254.9681	1.07	1052324
M293T2_1		-1.30	293.0992	1.57	4363179
M335T6		-1.30	335.0751	5.73	2567280
M337T1_1		-1.29	336.9712	1.07	6255844
M606T10	UDP-N-acetylglucosamine	-1.29	606.0741	10.11	12421116
M258T5_1		-1.28	258.0384	5.22	1327397
M253T1_1		-1.27	253.0931	1.47	1195515
M300T9_1		-1.27	300.0491	9.05	1033054
M335T15_1		-1.26	335.1404	14.82	20663222
M168T2_3		-1.24	168.0432	2.49	2215092
M171T6_1		-1.23	170.9970	6.34	1273588
M421T13		-1.22	420.8067	12.95	2447462
M681T1		-1.20	680.9577	1.07	2260923
M356T1_2		-1.19	356.1675	1.34	1529947
M132T5	Aspartate	-1.19	132.0303	5.12	29405800
M88T1	Alanine	-1.14	88.0400	1.27	9226026
M104T1	Serine	-1.14	104.0352	1.29	4883636
M419T1_1		-1.13	418.9743	1.07	8688000
M272T1_2		-1.12	272.1240	1.31	1969227
M421T1_1		-1.12	420.9722	1.08	1168154
M165T6_3		-1.10	165.0406	5.81	3232267
M330T2_2		-1.10	330.1229	1.67	4137866
M270T1_3		-1.09	270.1196	1.31	96748208
M298T11		-1.09	298.0698	11.31	1352523
M132T6_3		-1.08	132.0303	5.70	3881522

M489T1_3		-1.07	489.1828	1.33	2075253
M286T1_3		-1.07	286.1145	1.29	20437378
M377T1_4		-1.06	377.0859	1.39	4720705
M111T2_2		-1.05	111.0200	1.57	25610978
M341T1_2		-1.04	341.1088	1.38	4658293
M763T1		-1.03	762.9604	1.07	1789098
M403T1_2		-1.02	403.1354	1.38	3395439
M182T2_1		-1.02	182.0750	2.21	5099923
M161T10_2		-1.01	161.0456	10.39	22610286
M223T9		-1.01	222.9779	8.83	1998749
M239T12_1		-1.01	238.8918	11.80	1793810
M202T10_2		1.00	202.0357	10.39	2040698
M297T18_2		1.00	297.2437	17.61	1099620
M369T1		1.01	369.1880	1.48	17464832
M311T15_1		1.03	311.2229	14.97	1874469
M114T1_2		1.03	114.0309	1.39	2534882
M311T1_4		1.04	311.1164	1.39	1111258
M345T1_4		1.06	345.1596	1.30	1373626
M310T1_2		1.08	310.1509	1.37	3503932
M113T10_1		1.09	112.9856	9.73	3517168
M131T6_2		1.10	130.9443	6.04	1098913
M246T11_3		1.14	246.0621	10.75	31392984
M314T1_2		1.19	314.1108	1.13	3007662
M217T15_2		1.19	217.0484	15.30	1610720
M97T1_2		1.22	97.0041	1.40	1039078
M157T1_7		1.23	157.0368	1.39	70526968
M202T1_8		1.25	202.1085	1.42	10636324
M340T1_2		1.26	340.0933	1.37	3161663
M158T1_4		1.26	158.0402	1.39	3441986
M145T1_3	Lysine	1.27	145.0982	1.10	1627575
M187T1_5		1.27	187.1088	1.49	26427960
M216T10_1		1.28	216.0513	10.44	1076112
M346T10_1		1.30	346.0451	10.34	9188120
M246T11_2		1.31	246.0620	10.53	69132912
M173T11_6		1.31	173.0819	10.74	4039456
M215T10_3		1.35	214.9868	10.33	1118856
M231T7_2		1.36	231.0987	6.63	5673267
M386T1_2		1.37	386.1255	1.43	1483363
M325T9_2		1.37	325.1043	8.71	1961574
M214T6_1		1.37	214.0028	5.87	10704103
M228T11_1		1.38	228.0515	10.53	1305827
M339T1_2		1.39	339.1159	1.38	19689964
M405T1_3		1.39	405.1516	1.30	1443116
M182T1_4		1.48	182.0934	1.15	1791725
M711T9		1.51	711.2366	8.89	2467067
M136T1_2		1.55	136.0629	1.12	1188862

M166T1_3	1.55	166.0735	1.12	3739255
M294T11_1	1.56	293.9691	11.38	2949925
M246T11_1	1.57	246.0621	11.06	2922830
M121T1_2	1.59	121.0507	1.38	2446763
M238T1_2	1.61	238.0947	1.12	55705556
M220T1_3	1.62	220.0841	1.12	4287093
M298T1_1	1.63	298.1158	1.12	71811704
M642T1	1.63	642.2476	1.39	1068888
M328T1_3	1.65	328.1612	1.47	2660542
M168T1_4	1.67	168.0779	1.15	2321467
M249T10	1.76	249.0810	10.36	1776876
M284T1_3	1.97	284.1001	1.14	1540460
M315T1_2	2.08	315.0810	1.40	1077948
M421T1_3	2.17	421.2095	1.15	2128134
M274T1_2	2.26	274.0713	1.15	2299785
M295T17_3	2.29	295.2279	17.15	7519057
M281T1_3	2.37	281.1051	1.40	1443625
M261T9_3	2.42	261.0268	8.76	1447328
M211T1_3	2.58	211.1017	1.10	1281236
M477T1_2	2.69	477.1966	1.12	12865880
M644T8	2.88	644.2242	7.91	2166673
M297T17_4	3.02	297.2436	17.36	19855600
M252T1_3	3.74	252.1091	1.44	1023253
M141T2_3	4.26	141.0306	1.53	3986737
M227T13_4	4.46	227.0773	13.19	2363675
M218T9_2	5.08	218.1034	9.23	3644696
M218T10_3	5.28	218.0671	10.08	3230401
M119T15_3	5.65	118.9517	14.99	3358815
M119T15_2	5.71	118.9517	14.52	3417073
M218T11_1	5.77	218.1116	11.29	3386396
M192T1_3	6.02	192.0879	1.45	6389797
M150T1	6.87	150.0772	1.10	73117040
M210T1_2	7.09	210.0983	1.10	17182396
M160T7_6	7.92	160.0333	7.05	6585081
M389T1_4	10.42	389.1794	1.11	1507128

Table S2-30. BGCs affected in the gene expression level in *A. nidulans* Δ*nsdD*.

Compound Name	Backbone Gene ID	Backbone Gene name	Log2FC Vege	Log2FC Asex	Log2FC conidia
2,4-dihydroxy-3-methyl-6-(2-oxopropyl)benzaldehyde	AN7903	*pkeA*	-	5.33	-
Alternariol/isocoumarins	AN7071	*pkgA*	3.61	1.52	-
Arugosins	AN1050	*mdpG*	-	-1.15	-
Aspercryptins	AN7884	*atnA*	5.17	2.27	-
Aspercryptins	AN6448	*pkbA*	-	2.91	2.85
Asperfuranone	AN1036	*afoG*	-	-2.32	-
Aspernidine A	AN3230	*pkfA*	1.29	2.79	-
Aspyridone A and B	AN8412	*apdA*	-	-2.26	-1.49
Austinol/dehydroaustinol	AN8383	*ausA*	-2.98	-2.13	-
Cichorine	AN6448	*pkbA*	-	2.91	2.85
Citreoisocoumarin	AN7071	*pkgA*	3.61	1.52	-
Dehydroaustinol	AN8383	*ausA*	-2.98	-2.13	-
Emericellamides	AN2545	*easA*	-	-	-1.55
Emericellamides	AN2547	*easB*	-2.99	-	-
Emodin and analogs	AN1050	*mdpG*	-	-1.15	-
Ent-pimara-8(14),15-diene	AN1594		-	-3.93	-
F-9775 A and B/violaceol I and II/orsellinic acid	AN7909	*orsA*	-	3.58	-
Felinone A	AN7903	*dbaI*	-	5.33	-
Fellutamide B	AN3495	*inpA*	-	5.66	-
Fellutamide B	AN3496	*inpB*	3.32	3.78	-
Grey-brown conidiophore pigment	AN10576	*ivoA*	-	-2.86	-
Microperfuranone/dehydromicroperfuranone	AN3396	*micA*	-	2.12	-
Monodictyphenone	AN0150	*mdpG*	-1.13	-2.26	-
Orsellinic acid, diorcinol	AN7909	*orsA*	-	3.58	-
Penicillin	AN2621	*acvA*	-	-2.09	-1.52
Sanghaspirodins	AN7909	*orsA*	-	3.58	-
Sterigmatocystin	AN7825	*stcA/pksST*	-	1.75	-
Terrequinone A	AN8513	*tdiA*	-	-	-2.15
YWA1	AN8209	*wA*	-	-3.71	-

Table S2-31. BGCs affected in the gene expression level in *A. flavus* Δ*nsdD*.

Cluster name	Gene ID	Log2FC Vege	Log2FC Asex	Log2FC conidia
Aflatoxin	AFLA_139150	3.45	-	-
Aflatoxin	AFLA_139160	2.93	1.79	-
Aflatoxin	AFLA_139170	4.18	1.59	-
Aflatoxin	AFLA_139180	4.13	-	-
Aflatoxin	AFLA_139190	3.92	-	-
Aflatoxin	AFLA_139200	3.81	-	-
Aflatoxin	AFLA_139210	4.06	-	-
Aflatoxin	AFLA_139220	3.90	-	-
Aflatoxin	AFLA_139230	4.15	-	-
Aflatoxin	AFLA_139240	3.87	-	-
Aflatoxin	AFLA_139250	4.06	1.16	-
Aflatoxin	AFLA_139270	2.03	-	-
Aflatoxin	AFLA_139280	3.64	-	-
Aflatoxin	AFLA_139290	3.60	-	-
Aflatoxin	AFLA_139310	3.92	-	-
Aflatoxin	AFLA_139330	3.77	-	-
Aflatoxin	AFLA_139340	-	-	1.17
Aflatoxin	AFLA_139370	-	1.76	-
Aflatoxin	AFLA_139380	-	-	-1.12
Aflatoxin	AFLA_139390	3.48	-	-
Aflatoxin	AFLA_139400	3.79	-	-
Aflatoxin	AFLA_139410	4.10	-	-
Aflatoxin	AFLA_139420	1.32	-	-
Aflatrem(ATM2)	AFLA_045540	1.10	-1.20	-
AsparasoneA	AFLA_082160	-	2.06	-
Aspergillicins	AFLA_010480	-	-	-1.98
Aspergillicins	AFLA_010580	-	1.28	-
Aspergillicins	AFLA_010650	-	-1.61	-
BGC_06	AFLA_105410	-	1.74	2.64
BGC_06	AFLA_105420	-	3.26	3.45
BGC_06	AFLA_105430	-	4.25	-
BGC_06	AFLA_105440	-	3.94	-
BGC_06	AFLA_105450	-	3.31	1.16
BGC_06	AFLA_105490	-	-	-1.07
BGC_06	AFLA_105510	-	-1.45	-
BGC_06	AFLA_105520	-	-2.47	-
BGC_06	AFLA_105530	1.33	-	-
BGC_06	AFLA_105540	2.85	-	-
BGC_08	AFLA_004280	-	-	-2.51
BGC_08	AFLA_004300	-	7.71	-1.75
BGC_10	AFLA_005320	-	-	-3.05
BGC_10	AFLA_005350	-3.16	-	-
BGC_10	AFLA_005390	-	-	2.18
BGC_10	AFLA_005430	-	-	2.93

BGC_14	AFLA_042300	-	5.18	-
BGC_14	AFLA_042310	-	5.00	-
BGC_14	AFLA_042320	-	5.47	-
BGC_14	AFLA_042330	-	8.02	-
BGC_14	AFLA_042340	-	6.04	-
BGC_14	AFLA_042350	-	4.50	-
BGC_14	AFLA_042480	-	-	1.51
BGC_15	AFLA_126650	-	-	1.15
BGC_15	AFLA_126660	-	2.27	5.41
BGC_15	AFLA_126680	-	1.57	5.11
BGC_15	AFLA_126690	-	2.40	5.57
BGC_15	AFLA_126710	-	7.45	4.28
BGC_15	AFLA_126730	-1.42	4.56	4.36
BGC_15	AFLA_126760	-	-	-1.72
BGC_15	AFLA_126770	-	-	-2.18
BGC_20	AFLA_041610	-	-	1.92
BGC_20	AFLA_041650	-	-	-1.04
BGC_21	AFLA_102480	1.11	-	-
BGC_23	AFLA_064440	-	6.34	5.43
BGC_23	AFLA_064460	-	-	4.04
BGC_23	AFLA_064470	4.56	-	4.32
BGC_23	AFLA_064490	-	3.11	3.16
BGC_23	AFLA_064530	-	-	1.66
BGC_23	AFLA_064540	-	-	3.10
BGC_23	AFLA_064550	-	4.77	3.89
BGC_23	AFLA_064570	-	-	2.79
BGC_23	AFLA_064580	-	-	2.73
BGC_23	AFLA_064590	-	-	3.44
BGC_23	AFLA_064600	1.25	-	4.01
BGC_23	AFLA_064610	-	1.32	-2.38
BGC_24	AFLA_066580	-	2.16	-
BGC_24	AFLA_066690	-	-	1.87
BGC_24	AFLA_066710	-	1.77	1.08
BGC_24	AFLA_066770	1.57	-	-1.30
BGC_24	AFLA_066810	-	-	-1.84
BGC_35	AFLA_087820	-	-	1.43
BGC_35	AFLA_087830	-	-	-1.00
BGC_36	AFLA_127000	-	4.14	-
BGC_36	AFLA_127130	4.24	-	-
BGC_37	AFLA_002640	-	-1.44	-
BGC_39	AFLA_053160	-	3.91	-1.57
BGC_39	AFLA_053170	-	2.12	-1.17
BGC_39	AFLA_053180	-	-	-1.52
BGC_39	AFLA_053220	-	-	1.24
BGC_39	AFLA_053270	-	3.54	-
BGC_39	AFLA_053280	3.51	-	-

BGC_39	AFLA_053290	-	4.49	-
BGC_40	AFLA_105170	-	-	-1.80
BGC_40	AFLA_105220	-	-	-1.40
BGC_40	AFLA_105240	-3.86	-	-1.84
BGC_40	AFLA_105270	-	2.98	-
BGC_41	AFLA_107090	-	3.90	-
BGC_42	AFLA_009240	-	-	-1.43
BGC_42	AFLA_009250	-	3.91	-2.17
BGC_43	AFLA_135390	-	-	1.19
BGC_43	AFLA_135400	-	-	1.54
BGC_43	AFLA_135420	-	-	3.61
BGC_43	AFLA_135560	-	-	-1.16
BGC_44	AFLA_100270	-	-2.75	-
BGC_44	AFLA_100330	-3.34	-	-
BGC_45	AFLA_083220	-	-	-1.37
BGC_46	AFLA_054210	-	1.18	-
BGC_46	AFLA_054240	-	-	1.49
BGC_46	AFLA_054290	-	-1.56	-
BGC_46	AFLA_054300	-	-	-1.69
BGC_46	AFLA_054330	-	-	-4.94
BGC_46	AFLA_054340	-	1.43	-
BGC_46	AFLA_054360	-	-	-1.93
BGC_47	AFLA_028720	1.46	-	-
BGC_47	AFLA_028790	-	-	-1.03
BGC_48	AFLA_009950	-	-	-1.71
BGC_48	AFLA_010020	-	-	-1.13
BGC_48	AFLA_010030	-	2.53	-
BGC_48	AFLA_010050	-	-	-2.02
BGC_48	AFLA_010060	-	-	-2.03
BGC_48	AFLA_010080	-	-	-2.01
BGC_50	AFLA_018320	-	-	-1.29
BGC_50	AFLA_018350	-	-1.58	-
BGC_51	AFLA_119770	-	-	-2.00
BGC_51	AFLA_119780	-	-	-2.35
BGC_51	AFLA_119800	-	-1.12	-
BGC_51	AFLA_119810	-	-	-1.59
BGC_51	AFLA_119820	-	-2.03	-
BGC_51	AFLA_119830	-	-1.36	-1.57
BGC_51	AFLA_119860	-	-	-1.53
BGC_52	AFLA_017770	-	-	-1.08
BGC_52	AFLA_017860	-	-	1.10
BGC_53	AFLA_062470	-1.89	-2.77	1.40
BGC_54	AFLA_080430	-	4.33	1.67
BGC_54	AFLA_080490	-	-	-1.59
BGC_54	AFLA_080510	-	-1.01	-
BGC_55	AFLA_082490	-	-	-2.02

BGC_55	AFLA_082500	-	-	-1.34
BGC_56	AFLA_070250	-	2.30	-
BGC_56	AFLA_070280	-	-1.05	-
BGC_56	AFLA_070320	-	2.24	2.24
BGC_56	AFLA_070340	-	-	2.46
BGC_57	AFLA_090110	-	-	-1.18
BGC_57	AFLA_090160	-	-1.38	-
BGC_57	AFLA_090220	1.41	1.33	-
BGC_58	AFLA_105000	-	-	-1.55
BGC_58	AFLA_105020	-	-	-1.55
BGC_58	AFLA_105050	-	-	-2.40
BGC_58	AFLA_105060	-	1.02	-1.31
BGC_58	AFLA_105070	-	-	-3.40
BGC_59	AFLA_002840	-	-2.59	-
BGC_59	AFLA_002920	-1.63	-	1.29
BGC_60	AFLA_039200	2.90	-	-
BGC_60	AFLA_039220	2.34	-	-
BGC_60	AFLA_039250	2.18	-	-
BGC_61	AFLA_060640	-	-	-1.63
BGC_61	AFLA_060660	-	2.38	-
BGC_61	AFLA_060680	-	2.91	-
BGC_61	AFLA_060690	-	2.70	-
BGC_62	AFLA_006170	-	-2.61	-
BGC_62	AFLA_006180	-	-2.24	-
BGC_63	AFLA_054060	-	-2.49	-4.06
BGC_63	AFLA_054070	-	-	1.68
BGC_63	AFLA_054080	-2.01	-	-
BGC_63	AFLA_054120	-	-	-1.36
BGC_63	AFLA_054130	-	1.20	-
BGC_63	AFLA_054150	-	4.36	-
BGC_64	AFLA_027160	-	-	-1.56
BGC_64	AFLA_027200	-	-	-1.16
BGC_64	AFLA_027210	-	-	1.27
BGC_64	AFLA_027260	1.20	-	-
BGC_65	AFLA_112810	-	-1.05	-
BGC_65	AFLA_112840	-	2.35	-1.32
BGC_65	AFLA_112850	-	-	3.62
BGC_65	AFLA_112890	-	-	1.56
BGC_66	AFLA_004410	-	-4.01	-1.75
BGC_66	AFLA_004430	-	-	-2.86
BGC_66	AFLA_004460	-	-	-1.93
BGC_66	AFLA_004500	-	-	-1.09
BGC_67	AFLA_137780	-	1.42	-
BGC_67	AFLA_137820	-	-	-1.25
BGC_67	AFLA_137840	-	-	1.91
BGC_67	AFLA_137860	-	-	1.28

BGC_67	AFLA_137900	2.80	-	-
BGC_67	AFLA_137910	-	-2.78	-
BGC_68	AFLA_038260	-	7.66	-
BGC_68	AFLA_038300	-	1.95	-
BGC_68	AFLA_038370	-	-	-1.58
BGC_69	AFLA_053840	-	-1.53	-
BGC_69	AFLA_053930	-	-	1.21
BGC_69	AFLA_053950	1.81	-	-
BGC_70	AFLA_079280	-	-	-3.04
BGC_70	AFLA_079290	-2.48	-	-
BGC_70	AFLA_079420	-	-	1.08
BGC_71	AFLA_038570	-	-	-1.36
BGC_71	AFLA_038590	-	-2.98	-
BGC_71	AFLA_038630	1.38	-	-
BGC_71	AFLA_038650	-	-1.63	-
BGC_72	AFLA_062740	-	-	-1.10
BGC_72	AFLA_062760	-	-	1.03
BGC_72	AFLA_062800	-	-	1.30
BGC_72	AFLA_062880	-	-1.58	1.62
BGC_72	AFLA_062890	-	2.24	2.95
BGC_72	AFLA_062920	-	1.35	-
BGC_73	AFLA_089630	-	-	-2.57
BGC_73	AFLA_089670	-	-	2.03
BGC_73	AFLA_089690	-	-	-1.71
BGC_74	AFLA_118350	-	-	-1.69
BGC_74	AFLA_118360	-	1.22	-2.09
BGC_74	AFLA_118460	1.10	-	-
BGC_74	AFLA_118470	-	-1.10	-
BGC_75	AFLA_006800	-1.21	-	-
BGC_75	AFLA_006850	-	-1.36	-
BGC_75	AFLA_006920	-	-1.03	-1.84
BGC_76	AFLA_117740	-	-	-1.43
BGC_76	AFLA_117750	-1.29	-	-
BGC_76	AFLA_117820	-	-	-2.53
BGC_77	AFLA_008670	-	-	-1.19
BGC_77	AFLA_008680	-	-	1.21
BGC_77	AFLA_008720	-	-	-1.50
BGC_77	AFLA_008750	-	2.30	-1.65
BGC_77	AFLA_008830	-	-	-1.59
BGC_79	AFLA_102130	-	-	1.08
BGC_79	AFLA_102250	-	-1.66	-
BGC_79	AFLA_102260	-	-1.59	-
BGC_79	AFLA_102270	-	-	-1.21
BGC_79	AFLA_102340	-	1.95	-
BGC_79	AFLA_102360	-	-1.75	-
BGC_80	AFLA_070810	-	1.75	-

BGC_80	AFLA_070830	-	-1.16	-
BGC_80	AFLA_070870	-1.48	-1.85	-2.10
BGC_80	AFLA_070880	-1.67	-	-3.40
BGC_80	AFLA_070910	-	-	-1.26
BGC_80	AFLA_070920	-	-	-1.50
BGC_80	AFLA_070940	-	-	1.83
BGC_80	AFLA_070970	-	-	1.28
BGC_80	AFLA_070980	-	-	-1.64
BGC_81	AFLA_125690	-	-	1.37
BGC_81	AFLA_125700	-	-	2.33
BGC_81	AFLA_125750	-	2.93	-
BGC_81	AFLA_125770	-	3.08	2.16
BGC_81	AFLA_125810	-	-	1.80
BGC_82	AFLA_125610	-	-	-1.57
BGC_82	AFLA_125620	-	-	-1.92
BGC_83	AFLA_116140	-	-1.91	-
BGC_83	AFLA_116150	-	-	1.63
BGC_83	AFLA_116170	-	-3.04	-
BGC_83	AFLA_116180	-	-3.08	-
BGC_83	AFLA_116190	-	-3.19	-
BGC_83	AFLA_116210	-	-1.72	-
BGC_83	AFLA_116260	-	-	-3.64
BGC_83	AFLA_116270	-	-	-1.12
BGC_83	AFLA_116320	-	2.96	-
BGC_84	AFLA_059940	-	-3.56	-4.08
BGC_84	AFLA_059970	-	-1.92	-1.09
BGC_84	AFLA_059980	-	-1.49	-
BGC_84	AFLA_059990	-	-1.90	-
BGC_84	AFLA_060000	-	-1.92	-
BGC_84	AFLA_060070	-	-1.08	-2.49
BGC_84	AFLA_060080	-	1.91	-
BGC_85	AFLA_119040	-	4.61	-
BGC_85	AFLA_119080	-	-	1.21
BGC_85	AFLA_119130	-	-1.34	-
BGC_85	AFLA_119150	-	-	1.74
BGC_85	AFLA_119200	4.04	-	-
BGC_85	AFLA_119210	2.67	-	-
BGC_86	AFLA_118830	-	-	-2.77
BGC_86	AFLA_118840	-	-	-2.46
BGC_86	AFLA_118910	-	-2.50	-4.29
BGC_88	AFLA_109370	-	-	1.61
BGC_88	AFLA_109380	-1.70	-1.23	-
BGC_88	AFLA_109430	-	1.72	-
BGC_88	AFLA_109440	-	-	-2.79
BGC_88	AFLA_109450	-	-1.71	-
BGC_88	AFLA_109480	-	1.68	-

BGC_88	AFLA_109510	-	-	1.41
BGC_89	AFLA_114720	-	-	-1.46
BGC_89	AFLA_114750	-	-	1.30
BGC_90	AFLA_127980	-	-1.01	-
BGC_90	AFLA_128020	-	-	1.19
BGC_90	AFLA_128060	-	2.17	1.84
BGC_90	AFLA_128070	-	5.32	-
BGC_90	AFLA_128080	-	5.02	1.62
BGC_90	AFLA_128100	-	-	-1.01
BGC_90	AFLA_128140	-	-	-1.31
BGC_90	AFLA_128190	-	-	-1.29
BGC_90	AFLA_128240	-	-	-1.01
Ditryptophenaline	AFLA_005440	-2.73	-	-
Ditryptophenaline	AFLA_005460	-	-1.49	-
Imizoquin	AFLA_064240	-	-	1.54
Imizoquin	AFLA_064270	3.65	-	-
Imizoquin	AFLA_064290	3.71	-	-
Imizoquin	AFLA_064330	5.66	-	-
LeporinB	AFLA_066900	-	-	-2.04
LeporinB	AFLA_066910	-	-	-2.93
LeporinB	AFLA_066930	-	-	-1.33
Lovastatin-like	AFLA_096610	-2.35	1.51	-1.87
Lovastatin-like	AFLA_096680	-	3.04	-
Lovastatin-like	AFLA_096740	-	-	2.40
Lovastatin-like	AFLA_096750	-	-	2.51
Lovastatin-like	AFLA_096770	-	-	1.63
UstiloxinB	AFLA_094960	-	4.08	-
UstiloxinB	AFLA_094980	-	4.33	-
UstiloxinB	AFLA_094990	-	2.48	-
UstiloxinB	AFLA_095010	-	3.29	-
UstiloxinB	AFLA_095020	-	3.11	-
UstiloxinB	AFLA_095030	-	2.97	-
UstiloxinB	AFLA_095040	-	3.66	-
UstiloxinB	AFLA_095050	-	3.49	-
UstiloxinB	AFLA_095060	-	3.56	-
UstiloxinB	AFLA_095090	-	2.70	-
UstiloxinB	AFLA_095100	-	3.26	-

Table S2-32. Secondary metabolites affected in the abundance in *A. nidulans* Δ*nsdD*.

Metabolite ID	metabolite name	Log2FC	Median m/z ([M+H]-)	Median retention time	Maximum intensity
M332T13_2		-12.55	332.1391	12.90	2065733
M170T1		-7.89	170.0812	1.39	7734322
M170T2_3		-6.70	170.0812	1.67	4516782
M425T10_2		-6.54	425.1589	9.54	4896006
M304T5		-6.00	304.1904	5.01	1652436
M299T4_1		-5.69	299.1469	3.68	1160306
M203T5_2		-5.58	203.1100	4.69	1300237
M313T14_1		-5.45	313.0705	14.48	14975106
M317T7_2		-4.99	317.1858	7.44	1570643
M170T2_2		-4.59	170.0812	2.03	2131999
M372T13_1		-4.13	372.3108	13.21	1382823
M457T7_4		-3.93	457.2272	6.62	1940062
M217T5_1		-3.78	217.0859	4.90	1502774
M405T11_2		-3.70	405.2632	11.34	1403074
M425T8_2		-3.39	425.2152	8.31	2444718
M355T8_6		-3.27	355.2265	8.13	21771056
M328T3_3		-3.26	328.1904	2.72	1507962
M460T8_2		-3.11	460.2745	8.25	1623733
M403T9_3		-2.97	403.1922	8.99	3911172
M464T12_3		-2.75	464.3003	12.24	1874889
M425T11_1		-2.73	425.1712	10.67	3372959
M310T5_1		-2.71	310.1799	4.71	1252252
M397T7_3		-2.62	397.2370	7.11	16464707
M328T3_2		-2.57	328.1905	3.07	2227405
M443T9_5		-2.31	443.2285	8.54	9120525
M664T11		-2.24	664.3884	11.19	1041563
M539T11		-2.20	539.2536	11.23	1268112
M885T11		-2.11	885.4045	10.64	1038442
M460T9_5		-2.10	460.3045	8.68	2416892
M507T12_1	Asterriquinone	-2.05	507.2276	12.34	634591
M425T9_4		-2.01	425.2506	8.96	7543959
M403T12_4	Aspernidgulene B1	-1.98	403.2477	12.42	2944165
M429T13_2		-1.95	429.2635	12.51	1300781
M441T9_4		-1.94	441.2365	9.07	2573404
M317T8_3		-1.92	317.1857	7.83	1552310
M164T5_1		-1.91	164.1071	5.17	1338204
M429T11_3		-1.90	429.2629	11.21	2740450
M949T7		-1.90	949.3842	7.11	5028339
M370T9_1		-1.90	370.2011	9.48	4145790
M1395T9		-1.83	1395.0638	8.85	1217001
M459T13_2		-1.83	459.2014	12.65	1408002
M429T13_5		-1.82	429.3367	12.74	1186090

M460T8_3		-1.82	460.3048	8.27	1623733
M514T8		-1.80	514.3136	8.27	1884201
M931T9_1		-1.79	931.3735	8.57	1806234
M356T9_3		-1.78	356.2217	9.05	1095887
M402T8_2		-1.77	402.2272	8.28	2135204
M966T7		-1.76	966.4107	7.11	2669278
M378T15_4		-1.76	378.3233	15.31	1911252
M459T11_3		-1.76	459.2014	10.61	2776839
M370T8_3		-1.75	370.2010	8.28	1546998
M396T15_3		-1.74	396.3337	15.47	6112244
M642T11		-1.73	642.4065	11.18	1838441
M354T10_2		-1.72	354.2061	9.61	1197321
M439T7_4		-1.72	439.2801	6.84	1171707
M422T9_3		-1.71	422.2532	8.75	5432715
M457T15_1		-1.71	457.1853	15.30	1252086
M402T9_2		-1.71	402.2273	9.41	2901731
M235T2		-1.70	235.1077	2.13	1613447
M459T11_2		-1.70	459.2014	10.99	2572769
M490T9_2		-1.69	490.2430	8.96	1050665
M459T13_3	austinol (desacetylaustin)	-1.67	459.2013	12.95	1473186
M656T12_1		-1.66	656.4222	12.45	2595465
M404T8_2		-1.65	404.2428	7.80	1116279
M441T9_3		-1.65	441.2461	9.39	3029270
M899T9_1		-1.63	899.3841	9.10	1218715
M404T10_3		-1.63	404.2424	9.61	1481763
M446T9		-1.63	446.2533	8.53	1253701
M916T9_2		-1.62	916.3801	8.99	2009138
M386T10_2	Aspernidine B	-1.61	386.2324	9.62	1201865
M474T9_3		-1.60	474.2121	9.22	2030103
M439T11_2		-1.58	439.2088	10.74	1542217
M474T7_2		-1.57	474.2120	7.11	5068763
M427T12_1	isoaustinone	-1.55	427.2114	11.56	5134607
M401T12_1		-1.54	401.2321	11.99	5028938
M492T9_3		-1.54	492.3313	8.68	4689277
M429T11_1		-1.51	429.2173	11.15	4501063
M466T12_3		-1.51	466.3161	12.41	3670522
M445T12_3		-1.50	445.2581	11.66	1266094
M492T8_3		-1.50	492.3316	8.27	5614798
M369T7_1		-1.48	369.1694	7.11	1014771
M436T12_3		-1.48	436.2691	12.04	1382741
M399T9_3		-1.48	399.1798	8.66	3039235
M457T10_2	dehydroaustinol	-1.47	457.1853	9.90	6714085
M628T11_1		-1.46	628.3916	10.59	1978953
M431T11_2	protoaustinoid A	-1.46	431.2790	11.50	1513456
M422T13_4		-1.45	422.3262	13.41	5544654

M367T9_4		-1.45	367.1537	8.70	2666829
M449T10_3		-1.44	449.2531	10.43	3084429
M340T7_3		-1.41	340.1622	6.82	1312677
M420T9_1		-1.40	420.2377	9.05	9124952
M450T9_2		-1.39	450.2486	8.85	1406267
M371T9_1		-1.38	371.1851	8.66	3369342
M626T12_3		-1.38	626.4118	11.60	1459953
M299T8_2		-1.38	299.2368	7.88	1436157
M355T8_4		-1.37	355.1901	8.05	31620190
M317T8_4		-1.37	317.2473	7.88	1320483
M438T9		-1.36	438.2847	9.02	1284719
M367T8_4		-1.36	367.2090	8.33	1833249
M458T11_3		-1.35	458.1887	11.11	1361260
M457T7_2		-1.34	457.1853	6.82	6782742
M558T11_2		-1.34	558.3279	10.74	1641803
M514T9		-1.34	514.3133	8.67	2686537
M340T7_2		-1.32	340.1622	7.11	8202558
M450T9_3		-1.30	450.2482	9.48	2033168
M439T7_1		-1.30	439.1748	7.11	3966254
M341T7_3		-1.30	341.1653	7.11	1132243
M442T9_1		-1.30	442.1940	8.83	2571238
M472T7_1		-1.28	472.1962	7.01	7885794
M339T7_2		-1.28	339.1589	6.80	5729294
M477T9_2		-1.27	477.2473	8.78	2624690
M409T11_3	emericellin	-1.27	409.2008	11.07	1981536
M403T9_7	Aspernidgulene B2	-1.27	403.2477	9.02	3911172
M401T10_2		-1.24	401.2321	10.40	3723057
M644T9		-1.23	644.3681	8.91	1501143
M475T7_1		-1.23	475.1958	7.11	4498561
M425T11_2		-1.23	425.1956	10.60	3941589
M373T10_1		-1.22	373.0552	10.50	1791027
M423T13_6		-1.22	423.3254	12.75	3073661
M457T14_1		-1.21	457.1853	14.08	1550904
M459T7		-1.21	459.1899	7.11	2049344
M419T10_5	Aspernidgulene A1	-1.20	419.2427	10.08	1586601
M161T2_1		-1.19	161.0709	2.31	1688995
M368T9_2		-1.19	368.1936	9.25	1500680
M329T11_2		-1.19	329.1746	11.07	2069454
M345T8_5		-1.19	345.2422	8.05	1356149
M397T7_1		-1.18	397.1642	7.11	16464707
M387T7		-1.18	387.1799	7.11	5060756
M618T12_3		-1.16	618.3831	11.85	5396955
M375T9_2		-1.16	375.2163	9.35	2052060
M437T9_2		-1.16	437.2436	9.36	1051628
M382T9		-1.16	382.2090	9.24	1128209
M458T7		-1.15	458.1886	7.11	11476607

M441T9_1		-1.14	441.1906	8.83	8899863
M443T11_3		-1.14	443.3515	11.15	1082913
M443T11_1	austinolide	-1.14	443.2062	10.66	81595992
M622T12_3		-1.13	622.4167	12.41	9440215
M492T7_3		-1.13	492.2225	7.11	1486328
M386T9_2		-1.12	386.2043	9.24	1807806
M411T10_4		-1.12	411.3256	10.19	3711514
M459T11_5		-1.10	459.2375	10.75	1659392
M444T9_3		-1.10	444.2095	9.11	2153443
M459T9_1		-1.10	459.2010	8.83	935415168
M444T9_2		-1.09	444.2095	8.69	2507977
M343T10_2		-1.09	343.1902	10.48	2279009
M1298T14		-1.09	1297.8715	13.70	1060995
M558T9_2		-1.09	558.3264	8.70	1046162
M441T10_1		-1.08	441.1905	9.91	20813426
M917T9_2		-1.08	917.3943	8.83	71446584
M399T10_2	Preaspernidgulene A1	-1.07	399.2166	9.51	3401068
M596T12	emericellamide C/D	-1.07	596.4013	11.85	13134778
M339T7_1		-1.07	339.1589	7.11	36922272
M397T10_2		-1.07	397.2007	9.84	3381015
M383T10_1		-1.07	383.1849	9.84	1508659
M417T10_3		-1.06	417.2270	9.52	9668449
M371T9_3		-1.05	371.2215	9.00	1100493
M349T9		-1.05	349.1795	9.25	2237495
M369T10_2		-1.05	369.2058	9.83	2149169
M401T10_1		-1.04	401.1956	9.84	1099650
M641T12_2		-1.04	641.4305	12.24	3807898
M415T10_1	austinoneol	-1.03	415.2113	9.84	23628564
M337T9		-1.03	337.1432	9.23	1948325
M451T10_2		-1.03	451.2517	9.84	3351537
M434T9_2		-1.02	434.2537	9.25	6754963
M335T9		-1.02	335.1486	8.82	1118740
M207T8_1		Only in Δ*nsdD*	207.0045	7.73	29923
M320T7		Only in Δ*nsdD*	320.2664	6.85	110343
M333T8_4		Only in Δ*nsdD*	333.2786	7.76	54200
M381T4		Only in Δ*nsdD*	381.0789	3.55	64091
M491T14	Terrequinone A	Only in Δ*nsdD*	491.2325	14.02	4022153
M511T5		Only in Δ*nsdD*	511.1425	4.62	36117
M621T8_1		Only in Δ*nsdD*	621.3820	8.35	112904

M844T9	Only in Δ*nsdD*	844.4255	9.45	120889
M1006T8	Only in Δ*nsdD*	1005.6365	8.28	129598
M591T14	1.00	591.3363	14.00	1042068
M383T10_5	1.03	383.2579	9.86	1071578
M295T7_2	1.04	295.2056	6.51	1251259
M341T14_2	1.05	341.2321	13.57	6427064
M314T7	1.06	314.2194	6.51	1142223
M251T4_3	1.06	251.1852	3.87	2548217
M374T12_1	1.07	374.2536	11.90	1729871
M510T9_1	1.07	510.2789	9.33	3282540
M620T11_2	1.09	620.3438	10.85	1040804
M344T13_3	1.10	344.2794	13.22	16250805
M280T11_2	1.10	280.2482	11.10	1353090
M210T3	1.11	210.0759	3.06	4544307
M531T9_1	1.12	531.2576	9.35	2359932
M277T12_6	1.17	277.2162	12.45	2070908
M299T10_1	1.17	299.0549	9.94	3575485
M310T13_3	1.19	310.2456	13.21	1910436
M359T6_2	1.19	359.0758	5.88	14161303
M526T9	1.26	526.3020	9.32	2679671
M463T12_3	1.27	463.3415	11.60	1199450
M280T13_2	1.30	280.2351	13.06	2192961
M548T9_1	1.37	548.2867	9.34	1535700
M714T12_1	1.38	713.5162	12.13	1328803
M619T10_1	1.39	619.3312	9.56	1205029
M546T12	1.43	546.3072	11.86	1208161
M618T10_1	1.43	618.3280	9.55	2957640
M697T13	1.49	697.4850	13.11	5186537
M265T5	1.49	265.0695	4.81	1003359
M575T11	1.52	575.2972	10.57	2226050
M226T4_1	1.54	226.0709	3.95	1206845
M343T6_1	1.54	343.0810	6.40	2016693
M621T12	1.55	621.3469	12.27	17523694
M311T10_2	1.56	311.0548	10.33	37437468
M333T13_6	1.57	333.2422	12.64	1023093
M620T12_1	1.58	620.3436	12.27	44855824
M552T12_1	1.58	552.2810	12.27	4458615
M244T11	1.58	244.2271	11.05	7589361
M220T8_2	1.60	220.1365	7.74	1711360
M293T13_2	1.60	293.2110	13.47	5393532
M698T13	1.62	698.4883	13.11	1978178
M216T4	1.66	216.0629	4.15	1258350
M243T5	1.66	243.0877	4.81	26193378
M277T10_5	1.67	277.2162	9.61	2484619

M305T14		1.67	305.2110	13.90	2443213
M341T8	Isoversicolorin C	1.69	341.0654	8.14	14499366
M331T9_1		1.70	331.0810	8.52	6092195
M636T11_1		1.77	636.3381	11.22	3135762
M195T5	nidulol	1.80	195.0649	5.40	1022746
M325T13_3		1.81	325.2372	12.88	4877804
M606T12_1		1.83	606.3282	11.87	1025775
M283T12_1		1.85	283.0600	12.02	4494687
M332T10_2		1.88	332.1126	9.84	1922374
M373T8_4		1.91	373.0915	7.50	1617012
M620T13_1		1.95	620.3436	12.96	1065039
M957T12_1		1.96	956.5666	12.36	1323313
M267T13_3		1.96	267.1954	13.25	1455302
M373T8_3		1.98	373.0915	7.77	9395112
M618T11_1		2.00	618.3302	10.94	1141872
M665T11_1		2.10	665.1279	11.24	17793346
M683T12		2.10	683.4694	12.32	3107391
M618T12_1		2.11	618.3281	11.95	3109723
M303T6	2-ω-Dihydroxyemodin	2.13	303.0498	6.48	2951177
M548T9_2		2.14	548.2864	8.80	1145008
M195T4		2.15	195.0841	4.15	6459528
M681T13_1		2.21	681.1230	12.53	1012450
M459T10_5		2.26	459.2361	10.28	1118719
M345T10_1		2.35	345.0604	9.85	1605912
M355T8_1		2.36	355.0810	7.94	1184626
M742T12		2.44	741.5112	11.87	1482220
M580T10_2		2.45	580.2759	10.21	1297485
M508T10		2.47	508.2552	9.61	3574635
M742T13		2.47	741.5113	12.98	4431478
M785T12_2		2.48	784.5531	11.78	6831756
M355T8_5		2.52	355.2090	7.79	1296077
M343T7		2.52	343.0809	7.09	1457394
M287T11_1	citreorosein	2.54	287.0549	10.82	1297802
M807T12		2.54	806.5350	11.79	2391703
M618T10_2		2.61	618.3281	10.48	1714930
M239T4_1		2.63	239.1106	3.90	1914965
M260T4		2.63	260.0892	3.93	3347805
M576T14_1		2.66	576.3176	13.52	8624687
M492T4		2.68	492.1146	4.48	2973936
M634T13_1		2.71	634.3591	13.00	2784744
M303T7_1		2.74	303.0861	6.66	3446974
M293T12_1		2.76	293.1534	12.04	1693469
M508T14_2		2.76	508.2550	13.52	1152644
M329T8_2		2.82	329.0654	7.65	3330094
M302T8_2		2.84	302.0658	8.22	1197241
M359T6_1		2.85	359.0758	6.22	5788608

M643T14_1		2.92	643.3596	13.83	2377749
M216T8_2		2.93	216.1957	8.48	7734341
M238T4		2.93	238.1073	3.93	13721241
M446T8_2		3.02	446.2394	7.87	1030870
M331T11_1		3.05	331.2264	10.91	1351103
M345T6_1		3.06	345.0967	5.92	1167656
M933T12_1		3.11	932.5693	12.37	1154186
M387T4		3.24	387.1547	4.15	11309303
M514T12_1		3.27	514.3021	11.96	1054513
M548T11_1		3.30	548.2863	11.11	1956561
M941T13		3.36	940.5718	13.29	1392884
M465T10_2		3.37	465.2492	10.40	1370502
M363T4	Isosecosterigmatocystin	3.42	363.1072	4.49	1317672
M259T8_1	alternariol	3.44	259.0601	8.24	11582719
M317T5_1		3.46	317.1017	4.99	1673382
M317T11_1		3.46	317.0657	11.22	2373502
M919T13_2		3.60	918.5900	13.29	7911363
M440T12_3		3.73	440.2121	12.50	1267031
M441T11_2		3.75	441.1904	11.27	1145197
M504T10		3.95	504.2604	9.94	1065080
M459T14_7		3.97	459.3460	14.18	1291781
M403T13_1		4.04	403.1537	12.71	2261161
M239T12_1		4.19	239.0703	11.57	1345087
M473T10_3		4.35	473.1803	9.94	3910130
M421T12_2		4.37	421.1644	12.14	2132546
M438T7		4.53	438.2132	6.62	1270301
M203T11_2		4.59	203.1278	10.64	1064498
M343T8_1		4.61	343.0810	7.62	9984862
M439T10_3		4.68	439.1749	10.10	3720160
M510T10_2		4.69	510.3072	10.15	1151871
M455T10_1		5.34	455.1698	9.95	2210208
M394T7		5.40	394.0919	7.34	1738313
M771T11		5.52	770.5373	11.00	1806770
M439T12_2		5.53	439.1749	12.03	2144757
M497T4		5.73	497.1890	3.92	1017103
M339T9_1		5.77	339.0861	8.67	1217955
M249T10_2		5.82	249.1121	9.83	4425413
M387T10_5		5.92	387.2356	9.60	27510200
M278T2_1		6.20	278.1069	2.13	1076400
M592T11		6.24	592.3125	11.40	2565026
M425T11_3		6.24	425.1954	11.00	1319225
M339T12_2		6.44	339.1225	12.23	2653653
M405T12_2		6.52	405.1695	11.85	2343172
M327T13_2		6.62	327.1225	13.26	4118480
M371T12_2		6.78	371.1488	11.97	1071161
M439T12_1		6.83	439.1749	11.67	3601640

M441T15_1		6.87	441.1899	15.47	9779210
M423T14_1		6.95	423.1800	13.55	1898168
M355T15_2		6.98	355.1170	15.47	3130989
M263T8_1		6.99	263.1277	8.00	1341967
M423T15_1		7.07	423.1795	15.47	17270964
M440T13		7.20	440.1782	12.63	3133948
M421T15_2		7.21	421.1643	15.30	5458453
M423T11_1		7.40	423.1797	11.27	1168667
M441T13_4		7.40	441.1906	12.83	6926866
M351T14_2		7.63	351.1225	14.46	1057488
M473T12_1		7.78	473.1803	12.38	8263494
M905T13		7.85	904.5744	12.61	4301306
M421T13_2		7.85	421.1643	13.12	2412525
M387T14_3		7.89	387.1587	14.42	1853202
M421T10_2		7.93	421.1643	10.11	3721776
M301T14_1		7.96	301.0706	14.46	1033110
M717T8		8.10	717.1441	8.22	2180602
M421T15_1		8.24	421.1643	14.86	4185152
M339T12_3		8.40	339.1226	11.85	2783704
M353T12_1		8.60	353.1382	11.97	4226238
M421T13_1		8.63	421.1642	12.71	6216414
M425T15_1		8.63	425.1958	15.38	4025481
M423T12_3		8.72	423.1800	11.85	3494175
M337T10_1		8.74	337.1068	10.11	1469294
M285T12_1		8.78	285.0756	11.98	1827515
M421T14_1		8.84	421.1643	13.63	2127262
M425T14_2		8.87	425.1955	14.33	3570301
M425T15_3		8.90	425.1956	14.63	3049724
M405T15_1		9.01	405.1693	15.38	3945615
M387T15		9.02	387.1584	15.47	1651383
M407T15_3	shamixanthone or epishamixanthone	9.29	407.1851	14.99	8899095
M423T13_2		9.42	423.1801	12.51	3047515
M283T14_1		9.45	283.0600	14.46	1120154
M407T15_4	shamixanthone or epishamixanthone	9.46	407.1851	14.63	20764708
M423T12_2		9.61	423.1799	12.30	3848956
M423T13_3		9.75	423.1799	12.83	16944094
M355T10_2		9.87	355.1173	10.10	2522205
M425T15_2		9.97	425.1962	14.99	5205353
M321T12_1		10.39	321.1120	11.85	1083323
M373T13_1		10.43	373.1280	12.83	1534740
M373T15_1		10.47	373.1276	15.47	1101397
M355T13_1		10.47	355.1173	12.63	6231520
M421T12_1		10.58	421.1643	12.47	5218610
M405T14_1		10.86	405.1693	14.42	12450643

M356T13_1		11.15	356.1207	12.63	1348055
M323T13_2		11.20	323.1276	12.83	1994390
M455T12_2		11.27	455.1697	11.98	1427112
M654T13_2		11.52	654.3047	12.93	1893559
M349T14_1		11.73	349.1068	14.26	1777020
M455T12_1		11.98	455.1697	12.38	3801583
M339T15_2		12.99	339.1225	14.63	1614893
M202T4		Only in WT	202.4512	4.17	44320
M251T12		Only in WT	250.8823	11.67	50634
M255T13_1	chrysophanol	Only in WT	255.0651	13.03	1972494
M270T13_1		Only in WT	270.0761	13.38	321920
M285T11_1		Only in WT	285.0387	11.20	179424
M293T10_1		Only in WT	293.0211	9.60	117083
M302T14		Only in WT	302.0740	14.47	247158
M321T15		Only in WT	321.1112	14.63	306718
M322T12		Only in WT	322.1153	11.85	240909
M323T12_1		Only in WT	323.0913	12.08	334218
M324T13_1		Only in WT	324.0939	12.66	105254
M331T5_1		Only in WT	330.6144	4.81	27385
M333T15_1		Only in WT	333.1118	14.87	93410
M333T13_1		Only in WT	333.1119	12.53	203579
M333T12_1		Only in WT	333.1119	11.70	457091
M337T13_1		Only in WT	337.1067	12.83	569442
M337T13_2		Only in WT	337.1068	13.07	599018
M338T10_1		Only in WT	338.1102	10.11	416114
M339T14_2		Only in WT	339.1225	13.55	1216392

M339T15_1		Only in WT	339.1225	14.99	2286530
M339T11_1		Only in WT	339.1226	11.18	223231
M340T10		Only in WT	340.1259	10.04	210295
M341T13_2	Isoversicolorin C	Only in WT	341.0655	12.98	164984
M341T15_1		Only in WT	341.1019	15.00	91610
M342T13_2		Only in WT	342.1416	12.83	151328
M345T15_1		Only in WT	345.1110	15.48	72560
M347T13_1		Only in WT	347.1272	12.82	110401
M348T6_1		Only in WT	348.1438	6.44	68903
M349T15_1		Only in WT	349.1062	15.49	98625
M349T15_2		Only in WT	349.1431	14.63	421904
M350T14_1		Only in WT	350.1101	14.26	451218
M351T12_2		Only in WT	351.1225	11.70	1964963
M352T14_1		Only in WT	352.1258	14.46	237549
M353T15_2		Only in WT	353.1381	14.99	112012
M354T14_1		Only in WT	354.1051	13.56	176308
M356T10_2		Only in WT	356.1207	10.10	567571
M357T13_1		Only in WT	357.1234	12.63	183915
M357T15_2		Only in WT	357.1331	14.99	608208
M357T13_2	norsolorinic acid anthrone	Only in WT	357.1332	13.24	366322
M358T12_1		Only in WT	358.1365	12.08	102044
M363T10_1		Only in WT	363.1224	10.37	228242
M363T15_1		Only in WT	363.1225	14.51	85744

M365T8_1	Only in WT	364.7676	8.39	1317699
M365T14_2	Only in WT	365.1018	13.64	157009
M365T12_1	Only in WT	365.1018	12.06	260209
M365T13_2	Only in WT	365.1381	12.82	463196
M365T13_1	Only in WT	365.1382	13.45	221960
M365T12_2	Only in WT	365.1382	12.24	203985
M367T9_2	Only in WT	367.1174	8.58	252185
M367T12_2	Only in WT	367.1175	11.62	716366
M367T12_1	Only in WT	367.1175	12.47	536694
M367T13_1	Only in WT	367.1176	13.25	241959
M368T12_2	Only in WT	368.1209	11.61	187231
M368T10	Only in WT	368.1211	10.48	137859
M369T14_1	Only in WT	369.1331	14.47	498150
M369T8_2	Only in WT	369.1331	8.21	511459
M369T9_2	Only in WT	369.1332	9.23	753151
M370T13_1	Only in WT	370.1364	12.72	190559
M374T15_1	Only in WT	374.1310	15.47	158749
M380T12_1	Only in WT	380.1209	11.54	171296
M382T14_1	Only in WT	382.1366	14.26	70261
M383T13_2	Only in WT	383.1488	12.82	132902
M385T10_2	Only in WT	384.9565	10.33	238506
M385T14_2	Only in WT	385.1279	13.88	139212
M385T9_2	Only in WT	385.1281	9.12	813908

M387T6_1	Only in WT	387.1071	5.98	97649
M387T14_1	Only in WT	387.1224	13.57	95045
M387T14_2	Only in WT	387.1436	14.04	64838
M388T13_1	Only in WT	388.1470	12.72	71764
M389T15_1	Only in WT	389.1380	14.83	400173
M389T14_1	Only in WT	389.1381	13.77	121660
M392T15_1	Only in WT	392.1571	14.83	147472
M393T14_1	Only in WT	393.1329	14.48	94047
M394T15_1	Only in WT	394.1730	14.93	79042
M395T15_1	Only in WT	395.1487	14.59	78548
M396T7_1	Only in WT	396.0978	7.34	93665
M397T10_1	Only in WT	397.1279	9.94	335707
M397T8_1	Only in WT	397.1280	8.14	522006
M397T14_2	Only in WT	397.1280	13.62	164874
M397T11_1	Only in WT	397.1281	10.65	179853
M397T12_2	Only in WT	397.1281	11.55	225081
M398T12_1	Only in WT	398.1314	12.38	139426
M400T12_1	Only in WT	400.1471	11.93	169669
M403T9_2	Only in WT	403.1384	9.11	695716
M404T7_1	Only in WT	404.1338	7.32	104517
M404T15_1	Only in WT	404.1568	15.47	107310
M406T15_2	Only in WT	406.1727	14.93	1264109
M407T4	Only in WT	406.6296	4.15	210233

M407T15_1	Only in WT	407.1487	14.83	425752
M407T14_1	Only in WT	407.1488	13.77	182853
M407T12_1	Only in WT	407.1488	12.08	561046
M409T15_2	Only in WT	409.1913	14.99	416625
M409T15_3	Only in WT	409.1913	14.63	883130
M411T15_1	Only in WT	411.1799	14.93	113924
M413T12_1	Only in WT	413.1226	11.59	317874
M414T4	Only in WT	414.1143	4.15	157680
M414T2	Only in WT	414.1403	2.13	65900
M415T11_1	Only in WT	415.1387	10.97	1679265
M416T7_1	Only in WT	416.0737	7.34	158828
M417T9_1	Only in WT	417.1177	8.97	186349
M419T10_1	Only in WT	419.1335	10.00	173906
M419T14_1	Only in WT	419.1485	14.21	136420
M419T12_1	Only in WT	419.1486	12.40	201320
M421T11_1	Only in WT	421.1640	11.24	1654350
M422T12_1	Only in WT	422.1676	12.47	1447681
M422T12_2	Only in WT	422.1693	11.63	222005
M423T13_1	Only in WT	423.1436	12.61	155420
M423T12_1	Only in WT	423.1436	11.94	198825
M424T13_1	Only in WT	424.1833	12.52	969557
M424T12_2	Only in WT	424.1833	12.30	1172635
M424T12_1	Only in WT	424.1834	11.85	999052

M426T15_2	Only in WT	426.1989	14.62	884345
M430T4	Only in WT	430.1492	4.21	175477
M430T6	Only in WT	430.1493	5.72	113084
M437T12_1	Only in WT	437.1592	11.99	1172645
M440T15_2	Only in WT	440.1782	14.98	106314
M440T12_1	Only in WT	440.1783	11.68	1042433
M447T15	Only in WT	447.1775	15.48	263200
M451T14_2	Only in WT	451.1748	14.22	101978
M455T14_3	Only in WT	455.2060	14.16	75800
M463T15_2	Only in WT	463.1715	15.49	518766
M464T13_1	Only in WT	464.2064	12.82	259953
M469T12_1	Only in WT	469.1855	12.22	260855
M471T15_1	Only in WT	471.2011	14.73	178975
M471T13_1	Only in WT	471.2011	12.65	128788
M479T8	Only in WT	479.1674	8.48	227110
M480T10	Only in WT	480.1861	10.25	203396
M482T13_1	Only in WT	482.2170	12.82	147694
M487T12_2	Only in WT	487.1959	12.22	551037
M488T13	Only in WT	487.7590	13.29	120465
M489T12_1	Only in WT	489.1751	11.58	513120
M495T3	Only in WT	495.1332	3.29	41302
M496T6	Only in WT	496.2436	5.73	144874
M496T5	Only in WT	496.2437	4.93	292595

M499T4_1	Only in WT	499.1947	3.93	75447
M505T4	Only in WT	505.2743	4.50	79420
M532T5_2	Only in WT	532.2173	5.29	618985
M532T5_1	Only in WT	532.2173	4.72	963883
M535T8	Only in WT	535.1422	7.84	84486
M540T6	Only in WT	540.2201	5.67	88766
M544T6	Only in WT	544.1924	5.79	184850
M552T11	Only in WT	552.3683	11.17	123573
M555T7_1	Only in WT	555.2388	6.74	149934
M558T9_1	Only in WT	558.2319	8.93	178925
M561T14	Only in WT	561.4872	14.31	199280
M578T7	Only in WT	578.1653	7.48	88056
M579T14_2	Only in WT	579.3265	13.52	149952
M582T10_1	Only in WT	582.2474	9.99	117479
M583T9	Only in WT	583.1456	8.96	232271
M586T13	Only in WT	586.2423	12.92	224050
M592T4	Only in WT	592.1666	3.67	119707
M593T11	Only in WT	593.3160	11.41	1011523
M601T13_2	Only in WT	601.3421	12.59	143169
M607T9	Only in WT	607.3233	9.07	235863
M609T14	Only in WT	609.1022	13.58	139533
M611T14	Only in WT	611.1178	14.35	139878
M611T11	Only in WT	611.1179	11.22	448006

M616T10_1	Only in WT	616.1161	9.97	115488
M627T11_1	Only in WT	627.1126	10.74	281243
M639T8	Only in WT	639.2587	7.91	107111
M641T11	Only in WT	641.0919	10.74	210546
M644T10	Only in WT	644.1110	9.62	189916
M647T14	Only in WT	647.3542	13.69	114014
M651T7	Only in WT	651.0737	6.86	138871
M655T13_1	Only in WT	655.3080	12.93	744641
M656T6	Only in WT	656.1082	6.38	187395
M682T10_1	Only in WT	682.1264	10.24	241043
M686T11	Only in WT	686.1213	10.69	347629
M687T10	Only in WT	687.1333	10.38	149375
M696T8	Only in WT	696.1344	7.75	88415
M698T11	Only in WT	698.1212	10.54	285890
M702T9	Only in WT	702.1524	8.92	402355
M707T7	Only in WT	707.1361	7.46	219163
M709T12	Only in WT	709.1543	12.17	169301
M713T13	Only in WT	713.1855	13.21	70210
M719T8	Only in WT	719.1502	8.22	237937
M727T10	Only in WT	727.1647	10.40	213572
M727T13_1	Only in WT	727.1648	13.15	124772
M732T8	Only in WT	731.5096	7.51	342647
M733T8	Only in WT	733.1388	8.20	145205

M739T8	Only in WT	739.1257	8.23	229299
M741T10	Only in WT	741.1439	9.58	103397
M747T15	Only in WT	747.2426	14.98	90299
M750T12	Only in WT	750.4803	12.17	171965
M751T8	Only in WT	750.5086	8.40	788032
M755T13	Only in WT	754.5062	12.79	108659
M834T13	Only in WT	833.5364	12.51	123222
M845T13	Only in WT	845.3515	12.82	283977
M871T15	Only in WT	871.3651	14.63	202897
M903T13_1	Only in WT	902.5581	12.92	300783
M904T13_2	Only in WT	904.3577	12.82	176979
M909T10	Only in WT	909.4368	10.31	80194
M928T13	Only in WT	927.5593	12.61	442063
M967T10	Only in WT	967.3340	10.11	190111
M967T13	Only in WT	967.3350	12.64	315841
M1000T14	Only in WT	999.9141	13.52	60633
M1341T12	Only in WT	1340.9665	12.38	95592

Table S2-33. Secondary metabolites affected in the abundance in *A. flavus* Δ*nsdD*.

Metabolite ID	metabolite name	Log2FC	Median m/z ([M+H]-)	Median retention time	Maximum intensity
M623T2		-8.50	623.3221	2.29	2249091
M194T2_3		-7.96	194.1175	2.10	14866777
M349T7_3		-7.90	349.2372	6.81	39297400
M695T7_4		-7.81	695.3330	6.89	33629468
M180T2_3		-7.47	180.1382	2.10	4298009
M364T5_3		-5.76	364.1652	4.90	4732331
M350T7_1		-5.27	350.1497	6.90	4888573
M380T5_1		-5.25	380.1602	5.02	3964566
M416T11_4		-4.89	416.2328	10.61	1418835
M364T6_1		-4.56	364.1652	6.27	9090343
M402T6_2		-4.52	402.1420	5.88	1314207
M352T7_1		-4.44	352.1654	6.64	8843773
M364T6_2		-4.29	364.1652	5.81	2366134
M365T5_3		-4.20	365.2320	4.93	1086609
M348T7_1		-4.16	348.1704	6.89	174055648
M350T7_2		-4.10	350.1769	6.90	4888573
M319T2_3		-4.09	319.1728	2.26	1849245
M186T7_2		-4.07	186.1493	6.53	28862770
M380T6_3		-3.94	380.1602	5.87	9702164
M419T2		-3.92	419.1962	2.39	2985051
M327T3		-3.91	327.2065	2.87	3857458
M371T8		-3.85	371.1784	7.67	2885689
M180T2_1		-3.55	180.0880	2.11	4298009
M370T7		-3.34	370.1523	6.89	6329898
M299T2_4		-3.26	299.1753	1.59	4354093
M339T10_1		-3.13	339.1336	10.00	1134676
M300T2_1		-3.03	300.1595	2.08	2369888
M212T2_1		-2.96	212.0859	2.08	1503234
M194T2_2		-2.63	194.1035	2.11	14866777
M425T12_4		-2.62	425.3410	12.25	1599806
M352T11	Leporin B	-2.51	352.1905	11.18	2137933
M300T2_2		-2.44	300.1787	1.95	2555024
M194T3_2		-2.39	194.1035	2.60	2790770
M299T2_3		-2.32	299.1753	1.94	13024433
M352T9		-2.32	352.1903	9.35	1598217
M177T2_3		-2.22	177.1386	2.08	1338301
M326T2		-2.22	326.1624	2.10	7257931
M541T13		-2.19	541.4148	13.20	1915696
M334T6_1		-2.19	334.1548	5.91	3063606
M283T2_2		-2.18	283.1807	2.10	1948422
M208T2_3		-2.16	208.1193	2.33	30744026
M431T5_2		-2.12	431.1962	4.85	1111417

M493T10		-2.11	493.2803	10.14	1259154
M793T10		-2.11	793.3696	9.56	1418797
M341T10		-2.09	341.1494	10.21	3113312
M409T13_1		-2.08	409.2649	12.68	1361843
M353T8_3		-2.05	353.2107	8.02	1098411
M174T4_2		-2.05	174.1489	3.85	1561088
M170T5		-2.03	170.1539	5.26	9634653
M233T11_3		-2.01	233.1900	11.23	1009098
M510T10_2		-2.01	510.3073	10.14	2024626
M226T1_2		-1.93	226.1298	1.42	1760028
M188T5_2		-1.91	188.1644	5.26	46035836
M685T3		-1.89	685.3951	3.45	3247973
M309T12_3		-1.85	309.2060	11.50	1407396
M379T5		-1.84	379.2224	5.37	3420615
M651T2		-1.81	651.3169	2.10	2169215
M709T8_1		-1.78	709.3123	7.69	7818495
M212T2_2		-1.72	212.1140	2.10	1503234
M337T15_1	Cyclopiazonic acid	-1.70	337.1544	14.82	4288294
M309T7_5		-1.65	309.1596	6.94	4593456
M693T7		-1.63	693.3173	7.47	6271442
M725T9_2		-1.59	725.4222	9.40	28188646
M742T9_2		-1.52	742.4487	9.41	1531884
M344T10_1		-1.51	344.1386	10.40	2025263
M295T4_2		-1.50	295.1804	3.97	1033138
M433T4		-1.49	433.2118	3.88	2367139
M313T2_3		-1.48	313.1545	2.11	1924237
M725T11		-1.45	725.4877	11.47	1557043
M551T10_1		-1.39	551.3840	9.52	2668885
M741T12		-1.37	741.4828	12.23	4547388
M691T10_2		-1.32	691.3019	10.40	9095483
M691T9_1		-1.23	691.3018	8.55	14648388
M548T11		-1.16	548.2864	11.12	8376550
M1079T10		-1.15	1079.4903	9.66	1130312
M733T9_2		-1.15	733.3884	8.70	1837723
M336T6_1		-1.14	336.1340	5.70	1618529
M222T5_1		-1.12	222.1126	5.45	1085031
M711T9_2		-1.11	711.4065	8.70	2605785
M311T3_4		-1.10	311.1753	2.51	1047913
M747T9_3		-1.08	747.4042	9.42	6639795
M336T9_2		-1.07	336.1955	9.49	16334488
M340T11		-1.05	340.1906	11.15	5880605
M572T10		-1.04	572.2863	10.11	1599464
M346T9_1		-1.04	346.1542	8.66	2218446
M1257T9		-1.01	1256.5913	9.30	2218526
M173T10_2		Only in Δ*nsdD*	173.4526	9.63	166180

M282T12		Only in Δ*nsdD*	282.0843	12.07	155068
M324T2_1		Only in Δ*nsdD*	323.9626	1.90	9961
M347T7_7		Only in Δ*nsdD*	347.4790	6.90	133525
M368T7_1		Only in Δ*nsdD*	367.6455	6.89	406146
M378T5_2	Circumdatin J	Only in Δ*nsdD*	378.1446	5.41	171881
M412T6_2		Only in Δ*nsdD*	412.2672	6.06	191885
M435T2_1		Only in Δ*nsdD*	435.1059	2.11	261615
M437T2		Only in Δ*nsdD*	437.1039	2.12	122999
M449T2_1		Only in Δ*nsdD*	449.1213	2.11	371619
M451T2_1		Only in Δ*nsdD*	451.1195	2.11	159760
M470T7_1		Only in Δ*nsdD*	470.2046	6.82	143440
M473T3		Only in Δ*nsdD*	473.1064	3.25	155138
M623T12		Only in Δ*nsdD*	623.0755	12.07	155882
M645T7		Only in Δ*nsdD*	645.0152	6.84	104087
M675T3		Only in Δ*nsdD*	675.1978	3.27	118422
M695T7_2		Only in Δ*nsdD*	695.2975	6.74	171468
M698T7		Only in Δ*nsdD*	698.3420	6.90	649727
M717T7		Only in Δ*nsdD*	717.3149	6.90	927102
M781T2		Only in Δ*nsdD*	781.3469	2.02	65891
M870T7		Only in Δ*nsdD*	869.8948	6.85	95006
M372T12_1		1.00	372.3470	11.89	29944048
M510T9		1.04	510.2787	9.33	1477248
M243T7_1		1.05	243.0627	7.13	1043290
M741T9		1.06	741.4171	8.54	4408915
M457T1_1		1.06	457.1854	1.10	1745968
M461T9_3		1.07	461.3255	9.40	1349518

M457T7_1		1.07	457.1854	7.08	1356805
M221T7		1.08	221.0809	7.13	23061076
M308T5_1		1.10	308.1208	5.10	1378203
M443T10_2		1.11	443.3155	9.78	1423010
M249T7_1		1.11	249.1121	6.82	1864516
M373T12_3		1.17	373.3505	12.48	2198109
M307T5	(S)-(−)-6,8-di-O-methyl citreoisocoumarin	1.18	307.1174	5.11	7462174
M251T5_2		1.18	251.1617	5.45	1361533
M370T11_2		1.27	370.3313	11.02	1783442
M457T11_6		1.31	457.2945	10.91	3272716
M386T9_4		1.37	386.3261	9.02	2389852
M353T6_1		1.38	353.1688	6.07	1413831
M581T5	Imizoquin D	1.38	581.1888	5.10	1264146
M411T11_3		1.42	411.3255	10.98	1696608
M618T14_1		1.45	618.4205	14.09	3938953
M459T6		1.47	459.2923	5.55	1609461
M763T9_2		1.58	763.3989	8.54	3044610
M475T13_5		1.62	475.3414	13.41	1150400
M372T13_3		1.72	372.3470	13.03	3683651
M535T7		1.72	535.3085	7.07	1389521
M338T8_5		1.74	338.2403	8.06	1754927
M265T5_3		1.74	265.1070	5.11	4081857
M287T5_1		1.75	287.0888	5.35	1720268
M391T10_2		1.77	391.2992	9.50	1295208
M441T9_2		1.79	441.2996	8.80	1122769
M539T5_1		1.80	539.1784	5.33	1260844
M265T5_2		1.84	265.1070	5.35	19408578
M354T2_2		2.02	354.1809	2.11	1370783
M333T12_3		2.15	333.2423	12.16	1053381
M586T10_2		2.16	586.3804	9.91	2835000
M603T10_4		2.18	603.4070	9.91	1648205
M427T9_2		2.19	427.3206	9.50	1123226
M347T7_4		2.19	347.1962	6.52	1477601
M374T6_1		2.30	374.1472	6.08	4370424
M461T12_3		2.34	461.3258	11.85	2763166
M475T7_2		2.42	475.2874	6.51	2215672
M453T12_2		2.44	453.3354	12.25	1449761
M429T11_3		2.55	429.3361	10.98	1316619
M409T9_3		2.64	409.3098	9.50	2247244
M457T7_3		2.67	457.2767	6.50	1560809
M335T8_1		3.09	335.0258	7.96	2325472
M294T12_1		3.15	294.1546	11.84	1047021
M409T7_1		3.17	409.3098	7.48	1150038
M409T9_2		3.19	409.3098	9.10	7369927

M427T9_1	3.28	427.3204	9.11	7956289
M391T9_2	3.54	391.2990	9.10	4269479
M353T14_6	3.81	353.2795	13.85	1611667
M336T7_2	4.58	336.2028	7.36	5750099
M295T6_3	5.80	295.1441	6.06	149282096
M223T12	5.87	223.1440	12.04	1129254
M267T6_3	6.29	267.1491	6.04	1674143
M372T13_2	6.35	372.2742	12.68	6248744
M293T7_2	8.54	293.1284	6.99	2351363
M197T1	9.58	197.1645	1.49	1621017
M589T6	9.92	589.2803	6.04	6968820
M296T6_2	10.04	296.1028	5.99	29016000
M179T4_3	Only in WT	179.1544	3.64	88531
M179T1	Only in WT	179.1544	1.49	4316582
M208T8	Only in WT	208.1570	7.71	61067
M257T9_1	Only in WT	257.0387	9.09	128815
M296T6_1	Only in WT	295.6836	6.04	221896
M313T2_2	Only in WT	313.0229	2.06	46292
M350T9_5	Only in WT	350.2558	9.50	62231
M410T6_2	Only in WT	410.3132	5.73	78660
M462T6	Only in WT	461.6870	6.03	241665
M480T10	Only in WT	480.3682	9.87	74225
M495T2	Only in WT	495.2598	2.42	64601
M510T4	Only in WT	510.3419	4.09	62731
M510T5	Only in WT	510.3422	5.49	144499
M511T9	Only in WT	511.3990	8.76	97915
M537T6_2	Only in WT	537.4216	6.06	141679
M548T4_1	Only in WT	548.2249	4.43	97640
M562T14	Only in WT	562.4904	14.30	79878

M600T10_2	Only in WT	600.3959	10.32	89784
M640T10	Only in WT	640.4465	10.48	157547
M674T6	Only in WT	674.2354	6.02	194780
M693T10_4	Only in WT	693.4148	9.78	138629
M705T9_3	Only in WT	705.4571	9.16	133697
M722T7_2	Only in WT	722.4798	7.38	93730
M855T9	Only in WT	854.6364	9.11	400973
M872T9	Only in WT	871.6430	9.10	202856
M1101T6	Only in WT	1100.5565	5.96	129665

CHAPTER 3: Unraveling the Gene Regulatory Network of VeA and LaeA in *Aspergillus nidulans*

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3.1 Abstract

In the filamentous fungus *Aspergillus nidulans*, the Velvet family protein VeA and the global regulator of secondary metabolism LaeA play crucial roles in fungal development and secondary metabolism. VeA acts as a key light-responsive developmental regulator, which promotes sexual development but suppresses conidiation. In addition, it bridges VelB and LaeA in the absence of light to form the VelB-VeA-LaeA heterotrimeric velvet complex that regulates secondary metabolism and sexual development. LaeA is a global regulator of secondary metabolism as it is required for the proper expression of various secondary metabolite (SM) biosynthetic gene clusters and their corresponding SM production including mycotoxins. Along with metabolic regulation, LaeA also affects growth and asexual sporulation. The regulatory roles of these highly conserved proteins have been well identified, but the detailed mechanisms underlying these cellular and chemical regulations are not clearly elucidated yet. Thus, to unravel the gene regulatory networks of VeA and LaeA, we carried out network analyses on the transcriptome and protein-DNA interaction study integrating with the known *A. nidulans* protein-protein interaction data. In summary, VeA and LaeA play interdependent and distinct roles in governing fungal development and overall metabolism by directly regulating the expression of numerous genes involved in a variety of biological processes in *A. nidulans* vegetatively growing cells. The central parts of VeA- and LaeA-mediated gene regulatory networks are presented in the study.

3.2 Introduction

Fungi are of great importance in human lives and environments in that they play diverse roles in different aspects; the medical area (human pathogens and antibiotics producers), food industries (fermentation and process), agricultural fields (pathogens and growth aids), and environmental recycling. Most filamentous fungi reproduce mainly through asexual sporulation, which generates multicellular asexual reproductive organs and non-motile spores (Roper *et al*., 2010). Interestingly, in some fungi, this main reproductive system is tightly coupled with secondary metabolite production. Several studies in *Aspergillus* species have reported that developmental mutants defective in sexual and/or asexual developments coincidentally exhibited a loss of ability to produce mycotoxins such as sterigmatocystin and aflatoxin (Calvo *et al*., 2002; Bennett and Klich, 2003; Yu and Keller, 2005). This type of genetic link between development and metabolism has been observed in a variety of fungal species, but their full elucidation has not been established yet due to the complexity of gene regulatory networks (Calvo *et al*., 2002).

In the most ubiquitous fungi Aspergilli, few regulators govern development and metabolism at a bona fide upstream molecular level. These so-called global regulators directly and indirectly affect the expression of a vast array of genes including transcription factors that have roles in different biological functions and processes. Velvet family proteins (VeA, VosA, VelB, and VelC), LaeA, and NsdD are well-known global regulators of development and secondary metabolism in *A. nidulans* (Park and Yu, 2016; Bok and Keller, 2004; Lee *et al*., 2016). Among them, extensive studies have been done on VeA and LaeA since the 1960s. they have revealed that VeA is a key light-dependent developmental regulator that activates sexual development, yet represses asexual sporulation (Timberlake, 1990; Yager, 1992). Moreover, once VeA enters the nucleus in the dark, it physically interacts with other regulators and forms diverse complexes. These complexes including VelB-VeA-LaeA heterotrimeric complex play crucial roles in fungal development and secondary metabolism (Stinnett *et al*., 2007; Bayram *et al*., 2008). About two decades ago, Bok and Keller (2004) firstly identified LaeA through the mutagenesis screening of several mutants displaying loss of sterigmatocystin (ST) production but normal asexual sporulation. They elucidated that LaeA is required not only for the production of several secondary metabolites (SM) including ST, penicillin, and lovastatin but also for the proper expression of corresponding SM biosynthetic gene clusters. Despite the pivotal regulatory roles of VeA and LaeA in fungal biology having been well characterized, the detailed molecular mechanisms underlying how these upstream regulators govern fungal development and metabolism simultaneously are not clearly understood yet.

In this study, we elucidated the regulatory mechanisms of VeA and LaeA by integrating transcriptomic, protein-DNA interaction, and protein-protein interaction network analyses in *A. nidulans* vegetatively growing cells. Genome-wide direct and indirect target genes of VeA and LaeA were determined from RNA-seq and ChIP-seq analyses, and then they were subjected to the network analyses for constructing the VeA- and LaeA-mediated gene regulatory networks. Furthermore, we propose the core sections of these VeA- and LaeA-mediated networks demonstrating the central regulatory mechanisms of the two global regulators VeA and LaeA in the development and metabolism of *A. nidulans*.

3.3 Materials and Methods

3.3.1 *Aspergillus* strains and culture conditions

Aspergillus strains used in this study are listed in Table S3-1. The fungal strains were inoculated into liquid or solid 1% glucose minimal medium (GMM) with appropriate supplements and incubated at 37 °C. To collect vegetatively growing cells (Vege), 5×10^5 conidia/ml were inoculated into 100 ml liquid GMM and incubated for 24 hr at 37 °C, 220 rpm in the dark.

3.3.2 Construction of ***veA*** and ***laeA*** complemented strains

Sexual crosses of *A. nidulans* strains were conducted as described in Pontecorvo *et al*. (1953). The *laeA-FLAG* strain (TJW143) was created in TNO2A7 by inserting 3x FLAG-Afribo at C-terminus *laeA* ORF using modified double-joint PCR (Bok *et al*. 2013) consisting of the following: 1 kb DNA fragment upstream of the *laeA* stop codon (primers laeAFlag5F and laeAFlag5R), a 2.5 kb DNA fragment of 3x FLAG with *A. fumigatus ribo* (primers FlagF and FlagriboR) via a single joint PCR to fuse a 3x FLAG fragment from pHS13 (primers FlagF and FlagjointR) and *riboB* from *A. fumigatus* (primers FlagriboF and FlagriboR), and a 1 kb DNA fragment downstream of the *laeA* stop codon (primers laeAFlaf3F and laeAFlag3R). The first round PCR protocol started with initial activation of the Pfu Ultra fusion II polymerase (Agilent) at 95^o C for 3 min with hot start. For the next 36 PCR cycles, the temperature was held at 95^o C for 30 s denaturing, then ramped up to 58^o C for 30 s annealing, and to 68^o C for 2.5 min extension. Finally, a post extension step was performed at 68^o C for 10 min.The second round PCR utilized gel-purified DNA templates from the first round PCR with Pfu Ultra fusion II polymerase at 95^o C for 3 min with a hot start. For the next 12 PCR cycles, the temperature was held at 95^o C for 40 s denaturing, then ramped to 60^{o} C for 3 min annealing, and to 68^{o} C for 4 min extension. Finally, a post extension step was performed at 68^{o} C for 10 min. The third round PCR started with Expand long template PCR system (Roche) at 94^{o} C for 3 min. For the next 36 PCR cycles, the temperature was held at 94^{o} C for 20 s denaturing, then ramped to 59^{o} C for 30 s annealing, and to 68^{o} C for 4.5 min extension. Finally, a post extension step was performed at 68^{o} C for 10 min. 30 ml of G50 purified the third round PCR product was used for fungal transformation (Bok *et al*., 2013). *laeA-FLAG* transformants were confirmed by PCR (data not shown) and Southern blot (Fig. S3-1A) and one correct transformant, TJW143.2, was sexually crossed with RDIT2.1 to obtain the prototroph RJW302.11. The recombinants were confirmed by PCR (primers laeAFlagconfF and flagconfR, data not shown). Primers used in this study were listed in Table S3-2. The *veA-FLAG* strain (TJW191) was created in RJMP1.31 by inserting 3x FLAG-Afribo at C-terminus *veA* ORF using modified double joint PCR (Bok *et al*., 2013) as described in the *laeA-FLAG* tag construct consisting of the following: 1 kb DNA fragment upstream of the *veA* stop codon (primers veAflag5F and veAfalg5R), a 2.5 kb DNA fragment of 3x FLAG with *A. fumigatus ribo* (primers FlagF and FlagriboR) amplified from genomic DNA of *laeAFLAG* strain TJW143.2, and a 1 kb DNA fragment downstream of the *veA* stop codon (primers veAflag3F and veAflag3R). 30 ml of G50 purified the third round PCR product was used for fungal transformation (Bok *et al*., 2013). *veA-FLAG* transformants were confirmed by PCR (data not shown) and Southern blot (Fig. S3-1B) and one correct transformant, TJW191.2, was sexually crossed with RTMH207.13 to obtain the prototroph RJW324.3. The recombinants were confirmed with PCR (primers veAFlagconfF and flagconfR, data not shown). Primers used in this study were listed in Table S3-2.

3.3.3 Nucleic acid isolation and manipulation

The oligonucleotides used in this study are listed in Table S3-2. Genomic DNA isolation was carried out as described in Lee *et al*. (2017). A loopful of conidia (10^3-10^4/loop) from a solid culture were inoculated into 10 ml of liquid GMM on a sterile plate and incubated at 37 °C for 12-15 hr. Then semi-transparent mycelial mat was collected, squeeze-dried, and freeze-dried. Freeze-dried fungal tissues were ground by using a motor-spatula tool until they turn into a fine powder and high-quality genomic DNA was isolated. Genomic DNA and total RNA isolation were carried out as previously described (Seo *et al*., 2003; Park and Yu, 2012).

3.3.4 RNA sequencing analysis

Total RNA samples were submitted to Novogene company (Beijing, China) for sample quality check, library preparation, and mRNA sequencing. The quality of total RNA was validated thoroughly in multiple experimental confirmations using 1% agarose gel electrophoresis, Qubit 3.0 fluorometer (Thermo Fisher), and Agilent 2100 Bioanalyzer. During this step, RNA concentration (≥ 20ng/µl), purity (OD260/280 > 2.0), and integrating number (RIN ≥ 6.3) were verified to proceed to the library preparation. A strand-specific library was prepared using an Illumina TruSeq strand-specific RNA sample preparation system. The DNA library of 250-300 bp insert size was constructed and sequenced using an Illumina NovaSeq 6000 platform with a 150-bp paired-end sequencing strategy. Over 3.3×10^7 high-quality reads with 5×10^9 clean bases and less than 0.03% base error rate for all samples were achieved. The genome and gene annotations were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/; GCF_000149205.2 for *A. nidulans*).

Mapping of the clean reads to the genome was carried out using Hisat2 version 2.1 (Kim *et al*. 2019). More than 84.9% of total reads were mapped to the genome. Gene expression level was processed using FeatureCounts version 1.5.0 (Liao *et al*., 2013) and quantified as FPKM values covering all genes in each sample. For the differential expression analysis, DESeq2 version 1.6.3 (Love *et al*., 2014) was used to determine significantly differentially expressed genes. Briefly, genes were considered as differentially expressed genes (DEGs) when they exhibited an adjusted p-value of < 0.05 and more than two-fold changes of increase or decrease. The default parameter settings were used for programs unless indicated specifically.

3.3.5 Chromatin immunoprecipitation sequencing analysis

To collect samples for chromatin immunoprecipitation sequencing (ChIP-seq) analysis, vegetatively growing cells (24 hr) of RDIT9.32, RJW324.3, and RJW302.11 were cross-linked with 1% formaldehyde, resuspended in lysis buffer, and homogenized by a mini-beadbeater with 0.5mm zirconia/silica beads. The lysates were then sonicated for five to seven cycles (60 s on, 60 s off) with a sonifier to achieve 150-200 bp size DNA fragments (Jeong and Yu, 2012). After centrifugation, the lysates were diluted in ChIP dilution buffer and then were applied for ChIP assays according to the manufacturer's instructions using the MAGnify Chromatin Immunoprecipitation System (Invitrogen) with a modest modification. The diluted chromatin extracts were incubated with 1 μg of mouse monoclonal Anti-FLAG antibody (Sigma-Aldrich). As negative controls, the chromatin extracts were reacted with 1 μg of Anti-rabbit IgG. Initial input DNAs before immunoprecipitation were used as positive controls. The enriched DNA fragments were retrieved and used as a template for ChIP-seq.

ChIP DNA samples were sent to ProteinCT (Madison, WI) for library preparation and sequencing. DNA libraries were prepared using TruSeq ChIP library Preparation Kit (Illumina) and sequenced using an Illumina HiSeq2500 platform. More than 8 million reads per sample were achieved. The read sequences were mapped to the genome using bowties2 (Langmead *et al*., 2012) and Homer (version 4.11, Heinz *et al*., 2010) was utilized to call peaks. To make peak calls, high sensitivity settings were used: more than two-fold changes and a p-value less than 0.001. Identification of VeA and LaeA direct targets was done by selecting genes, in which peaks are found in their promoter regions within the 1.5kb upstream range from the translation start site (TSS). Then the response elements of VeA and LaeA were analyzed using the Homer *de novo* motif enrichment. To figure out the overlapping peaks between VeA and LaeA ChIP-seq data, the 'merge peaks' function of Homer was used. Then the overlapping peaks were analyzed using the Homer *de novo* motif enrichment to predict the VeA/LaeA common response elements.

3.3.6 Functional enrichment analysis

Gene Ontology enriched terms were identified using the tools available at FungiDB (Stajich *et al*., 2012). The parameters used in this study were biological process for the ontology, no limit to GO Slim terms, and a 0.05 p-value for the cutoff. Then enriched terms were sorted by p-values in ascending order.

3.3.7 Gene regulatory network analysis

We defined VeA- and LaeA-mediated gene regulatory networks as a protein-protein interaction network (PPIN), which consists of VeA or LaeA, its direct targets identified by overlapping ChIP-seq and RNA-seq analyses, and its putative direct target genes identified from ChIP-seq exclusively. The known protein-protein interaction information of *A. nidulans* was obtained from the gene network database STRING (version 11.5, Franceschini *et al*., 2013), by matching the protein ID and gene ID using the 'protein.aliases' table provided by the database.

We remained only the edges supported by protein-protein interaction from the whole STRING network with a threshold for confidence score of 150. Then we selected out the edges which have both nodes belonging to either the direct and/or putative direct target genes. These nodes and edges were used to construct a gene regulatory network. To investigate a core section of a network, we analyzed the network by applying the “guilt-by-association (GBA)” principle, which was fulfilled by examining the first neighbors of the gene/protein of interest in the network. For the visualization of the core networks, direct targets were represented in a rectangle shape and putative direct targets were represented in an ellipse shape. Then shapes were colored based on their functional categories; vegetative growth (pale green), asexual development (green), sexual development (deep saffron), primary metabolism (blue), secondary metabolism (magenta), and transcription regulation (red). The network visualization was performed using Cytoscape software (version 3.9.1, Shannon *et al*., 2003).

3.4 Results

3.4.1 VeA- and LaeA-mediated gene regulation in *A. nidulans* Vege

To understand the regulatory roles of VeA and LaeA in *A. nidulans*, we carried out genome-wide gene expression analyses in WT and null mutants' vegetative cells (24 hr). Totals of 29.74% (3,268/10,988) and 21.25% (2,338/10,988) of genes were differentially regulated in the Δ*veA* and Δ*laeA* mutant Vege, respectively (Table 3-1). Among the differentially expressed genes (DEGs), 39% and 39.7% were up-regulated and 61% and 60.3% were down-regulated in Δ*veA* and Δ*laeA* Vege, respectively, suggesting that VeA and LaeA tend to activate the expression of genes.

To gain an understanding on the functional roles of VeA and LaeA, functional category enrichment analyses were performed utilizing Gene Ontology (GO) terms (Table 3-2). The results of the GO analyses demonstrated that in Δ*veA* Vege, primary metabolic processes such as translation, peptide metabolic process, cellular amino acid metabolic process, cellular nitrogen compound biosynthetic process, and cellular amide metabolic process were up-regulated, while secondary metabolic processes including phenol-containing compound, organic heteropentacyclic compound, melanin, monodictyphenone, and toxin were down-regulated. These results suggest that VeA plays an activating role in secondary metabolism, but negatively regulates primary metabolism. Some similar and distinct regulatory patterns were observed in the Δ*laeA* Vege. Down-regulated genes in Δ*laeA* Vege were mostly related to secondary metabolic processes including austinol, ketone, and alkaloid metabolic processes similar to the case of VeA. On the other hand, up-regulated genes in Δ*laeA* were implicated in transmembrane transport including xenobiotic, carbohydrate, and amide transport, some secondary metabolic processes, and nitrate metabolic processes. Taken together, these results imply that both VeA and

LaeA positively regulate secondary metabolism yet repress some primary metabolism-related processes in an analogous manner in *A. nidulans* Vege.

3.4.2 Identification of potential direct targets of VeA and LaeA in Vege

To identify the direct target genes of VeA and LaeA, ChIP experiments followed by high-throughput sequencing were carried out using anti-FLAG antibody and strains that express FLAG epitope-tagged VeA and LaeA (Fig. 3-1). Totals of 3,190 and 1,834 genes were identified as VeA and LaeA peak-associated genes, respectively (determined as putative direct targets in this study). To identify the VeA and LaeA response elements (VRE and LRE), totals of 5,502 and 3,333 peaks were subjected to the Homer *de novo* Motif elicitation analysis, respectively (Fig. 3-2). Interestingly, we observed a high similarity between the predicted VREs and LREs. The top two predicted VREs were 5'-GTCACGTGAC-3' (6.14% of input peaks with a p-value of $1e^{-81}$) and 5'-TGATTGGCTG-3' (16.52% of input peaks with a p-value of $1e^{-65}$) (Fig. 3-2A) and the top two LREs were 5'-TGATTGGCTG-3' (10.83% of input peaks with a p-value of $1e^{-39}$) and 5'-GTCACGTGA-3' (9.39% of input peaks with a p-value of $1e^{-35}$) (Fig. 3-2B). They commonly had 5'-TGATTGGCTG-3' and 5'-TCACGTGA-3'. Thus, we decided to perform an additional *de novo* Motif elicitation analysis on the overlapping peaks between VeA and LaeA to determine whether both response elements are conserved binding motifs of the VeA-LaeA complex. A total of 1,186 overlapping peaks was subjected to the Motif elicitation analysis and the top two predicted common response elements of VeA and LaeA were 5'-TGATTGGCTG-3' (26.31% of input peaks with a p-value of $1e^{-47}$) and 5'-TCACGTGAC-3' (14.92% of input peaks with a p-value of $1e^{-40}$), suggesting that both 5'-TGATTGGCTG-3' and 5'-TCACGTGA-3' are binding motifs of VeA and LaeA and they may recognize these sequences as a VeA-LaeA complex in *A. nidulans* Vege.

Then the results of the ChIP-seq and RNA-seq analyses were compared to identify direct target genes of VeA and LaeA. We identified totals of 978 (8.9% of 10,988 genes) and 418 (3.8% of 10,988 genes) direct target genes that were bound by VeA and LaeA in their promoter regions and were differentially expressed in Δ*veA* and Δ*laeA*, respectively (Fig. 3-1A and B). Furthermore, common direct targets between VeA and LaeA were identified by comparing their direct target genes; 178 genes were co-regulated by VeA and LaeA. Interestingly, 93.26% (166/178) of these common targets were regulated by VeA and LaeA in the same pattern: 63 genes were up-regulated, and 103 genes were down-regulated in both deletion mutants, whereas only 6.74% (12/178) of them displayed an opposite regulation trend between VeA and LaeA (Fig. 3-1C). These results may indicate that VeA and LaeA directly regulates these 166 common target genes as a complex. The whole direct target genes and common targets of VeA and LaeA are listed in Table S3-3, 4, and 5, respectively.

To further investigate the roles of direct targets of VeA, LaeA, or both in *Aspergillus* biology, functional category analysis was carried out by determining GO terms (Table 3-3). Among the VeA direct target genes, several genes involved in translation, peptide metabolism, cellular nitrogen compound biosynthesis, and amide biosynthesis were up-regulated, whereas the expression of some genes related to glycogen metabolism, cellular glucan metabolic process, syncytium formation, and cleistothecium formation was decreased in Δ*veA*. These results suggest that VeA directly activates polysaccharide metabolic processes, syncytium formation, and sexual structure formation, yet negatively regulates primary metabolic processes including translation, peptide, amide, and nitrogen. In *A. nidulans* Δ*laeA*, the expression of LaeA direct target genes associated with cellular response to diverse stressors, carbohydrate transmembrane transport, glycolysis including NADH regeneration were enhanced, but the mRNA levels of some LaeA direct target genes involved in syncytium formation, anatomical structure formation, and diverse regulations such as cell differentiation, asexual sporulation, and sexual development were decreased. These results indicate that LaeA directly activates syncytium formation and regulations of asexual and sexual developmental processes, while it directly represses stress responses, carbohydrate transmembrane transports, and some processes in glycolysis. The up-regulated VeA/LaeA common target genes in both Δ*veA* and Δ*laeA* were associated with cellular response to osmotic stress, regulation of defense response, modulation by symbiont of host defense response, and purine-containing compound metabolism. However, the down-regulated common targets in both Δ*veA* and Δ*laeA* were predicted to regulate syncytium formation, cell-to-cell fusion, regulation of cell differentiation and developmental process, glycogen biosynthesis, manganese ion homeostasis, and RNA interference implying that by acting in concert, VeA and LaeA directly activate syncytium formation, manganese ion homeostasis, glycogen biosynthesis, RNA interference, and regulation of developmental processes, whereas they directly inhibit purine-containing compound biosynthesis and cellular stress and defense responses.

3.4.3 VeA-mediated gene regulatory network

Previous studies have characterized diversified regulatory roles of VeA and LaeA in development and secondary metabolism, however, their regulatory mechanisms are not clearly identified yet. To elucidate the detailed regulatory mechanisms of VeA and LaeA, the network analysis was conducted by integrating the results of ChIP-seq and RNA-seq, and the protein-protein interaction database.

From the ChIP-seq and RNA-seq analyses, the 978 direct target genes and 2,212 putative direct targets of VeA were selected out and then overlaid with the *A. nidulans* PPI database, which led to the formation of VeA regulatory network consisting of 2,210 nodes (either genes or proteins) and 83,844 edges (interactions between nodes). To elicit the core section of the VeA-mediated GRN, we analyzed the first neighbors of VeA within the network and figured out that the core section is composed of 8 direct targets and 22 putative direct targets including several well-known genes encoding developmental and metabolic regulators of *Aspergillus* such as *flbA·B·C*, *velB·C*, *mpkB*, *laeA*, *areA*, and *hogA* (Fig. 3-3; Table 3-4).

In *A. nidulans*, the *fluG*, *flbA*, *flbB*, and *flbC* are required for the proper transition from hyphal growth to conidiophore development (Wieser *et al*., 1994). They act as upstream activators of *brlA*, which encodes C_2H_2 zinc finger TF initiating the development of conidiophore (Adams *et al*., 1988; Chang and Timberlake, 1993). Moreover, the *lreB* (light response) gene, encoding a putative zinc-finger transcription factor, is involved in the morphological and physiological differentiation of *A. nidulans*. The LreB is known to interact with VeA, FphA, and LreA and form a large protein complex in the nucleus, which plays a crucial role in red- and blue-light responses (Purschwitz *et al*., 2008). These results suggest that VeA regulates asexual development by controlling the upstream regulators of conidiation and interacting with blue- and red-light sensors. Furthermore, some VeA direct targets such as *velC*, *steA*, and *esdC* are necessary for proper sexual development. The deletion of *velC*, one of the Velvet family genes, resulted in reduced production of sexual fruiting bodies (cleistothecia), whereas the overexpression of *velC* led to enhanced formation of cleistothecia (Park and Yu, 2016). The *steA* null mutant exhibited the complete absence of cleistothecium production (Vallim *et al*., 2000). The *esdC* (early sexual development) gene is necessary for proper sexual fruiting body formation, however, its overexpression does not enhance this process. Throughout development, VeA is known to positively regulate the expression of *esdC* (Han *et al*., 2008). Along with the roles of these genes, the expression of *velC*, *steA*, and *esdC* was all down-regulated in Δ*veA* Vege from the RNA-seq analysis, implying VeA activates sexual development by positively regulating several genes involved in the sexual structure formation of *A. nidulans*. Moreover, *kapA*, *velB*, and *laeA* genes appeared in the core network of VeA. KapA is the importin-α, which mediates the nuclear import pathway of the VeA-VelB heterodimer (Stinnett *et al*., 2007). Once the VeA-VelB complex enters the nucleus, VeA interacts with LaeA and forms the VelB-VeA-LaeA heterotrimeric complex in the dark; this complex plays crucial roles in not only sexual development but also secondary metabolism (Bayram *et al*., 2008). Similarly, MpkB, a mitogen-activated protein kinase, affects diverse biological processes: germination and conidiation through regulating the expression of *vosA* and *brlA* as well as secondary metabolism through controlling the expression of *laeA* and secondary metabolism gene clusters including *aflR* and *tdiB* (Kang *et al*., 2013; Atoui *et al*., 2008). Interestingly, a large portion of the core network was composed of genes involved in primary metabolism. They are associated with various primary metabolic processes: gluconeogenesis/glycolysis (*gpdA*), trehalose metabolism (*tpsA*, *treA*, *treB*, *orlA*), amino acid metabolism (*metG*, *glnA*), and nitrogen metabolism (*areA*, *nmrA*). Among these genes, *areA* is the only gene encoding a GATA-type transcription factor, suggesting that VeA directly controls primary metabolic processes. The *hogA* gene encodes a mitogen-activated protein kinase, which plays vital role in the high-osmolarity glycerol (HOG) response MAPK signaling pathway. In response to fludioxonil and osmotic stress, HogA activates the expression of *atfA* gene encoding a bZip-type transcription factor, which in turn alters the expression of numerous downstream genes involved in osmotic stress and fludioxonil responses (Hagiwara *et al*., 2009). Taken together, the network analysis of the VeA-mediated GRN demonstrates that VeA governs *Aspergillus* biology by directly regulating specific key genes of conidiation, sexual development, primary metabolism, and secondary metabolism.

3.4.4 LaeA-mediated gene regulatory network

To perform the network analysis on LaeA, the 418 direct target genes and 1,416 putative direct targets of LaeA were selected out from the RNA-seq and ChIP-seq analyses. Then they were combined with the *A. nidulans* PPI database, resulting in the formation of LaeA regulatory network consisting of 1,206 nodes and 24,334 edges. We elucidated the core section of LaeA-mediated GRN in the same way with the VeA network analysis described above; in the core section, we found 9 direct targets and 13 putative direct target genes including *flbA*, *flbC*, *kapA*, *trxA*, *veA*, *velB*, *velC*, *stuA*, *niiA*, *hogA*, and *pkaR* (Fig. 3-4; Table 3-5). In the core network of LaeA, the 10 genes, *flbA*, *flbC*, *kapA*, *velB*, *velC*, AN5055, *gpdA*, *tdiB*, *hogA*, and *pacC*, did also appear in the core network of VeA. This suggests that VeA and LaeA as a complex may co-regulate these key genes of development and metabolism in *A. nidulans*.

The *trxA* gene encodes thioredoxin A containing a thioredoxin active site motif (WCGPC). By working together with the thioredoxin reductase (TrxR), thioredoxin A plays a significant role in redox regulation of *A. nidulans*. These thioredoxin systems not only coordinate protein disulfide reduction, sulfur assimilation, detoxification of reactive oxygen species, and redox regulation of enzymes but also affect the growth and development of *A. nidulans* (Thön *et al*., 2007). The StuA (stunted) is a transcription activator classified as a spatial modifier of conidiophore morphogenesis in *A. nidulans*. The deletion of *stuA* resulted in the production of greatly shortened conidiophores with a lack of normal metulae and phialides (Miller *et al*., 1992). The effect of StuA in secondary metabolism of *A. nidulans* has not been characterized yet, but in other fungi including *A. fumigatus*, StuA orthologs regulate the expression of secondary metabolite biosynthetic genes, suggesting a similar role of StuA in the regulation of secondary metabolism in *A. nidulans* (reviewed in Yin and Keller, 2011). The *imeB* gene encodes a protein kinase, which is necessary for light-mediated inhibition of sexual development and mycotoxin production. The *imeB* null mutant exhibited reduced growth but greatly enhanced production of fertile cleistothecia under the light condition where the sexual development usually does not occur. Moreover, ImeB is required for the proper expression of the sterigmatocystin gene cluster (Bayram *et al*., 2009). In addition to the genes involved in *Aspergillus* development, several genes associated with primary and secondary metabolism were found in the core network of LaeA: *gpdA* (gluconeogenesis/glycolysis), *niiA* (nitrogen), *afoA* (asperfuranone), *ausA* (austinol/dehydroaustinol), *aatA* (penicillin), and *tdiB* (asterriquinone). Furthermore, some genes function in the upstream level of diverse biological processes such as *hogA*, *pkaR*, *nkuA*, and *dot1*. HogA and PkaR are critical components of HOG MAPK and PKA pathway, respectively. NkuA is the homolog of the human KU70 that is essential for DNA nonhomologous end-joining during double-strand break repair. As the deletion of *nkuA* drastically reduces the frequency of nonhomologous integration of designated DNA fragments during fungal transformation, it is widely used for gene targeting techniques (Nayak *et al*., 2006). The function of *dot1* gene is not identified in *A. nidulans* yet, but Liang *et al*. (2017) revealed that the Dot1 in *A. flavus* has a H3K79-specific histone methyltransferase activity that plays a vital role in heterochromatin formation, which in turn affects development, aflatoxin production and virulence. Put together, these results demonstrate that LaeA directly controls the expression of upstream genes in development, metabolism, and general transcription regulation, enabling the regulation of diverse biological processes in *A. nidulans*.

3.4.5 Gene regulatory network co-regulated by VeA and LaeA

Although previous studies have elucidated that VeA acts as a bridge between VelB and LaeA to form the VelB-VeA-LaeA heterotrimeric complex in the nucleus and this complex plays crucial regulatory roles in *Aspergillus* development and secondary metabolism (Bayram *et al*., 2008; Atoui *et al*., 2010; Reyes-Dominguez *et al*., 2010), the detailed molecular mechanisms of action have yet to be uncovered. To understand the regulatory mechanisms of VeA-LaeA complex, we decided to figure out the common direct targets of both VeA and LaeA in this study. Although the common target genes of VeA and LaeA may vary from actual direct targets of the VeA-LaeA complex, we speculated that they represent the actual targets of the complex with high probability based on the RNA-seq, ChIP-seq, and motif analyses of the present study. The network analysis was performed by integrating the ChIP-seq data of VeA and LaeA and PPI database. Total of 1,084 putative common direct targets were selected out of overlapping 3,190 and 1,834 putative direct target genes of VeA and LaeA, respectively. These putative common targets then were overlaid with the *A. nidulans* PPI database, resulting in the formation of the gene regulatory network co-regulated by VeA and LaeA (called as the common network afterward) consisting of 693 nodes and 9,467 edges. To elucidate the core section of the common network, the same method from the previous analyses was applied and we found 21 genes including 6 direct target genes and 15 putative direct targets (Fig. 3-5; Table 3-6). As we combined two different networks here, the definition of 'direct target' and 'putative direct target' may slightly differ from previous ones; when genes are a direct target of both VeA and LaeA, they are determined as a direct target in the common network, otherwise, they are a putative direct target.

In the core section of the common network, the genes, *flbA*, *flbC*, *velB*, *velC*, *kapA*, AN5055, *gpdA*, *tdiB*, *pacC*, and *hogA*, surrounding VeA and LaeA were found in both VeA and LaeA core networks. The 5 genes displayed at the top, *flbB*, *esdC*, *treA*, *treB*, and *nmrA*, were found in the VeA core network and the 6 genes displayed at the bottom, *trxA*, *niiA*, *png1*, *aatA*, *nkuA*, and *dot1*, appeared in the LaeA core network. Put together, these results suggest that VeA and LaeA may be able to function individually, but they tend to act as a complex in *A. nidulans* Vege.

3.5 Discussion

The conserved upstream regulators VeA and LaeA govern developmental and metabolic processes in *Aspergillus* species. The regulatory roles of VeA and LaeA have been extensively studied so that researchers now have a better understanding on how important these regulators are in *Aspergillus* biology, however, we still have a lack of knowledge on their regulatory mechanisms. Although previous studies revealed that VeA and LaeA form a complex together and regulate the expression of various downstream genes in the nucleus, their genome-wide target genes, fundamental elements for elucidating the regulatory mechanisms, have not been identified yet. In this study, we proposed the direct and putative direct targets of VeA and LaeA and their gene regulatory networks for the first time by integrating RNA-seq, ChIP-seq, and PPI analyses in *A. nidulans* Vege.

The *veA* and *laeA* genes and their respective proteins are constitutively expressed during vegetative growth. However, the localization pattern of the proteins is distinct; LaeA is constitutively nuclear, while VeA is mostly localized in the nucleus in the dark and partially nuclear in the light (Bayram *et al*., 2008; Bayram *et al*., 2010). Thus, we expected that most of expressed LaeA and VeA are in the nucleus, interact with each other to form the VelB-VeA-LaeA heterotrimer complex, and actively regulate downstream genes in our 24 hr Vege samples grew in the dark. Although we presumed that Vege 24 hr is the most representative condition for investigating the targets of VeA and LaeA, the target genes of VeA-LaeA complex may be variable to some extent depending on the cell types such as asexual and sexual cells and the growth conditions such as time and light. To understand the target gene information of VeA and LaeA in other conditions, further studies will be needed.

Our functional enrichment analyses suggested not only consistent results with previous findings of the regulatory roles but also novel characteristics of VeA and LaeA. Enriched terms of DEGs suggested the broad effects of VeA and LaeA in *Aspergillus* biology; most down-regulated genes in Δ*veA* and Δ*laeA* Vege were associated with secondary metabolite biosynthesis, whereas most up-regulated genes in Δ*veA* and Δ*laeA* were related to primary metabolism (Table 3-2). To dissect the direct regulatory roles of VeA and LaeA, we performed GO term analyses on direct targets of these proteins and obtained interesting results. Similar to the results from those of DEGs, the up-regulated direct target genes in Δ*veA* and Δ*laeA* were associated with mostly primary metabolic processes. However, the direct targets down-regulated in Δ*veA* were involved in the growth, sexual development, and metabolic processes of complex polysaccharides and the direct targets down-regulated in Δ*laeA* were associated with the regulation of asexual and sexual developmental processes (Table 3-3). First, these results indicate that VeA and LaeA may play a crucial role in primary metabolism as well. Previous studies focused on their functions in development and secondary metabolism without knowing whether these processes are likely to be affected by the alteration of primary metabolism. Thus, further studies are needed to understand the relationship between primary metabolic changes and the known cellular and chemical phenotypes in *A. nidulans* Δ*veA* and Δ*laeA*. In addition, LaeA is generally recognized as a global regulator of secondary metabolism in *A. nidulans*, but the effect of LaeA in fungal development can be as significant as the role of LaeA in secondary metabolism. In *A. nidulans*, the Δ*laeA* strain showed a similar radial growth on solid media but exhibited greatly reduced conidia production in the light and enhanced cleistothecia formation in the dark compared to the wild type (Bayram *et al*., 2010) and in other *Aspergillus* species, the roles of LaeA in development are also well studied (Bok *et al*., 2005; Amaike and Keller, 2009).

Consistent with the results from the GO term analyses, these results suggest that LaeA is an upstream regulator of *Aspergillus* development. Furthermore, the VeA/LaeA common direct targets up-regulated in both Δ*veA* and Δ*laeA* were mostly associated with stress responses. Baidya *et al.* (2014) revealed that VeA plays an important role in the regulation of oxidative stress response in *A. flavus*. Taken together, these results suggest that *A. nidulans* VeA may also be important in the response to environmental stresses.

The renowned gene network database STRING provides known PPI information of all proteins in *A. nidulans*. The definition of PPI was limited to physical interaction in the early 2000s, however, it has been more inclusive as defined through seven evidence channels: experiments, database, text mining, gene coexpression, neighborhood, fusion, and co-occurrence. The STRING database generated a combined score for interaction by measuring all these channels so that it refers to all types of interactions such as protein-protein, protein-DNA, and gene-gene (Szklarczyk *et al*., 2021). The gene regulatory networks proposed in this study were defined as a protein-protein interaction network (PPIN). Utilizing a PPIN is more suitable to perform genome-wide network analysis, although it does not generally indicate directionality of regulation between genes (or proteins) and a type of proteins such as transcription factor, unlike a traditional gene regulatory network. From the STRING database, we obtained 67 and 75 reported PPIs that VeA and LaeA have with other genes or proteins in *A. nidulans*, respectively. These numbers of PPIs merely constitute less than 1.2% of the VeA and LaeA interactions that we are proposing in this study (Fig. S3-2), demonstrating that the regulatory mechanisms of VeA and LaeA have been poorly studied and the present study provides valuable information filling the gap in research. Through the network analyses, we found that VeA and LaeA directly regulate multiple upstream regulators that govern all different biological functions: development, metabolism, and transcription regulation such as MAPK and PKA pathways. These results may provide possible explanations at the genetic level for the previous cellular and chemical phenotypic alterations that occurred by the deletion of *veA* and *laeA* genes in *A. nidulans*. For example, VeA is known to positively regulate sexual development in that the deletion of *veA* resulted in the absence of sexual fruiting body formation, even under sexual development-promoting conditions, while the overexpression of *veA* increased the production of cleistothecia (Kim *et al*., 2002). The core section of the VeA network displayed that *esdC*, *steA*, *velC,* and *mpkB* genes necessary for the proper sexual development are direct targets of VeA and they all are down-regulated in Δ*veA* Vege, demonstrating that VeA regulates sexual development by playing an activating role in the expression of genes necessary for sexual development. In addition, the core networks shed light on the regulatory roles of VeA and LaeA in primary metabolism, which have not been thoroughly characterized yet. To further understand these roles, primary metabolite analysis is needed to be performed. Moreover, although most genes associated with primary metabolism in the core networks were well-characterized, AN5055 and AN5199 are not identified yet even though they seem to play an important role in VeA- and LaeA-mediated primary metabolism. AN5055 and AN5199 genes are predicted to encode methionine aminopeptidases, which show 66% and 69% similarity of amino acid sequences with human methionine aminopeptidase 1 (MetAP1), respectively, and share 73% similarity with *Saccharomyces cerevisiae* MAP1, but not similar with MetAP2 and MAP2. MetAPs cleave the initiator methionine residue from newly synthesized proteins and thus are essential components of the N-terminal methionine excision pathway. This posttranslational modification is known as an essential process conserved from eubacteria to higher eukaryotes. Deletion of both *MAP1* and *MAP2* is lethal in yeast, while Δ*map1* yeast exhibited greatly slow growth and Δ*map2* yeast displayed no phenotypic change (reviewed in Upadhya *et al*., 2006). Orthologs of AN5055 and AN5199 were found in 71 and 78 *Aspergillus* species with over 59.84% and 67.52% identity from the NCBI protein blast search, respectively, validating that these genes are highly conserved in Aspergilli. Given the foreseen crucial role and prevalence of AN5055 and AN5199 in *Aspergillus* species and other fungi (data not shown), understanding the actual roles of these genes in fungal development and metabolism will improve our knowledge of fungal biology.

In conclusion, this study unravels the VeA- and LaeA-mediated gene regulatory networks and their regulatory mechanisms. In Vege, VeA and LaeA directly regulate the expression of genes encoding upstream regulators of diverse biological processes including development and metabolism and these upstream regulators control downstream genes, thus inducing an array of cellular and chemical developmental traits in *A. nidulans*. These results provide an advance in the knowledge of the global regulators VeA and LaeA by filling the gap in understanding of the regulatory mechanisms of VeA- and LaeA-mediated regulations in *A. nidulans* and will provide insights into other *Aspergillus* species.

3.6 References

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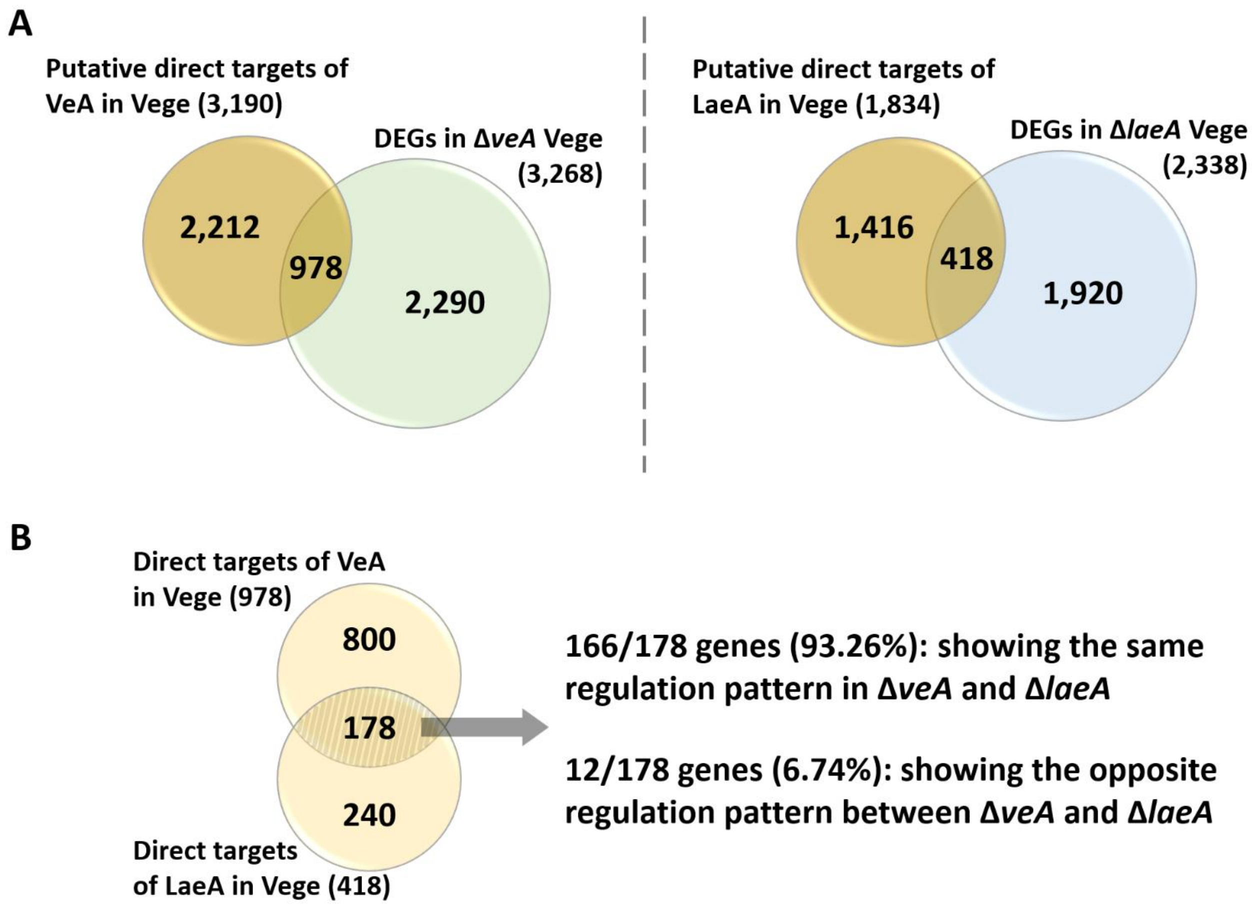

Figure 3-1. Identification of VeA and LaeA direct targets in *A. nidulans* Vege. (A) The Venn diagram displays the number of putative direct targets of VeA and DEGs in Δ*veA* Vege. The overlapped part in the Venn diagram indicates the number of direct targets of VeA. (B) The Venn diagram displays the number of putative direct targets of LaeA and DEGs in Δ*laeA* Vege. The overlapped part in the Venn diagram indicates the number of direct targets of LaeA. (C) The Venn diagram depicts the number of overlapped direct targets of VeA and LaeA.

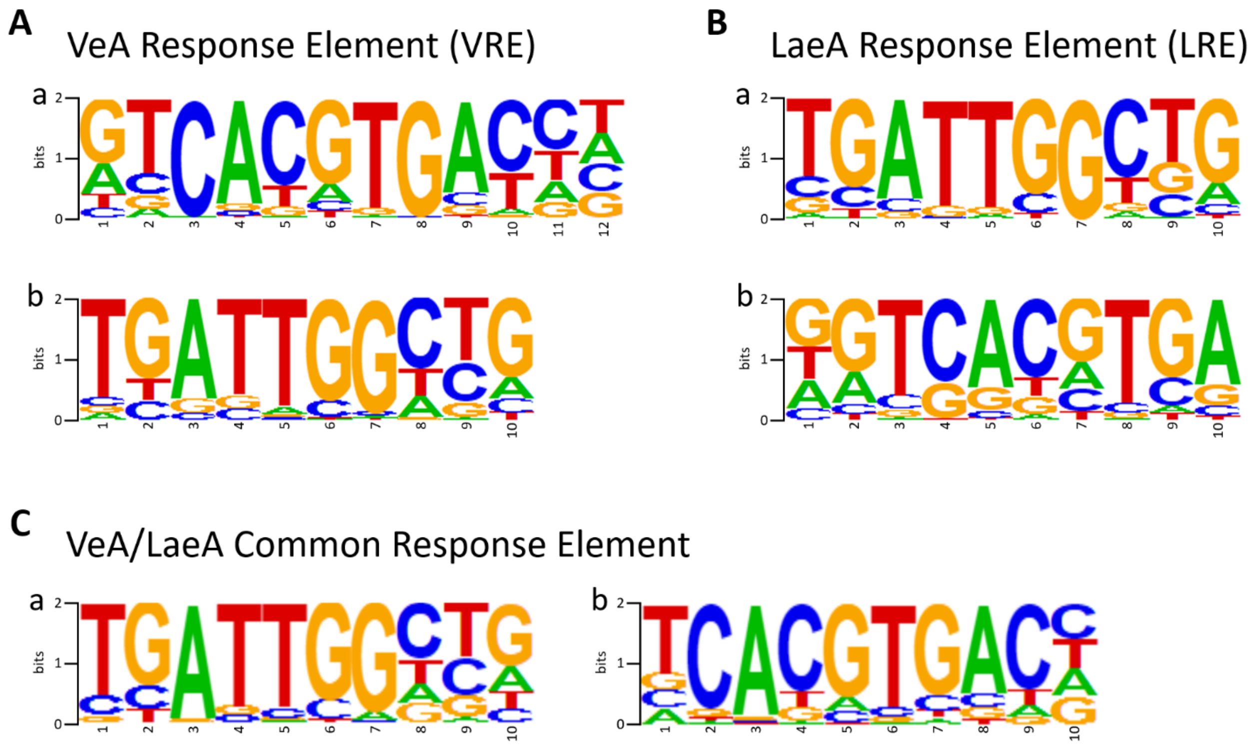

Figure 3-2. Identification of VeA and LaeA response elements. (A) VeA response elements (VREs) are 5'-GTCACGTGAC-3' and 5'-TGATTGGCTG-3'. (B) LaeA response elements (LREs) are 5'-TGATTGGCTG-3' and 5'-GTCACGTGA-3'. (C) the common (overlapped) response elements of VeA and LaeA are 5'-TGATTGGCTG-3' and 5'-GTCACGTGA-3'. a: response element appeared as the first rank from the motif elicitation analysis. b: response element appeared as the second rank from the motif elicitation analysis.

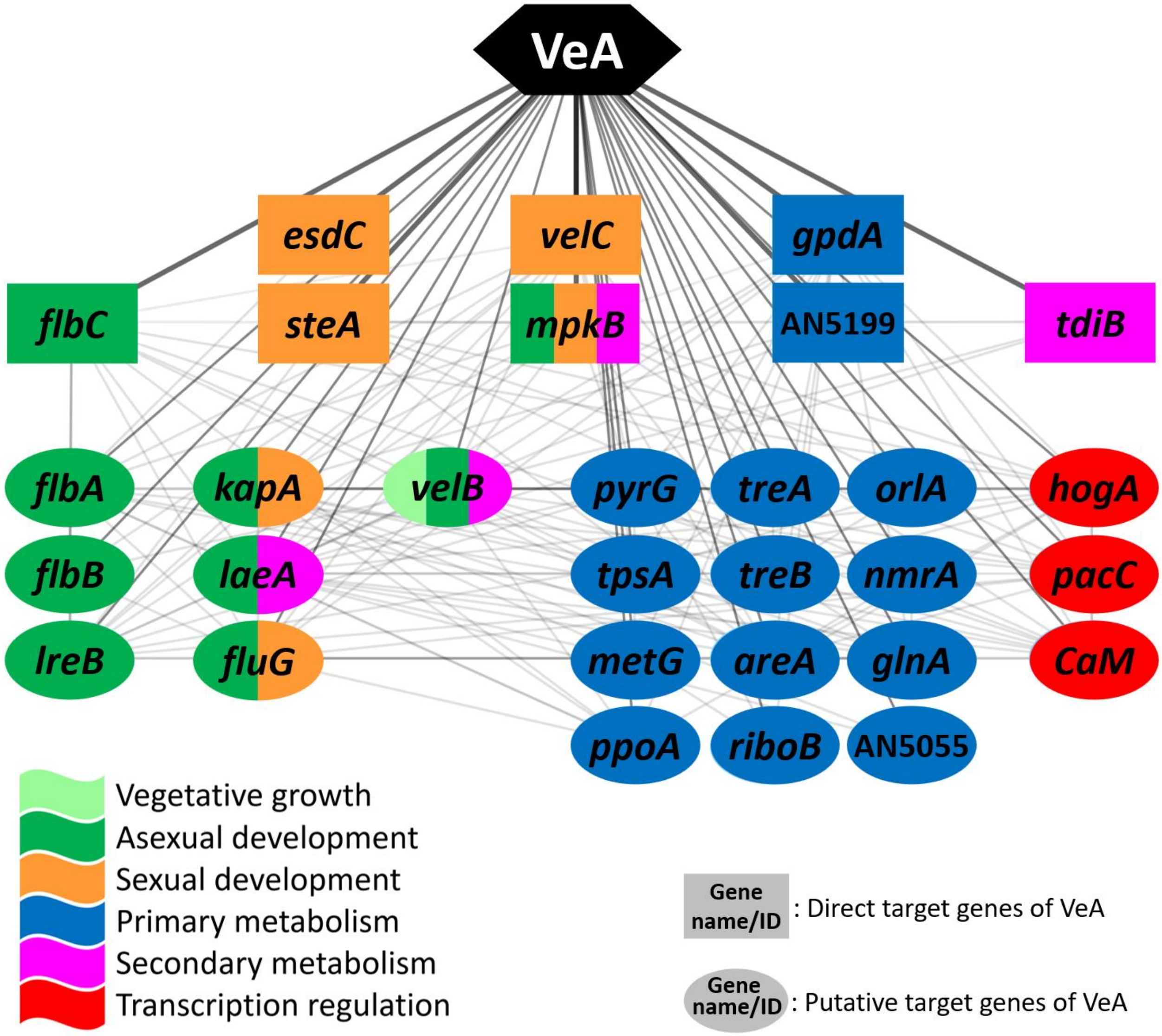

Figure 3-3. The core section of VeA-mediated gene regulatory network. See the "3.4.3 VeA-mediated gene regulatory network" section for the detailed description. In the network, genes are presented in different shapes: rectangle for direct target genes and ellipse for putative direct target genes of VeA. These shapes are colored depending on the predicted functions: vegetative growth, asexual development, sexual development, primary metabolism, secondary metabolism, and transcription regulation. Each line indicates the interaction between two genes/proteins.

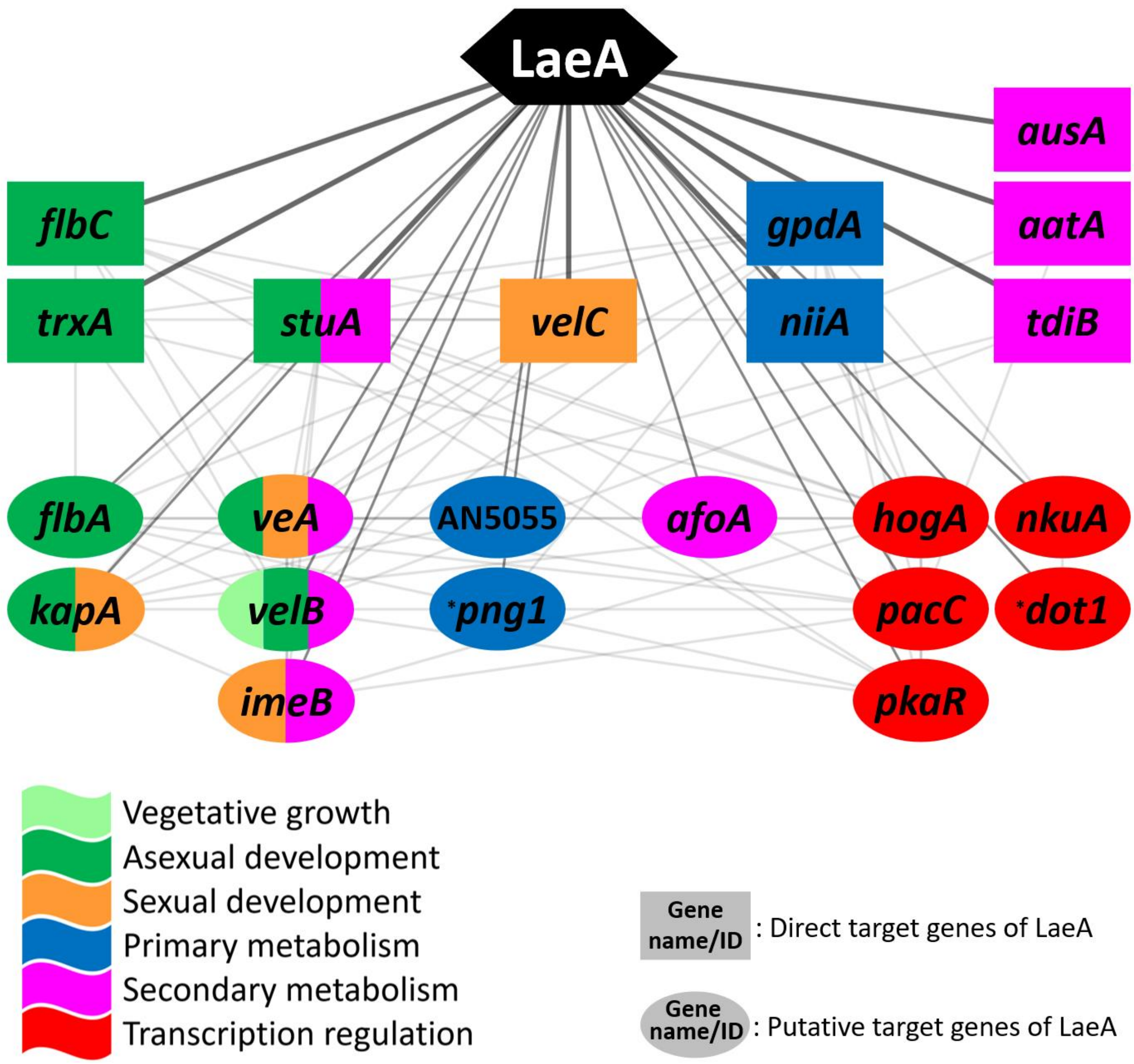

Figure 3-4. The core section of LaeA-mediated gene regulatory network. See the "3.4.4 LaeA-mediated gene regulatory network" section for the detailed description. In the network, genes are presented in different shapes: rectangle for direct target genes and ellipse for putative direct target genes of VeA. These shapes are colored depending on the predicted functions: vegetative growth, asexual development, sexual development, primary metabolism, secondary metabolism, and transcription regulation. Each line indicates the interaction between two genes/proteins.

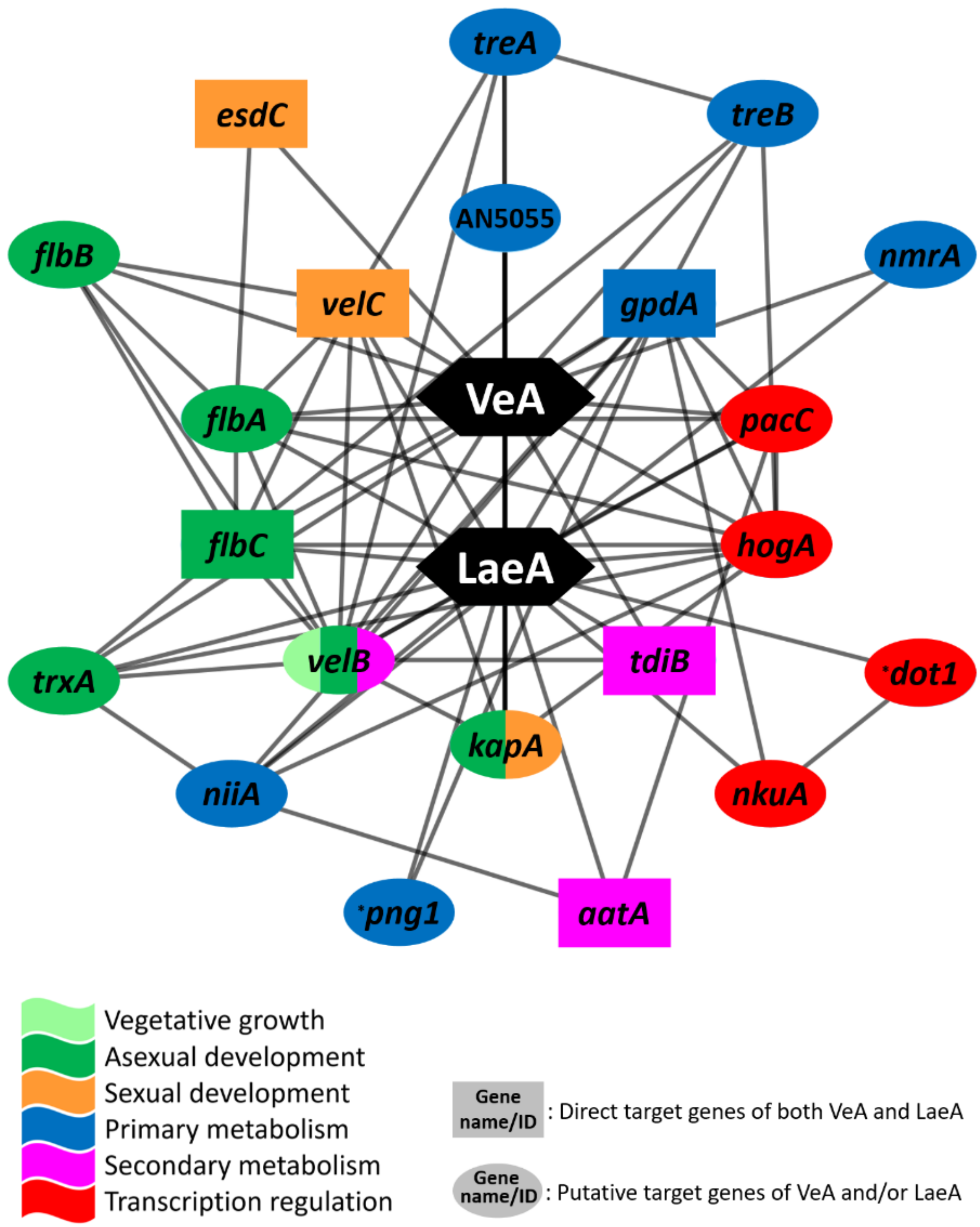

Figure 3-5. The core section of VeA/LaeA-mediated GRN. See the "3.4.5 Gene regulatory network co-regulated by VeA and LaeA" section for the detailed description. Genes in a rectangle shape are direct targets of both VeA and LaeA and genes in an ellipse shape are either direct or putative direct targets of VeA and LaeA, but not both.

Table 3-1. Summary of DEGs in *A. nidulans* ∆*veA* and ∆*laeA* Vege

	No. (%) of genes	
Category	**∆*veA* Vege**	**∆*laeA* Vege**
Unaffected genes	7,720 (70.26%)	8,650 (78.72%)
Differentially expressed genes	3,268 (29.74%)	2,338 (21.28%)
Up-regulated genes in DEGs	1,274 (11.59%)	928 (8.45%)
Down-regulated genes in DEGs	1,994 (18.15%)	1,410 (12.83%)
Total	10,988 (100%)	

Table 3-2. The functional enrichment analyses on DEGs in ∆*veA* and ∆*laeA* Vege

DEGs in ∆*veA* Vege	**DEGs in ∆*laeA* Vege**
Biological processes up-regulated in ∆veA (1,274) or ∆laeA (928)	
translation	transmembrane transport
peptide biosynthetic process	obsolete oxidation-reduction process
amide biosynthetic process	secondary metabolic process
organonitrogen compound biosynthetic process	xenobiotic transmembrane transport
peptide metabolic process	xenobiotic transport
cellular amide metabolic process	secondary metabolite biosynthetic process
cellular nitrogen compound biosynthetic process	amide transport
biosynthetic process	reactive nitrogen species metabolic process
cellular biosynthetic process	methionine biosynthetic process
organic substance biosynthetic process	nitrate metabolic process
organonitrogen compound metabolic process	nitrate assimilation
alpha-amino acid biosynthetic process	one-carbon compound transport
cellular amino acid biosynthetic process	monosaccharide transmembrane transport
metabolic process	nitrogen cycle metabolic process
cellular amino acid metabolic process	carbohydrate transmembrane transport
Biological processes down-regulated in ∆veA (1,994) or ∆laeA (1,410)	
secondary metabolic process	secondary metabolic process
secondary metabolite biosynthetic process	secondary metabolite biosynthetic process
phenol-containing compound metabolic process	phenol-containing compound metabolic process
phenol-containing compound biosynthetic process	phenol-containing compound biosynthetic process
organic heteropentacyclic compound biosynthetic process	monodictyphenone metabolic process
benzene-containing compound metabolic process	monodictyphenone biosynthetic process
organic heteropentacyclic compound metabolic process	benzene-containing compound metabolic process
monodictyphenone biosynthetic process	alkaloid metabolic process
monodictyphenone metabolic process	acetate metabolic process
melanin metabolic process	ketone biosynthetic process
melanin biosynthetic process	obsolete oxidation-reduction process
toxin metabolic process	austinol metabolic process
toxin biosynthetic process	austinol biosynthetic process
aflatoxin biosynthetic process	cellular ketone metabolic process
aflatoxin metabolic process	alkaloid biosynthetic process

Table 3-3. The functional enrichment analyses on VeA and/or LaeA direct target genes

VeA direct targets	LaeA direct targets	VeA/LaeA common targets
Biological processes up-regulated in ∆veA (417), ∆laeA (176), or both Vege (63)		
translation	cellular response to chemical stress	cellular response to osmotic stress
organonitrogen compound biosynthetic process	monosaccharide transmembrane transport	purine-containing compound biosynthetic process
peptide biosynthetic process	carbohydrate transmembrane transport	response to osmotic stress
cellular amide metabolic process	cellular response to osmotic stress	purine-containing compound metabolic process
amide biosynthetic process	methionine biosynthetic process	positive regulation of defense response
peptide metabolic process	NADH regeneration	induction by symbiont of host defense response
cellular nitrogen compound biosynthetic process	glucose catabolic process to pyruvate	adhesion of symbiont to host
cellular biosynthetic process	glycolytic process through fructose-6-phosphate	regulation of defense response
organic substance biosynthetic process	glycolytic process through glucose-6-phosphate	biological process involved in symbiotic interaction
biosynthetic process	canonical glycolysis	modulation by symbiont of host defense response
Biological processes down-regulated in ∆veA (561), ∆laeA (242), or both Vege (103)		
glycogen metabolic process	regulation of cell differentiation	syncytium formation
energy reserve metabolic process	syncytium formation by plasma membrane fusion	syncytium formation by plasma membrane fusion
glucan biosynthetic process	syncytium formation	cell-cell fusion
syncytium formation by plasma membrane fusion	regulation of sporulation	regulation of cell differentiation
syncytium formation	regulation of developmental process	glycogen biosynthetic process
glycogen biosynthetic process	regulation of sporulation resulting in formation of a cellular spore	manganese ion homeostasis
cellular glucan metabolic process	anatomical structure formation involved in morphogenesis	cellular manganese ion homeostasis
cleistothecium formation	regulation of sexual sporulation resulting in formation of a cellular spore	regulation of developmental process

cellular polysaccharide biosynthetic process	regulation of sexual sporulation	production of siRNA involved in RNA interference
polysaccharide biosynthetic process	regulation of asexual sporulation	RNA interference

Table 3-4. The genes forming the core section of the VeA-mediated GRN in *A. nidulans*

Gene ID	Gene Name	Description
AN1052	*veA*	Protein involved in light-sensitive control of differentiation and secondary metabolism; localizes to the nucleus in dark and to both nucleus and cytoplasm in the light; induced by light; AspGD sequence represents the veA1 mutant allele
AN2421	*flbC*	Putative C2H2 zinc finger transcription factor; involved in regulation of conidiophore development; required for light-dependent activation of brlA transcription
AN9121	*esdC*	Protein with a glycogen binding domain involved in sexual development; regulated by VeA and FlbA
AN2290	*steA*	STE-like transcription factor with homeobox and zinc finger domains; null mutation blocks sexual cycle but not asexual development, forms Hulle cells but no ascogenous tissue nor cleistothecia
AN2059	*velC*	Velvet family protein with homology to VeA, involved in regulation of sexual development
AN3719	*mpkB*	MAP kinase, component of a signaling module SteD-SteC-MkkB-MpkB that controls coordination of development and secondary metabolism; phosphorylates VeA in vitro; mutant has moderate growth defect and arrested sexual development
AN8041	*gpdA*	Glyceraldehyde-3-phosphate dehydrogenase with a predicted role in gluconeogenesis and glycolysis; the gpdA promoter is a commonly used regulatory sequence for driving constitutive heterologous gene expression
AN5199		Ortholog(s) have cytosol, nucleolus localization
AN8514	*tdiB*	Asterriquinone prenyltransferase; member of the tdi gene cluster; required for terrequinone A production; catalyzes the reverse prenylation event during terrequinone A biosynthesis; lacks canonical prenyl diphosphate binding motif (D/N)DXXD
AN5893	*flbA*	RGS (regulator of G-protein signaling) family member; negative regulator of G-protein signaling promoting conidiophore development; required for light-dependent activation of brlA transcription
AN7542	*flbB*	Basic leucine zipper transcription factor involved in regulation of conidiophore development; localizes to the most apical nucleus and the tip of mature vegetative hyphae; required for light-dependent activation of brlA transcription
AN3607	*lreB*	Putative zinc-finger transcription factor involved in blue-light responsive differentiation; interacts with VeA, FphA, and LreA; similar to N. crassa blue-light-sensing component WC-2
AN2142	*kapA*	Karyopherin (importin) alpha, involved in protein import into nucleus
AN0807	*laeA*	Methyltransferase-domain protein; velvet complex component composed of VelB, VeA and LaeA; self-methylates; coordinates asexual development in response to light; regulates secondary metabolism and is required for Hulle cell formation

AN4819	*fluG*	Cytoplasmic protein involved in regulation of conidiation and sterigmatocystin production; related to prokaryotic glutamine synthetases; expression upregulated after exposure to farnesol
AN0363	*velB*	Component of the velvet complex composed of VelB, VeA, and LaeA that coordinates development and secondary metabolism in response to light
AN6157	*pyrG*	Orotidine-5'-phosphate decarboxylase; enzyme of the pyrimidine biosynthesis pathway; pyrG89 mutant induces sexual development and is sensitive to excess uracil
AN5523	*tpsA*	Putative alpha,alpha-trehalose-phosphate synthase (UDP-forming) with a role in trehalose biosynthesis; transcriptionally induced during spore germination and exponential growth; required for viability of conidia during prolonged storage
AN7051	*metG*	Cystathionine beta-lyase, enzyme of the methionine biosynthesis pathway; mutants show a reduced rate of DNA damage repair
AN1967	*ppoA*	Putative fatty acid dioxygenase; responsible for the formation of the oxylipin psiB-alpha; PpoA localizes to lipid bodies in Hulle cells and metulae
AN9340	*treA*	Alpha,alpha-trehalase with a role in trehalose hydrolysis; localized to the conidial cell wall; expression upregulated after exposure to farnesol
AN5635	*treB*	Putative alpha, alpha-trehalase with a predicted role in trehalose hydrolysis
AN8667	*areA*	Wide-domain GATA-type transcription factor; mediates nitrogen metabolite repression; transcript induced by nitrogen starvation and degraded in response to ammonium or glutamine; AreA accumulates in the nucleus during nitrogen starvation
AN0670	*riboB*	Protein required for riboflavin biosynthesis; putative GTP cyclohydrolase; A. fumigatus riboB complements the riboB2 mutant
AN3441	*orlA*	Trehalose 6-phosphate phosphatase, predicted subunit of the trehalose-6-phosphate synthase/phosphatase complex; involved in chitin synthesis at elevated temperatures
AN8168	*nmrA*	Regulatory protein involved in nitrogen metabolite repression
AN4159	*glnA*	Putative glutamate-ammonia ligase with a predicted role in glutamate and glutamine metabolism; intracellular; transcript upregulated by nitrate limitation; protein abundance decreased by menadione stress and induced by farnesol
AN5055		Has domain(s) with predicted aminopeptidase activity, metalloexopeptidase activity and role in cellular process, proteolysis
AN1017	*hogA*	Putative mitogen-activated protein kinase (MAPK) involved in osmotic stress response; highly up-regulated under osmotic stress conditions; required for sexual development and sporulation; mutant sensitive to NaCl
AN2855	*pacC*	C2H2 finger domain transcription factor; undergoes proteolytic activation in response to alkaline ambient pH; physically interacts with PalA by two-hybrid analysis

AN2047	*CaM*	Calmodulin; EF-hands containing calcium binding protein; required for normal progression through the cell-cycle; localizes to hyphal tips and transiently to sites of septation; transcript upregulated in response to camptothecin

Table 3-5. The genes forming the core section of the LaeA-mediated GRN in *A. nidulans*

Gene ID	Gene Name	Description
AN0807	*laeA*	Methyltransferase-domain protein; velvet complex component composed of VelB, VeA and LaeA; self-methylates; coordinates asexual development in response to light; regulates secondary metabolism and is required for Hulle cell formation
AN2421	*flbC*	Putative C2H2 zinc finger transcription factor; involved in regulation of conidiophore development; required for light-dependent activation of brlA transcription
AN0170	*trxA*	Thioredoxin; predicted role in cell redox homeostasis; required for conidiation; expression upregulated after exposure to farnesol
AN5836	*stuA*	APSES domain transcription factor involved in regulation of conidiophore development; represses abaA and other developmentally regulated genes; locus consists of stuA-alpha and stuA-beta transcriptional units; stuA-alpha contains a uORF
AN2059	*velC*	Velvet family protein with homology to VeA, involved in regulation of sexual development
AN8041	*gpdA*	Glyceraldehyde-3-phosphate dehydrogenase with a predicted role in gluconeogenesis and glycolysis; the gpdA promoter is a commonly used regulatory sequence for driving constitutive heterologous gene expression
AN1007	*niiA*	Putative nitrite reductase with a predicted role in nitrogen metabolism; transcript stabilized by intracellular nitrate
AN8383	*ausA*	Polyketide synthase (PKS); produces 3,5- dimethyl orsellinic acid, the first intermediate in the biosynthesis of austinol and dehydroaustinol; aus secondary metabolism gene cluster member
AN2623	*aatA*	Isopenicillin-N N-acyltransferase; null produces reduced levels of penicillin; partially redundant with aatB
AN8514	*tdiB*	Asterriquinone prenyltransferase; member of the tdi gene cluster; required for terrequinone A production; catalyzes the reverse prenylation event during terrequinone A biosynthesis; lacks canonical prenyl diphosphate binding motif (D/N)DXXD
AN5893	*flbA*	RGS (regulator of G-protein signaling) family member; negative regulator of G-protein signaling promoting conidiophore development; required for light-dependent activation of brlA transcription
AN2142	*kapA*	Karyopherin (importin) alpha, involved in protein import into nucleus
AN1052	*veA*	Protein involved in light-sensitive control of differentiation and secondary metabolism; localizes to the nucleus in dark and to both nucleus and cytoplasm in the light; induced by light; AspGD sequence represents the veA1 mutant allele
AN0363	*velB*	Component of the velvet complex composed of VelB, VeA, and LaeA that coordinates development and secondary metabolism in response to light
AN6243	*imeB*	Putative serine/threonine protein kinase involved in light-mediated regulation of sexual development; required for sterigmatocystin production; mutant has a moderate growth defect

AN5055		Has domain(s) with predicted aminopeptidase activity, metalloexopeptidase activity and role in cellular process, proteolysis
AN3787	*png1*	Ortholog(s) have glycoprotein binding, metal ion binding, misfolded protein binding, peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase activity, structural constituent of cell wall activity
AN1029	*afoA*	Protein with homology to CtnR, citrinin biosynthesis transcriptional activator; contains a Zn(2)Cys(6) domain; involved in asperfuranone biosynthesis; overexpression induces expression of the asperfuranone biosynthesis gene cluster
AN1017	*hogA*	Putative mitogen-activated protein kinase (MAPK) involved in osmotic stress response; highly up-regulated under osmotic stress conditions; required for sexual development and sporulation; mutant sensitive to NaCl
AN2855	*pacC*	C2H2 finger domain transcription factor; undergoes proteolytic activation in response to alkaline ambient pH; physically interacts with PalA by two-hybrid analysis
AN4987	*pkaR*	Putative protein kinase A (PKA) regulatory subunit
AN7753	*nkuA*	ATP-dependent DNA helicase II; 70 kDa subunit of Ku70/Ku80; mutants display a dramatic increase in homologous integration
AN0091	*dot1*	Ortholog(s) have histone methyltransferase activity (H3-K79 specific), nucleosomal histone binding activity

Table 3-6. The genes forming the core section of the VeA/LaeA-mediated GRN in *A. nidulans*

Gene ID	Gene Name	Description
AN1052	*veA*	Protein involved in light-sensitive control of differentiation and secondary metabolism; localizes to the nucleus in dark and to both nucleus and cytoplasm in the light; induced by light; AspGD sequence represents the veA1 mutant allele
AN0807	*laeA*	Methyltransferase-domain protein; velvet complex component composed of VelB, VeA and LaeA; self-methylates; coordinates asexual development in response to light; regulates secondary metabolism and is required for Hulle cell formation
AN5893	*flbA*	RGS (regulator of G-protein signaling) family member; negative regulator of G-protein signaling promoting conidiophore development; required for light-dependent activation of brlA transcription
AN2421	*flbC*	Putative C2H2 zinc finger transcription factor; involved in regulation of conidiophore development; required for light-dependent activation of brlA transcription
AN2059	*velC*	Velvet family protein with homology to VeA, involved in regulation of sexual development
AN0363	*velB*	Component of the velvet complex composed of VelB, VeA, and LaeA that coordinates development and secondary metabolism in response to light
AN2142	*kapA*	Karyopherin (importin) alpha, involved in protein import into nucleus
AN5055		Has domain(s) with predicted aminopeptidase activity, metalloexopeptidase activity and role in cellular process, proteolysis
AN8041	*gpdA*	Glyceraldehyde-3-phosphate dehydrogenase with a predicted role in gluconeogenesis and glycolysis; the gpdA promoter is a commonly used regulatory sequence for driving constitutive heterologous gene expression
AN8514	*tdiB*	Asterriquinone prenyltransferase; member of the tdi gene cluster; required for terrequinone A production; catalyzes the reverse prenylation event during terrequinone A biosynthesis; lacks canonical prenyl diphosphate binding motif (D/N)DXXD
AN2855	*pacC*	C2H2 finger domain transcription factor; undergoes proteolytic activation in response to alkaline ambient pH; physically interacts with PalA by two-hybrid analysis
AN1017	*hogA*	Putative mitogen-activated protein kinase (MAPK) involved in osmotic stress response; highly up-regulated under osmotic stress conditions; required for sexual development and sporulation; mutant sensitive to NaCl
AN7542	*flbB*	Basic leucine zipper transcription factor involved in regulation of conidiophore development; localizes to the most apical nucleus and the tip of mature vegetative hyphae; required for light-dependent activation of brlA transcription
AN9121	*esdC*	Protein with a glycogen binding domain involved in sexual development; regulated by VeA and FlbA
AN9340	*treA*	Alpha,alpha-trehalase with a role in trehalose hydrolysis; localized to the conidial cell wall; expression upregulated after exposure to farnesol

AN5635	*treB*	Putative alpha, alpha-trehalase with a predicted role in trehalose hydrolysis
AN8168	*nmrA*	Regulatory protein involved in nitrogen metabolite repression
AN0170	*trxA*	Thioredoxin; predicted role in cell redox homeostasis; required for conidiation; expression upregulated after exposure to farnesol
AN1007	*niiA*	Putative nitrite reductase with a predicted role in nitrogen metabolism; transcript stabilized by intracellular nitrate
AN3787	*png1*	Ortholog(s) have glycoprotein binding, metal ion binding, misfolded protein binding, peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase activity, structural constituent of cell wall activity
AN2623	*aatA*	Isopenicillin-N N-acyltransferase; null produces reduced levels of penicillin; partially redundant with aatB
AN7753	*nkuA*	ATP-dependent DNA helicase II; 70 kDa subunit of Ku70/Ku80; mutants display a dramatic increase in homologous integration
AN0091	*dot1*	Ortholog(s) have histone methyltransferase activity (H3-K79 specific), nucleosomal histone binding activity

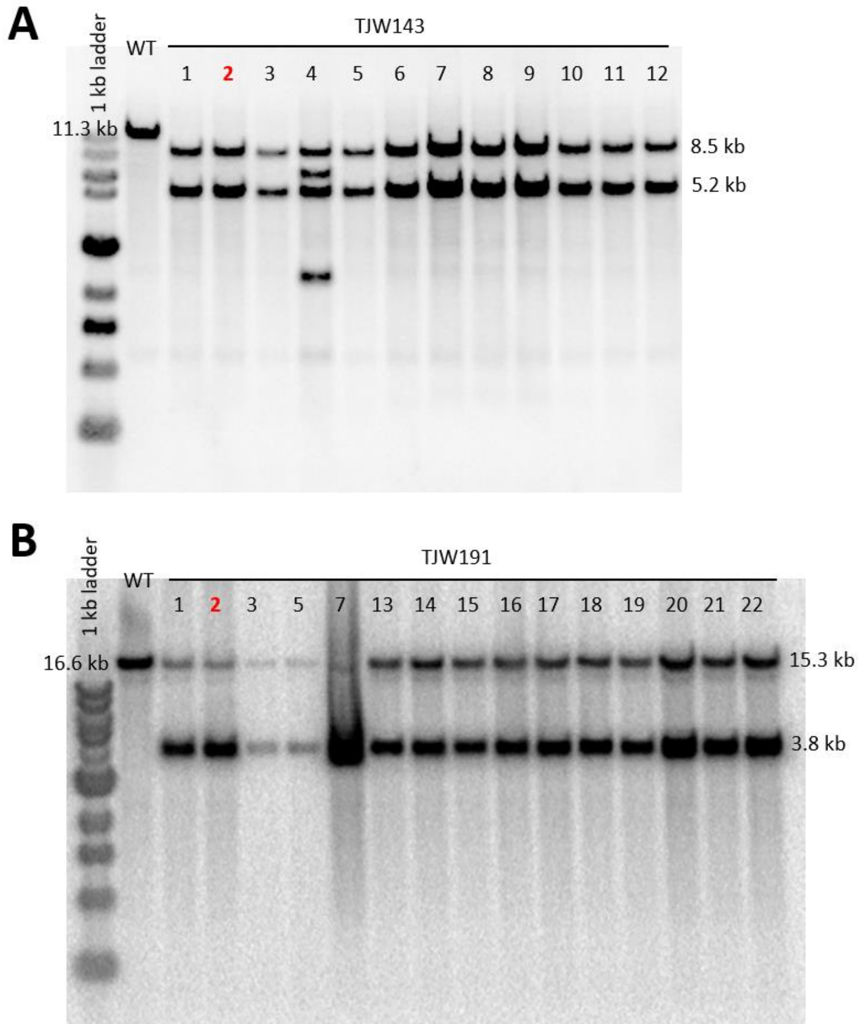

Figure S3-1. Southern confirmation of *laeA*::FLAG (A) and *veA*::FLAG (B) mutants. (A) Genomic DNA was digested by *EcoRI*. Wild type (WT, 11.3 kb), and *laeA*::FLAG (5.2 and 8.5 kb). TJW143.2 was chosen for the subsequent experiment. (B) Genomic DNA was digested by *Xba*I. Wildtype (WT, 16.6 kb), and *veA*::FLAG (3.8 and 15.3 kb). TJW191.2 was chosen for the subsequent experiment.

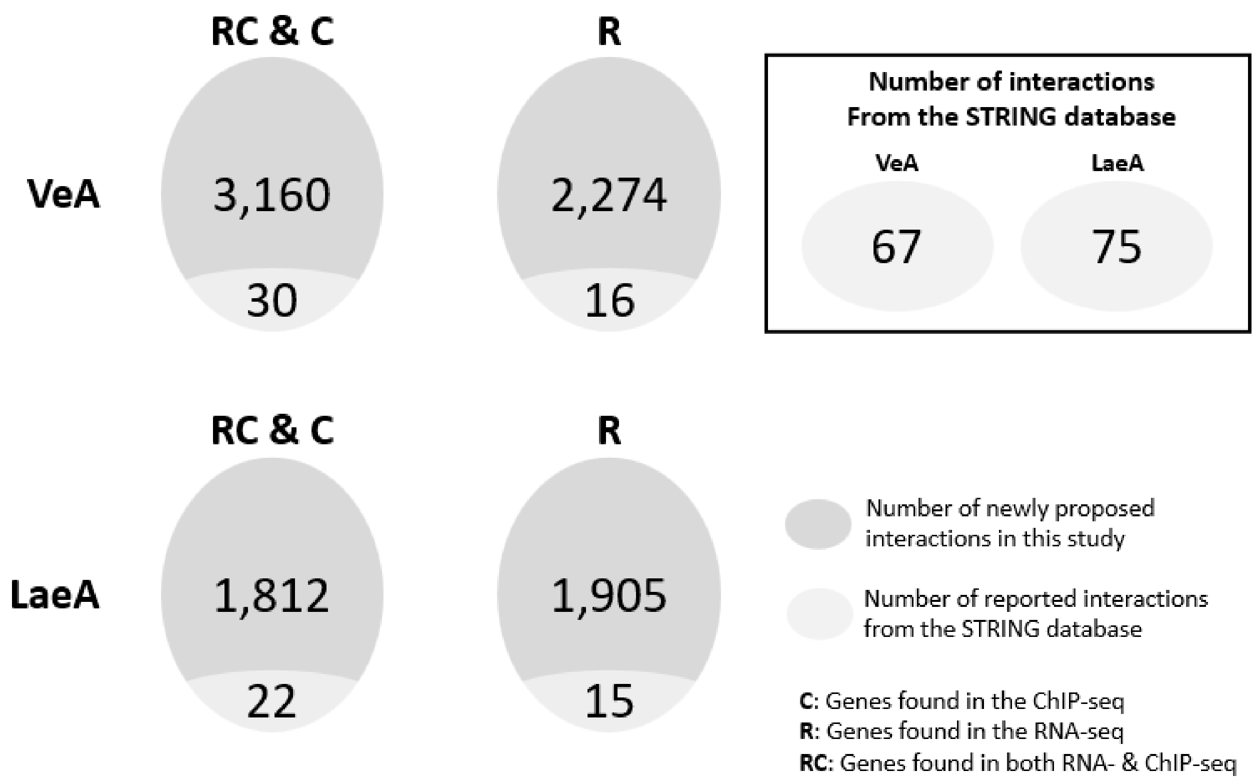

Figure S3-2. The known interactions of VeA and LaeA from the STRING database and novel interactions of VeA and LaeA proposed in this study.

Table S3-1. *Aspergillus* strains used in this study

Strain name	Relevant genotype	References
RDIT9.32	*A. nidulans* wild type	Tsitsigiannis *et al*., 2004
TNO2A7	*pyroA4, riboB2, nku70, veA1*	Bok *et al*., 2014
RDIT2.1	*metG1*	Bok *et al*., 2014
RTMH207.13	*pyrG89, veA1*	Bok *et al*., 2014
TJW143.2	*laeA::FLAG::AfriboB, pyroA4, nku70, veA1*	this study
TJW191.2	*veA::FLAG::AfriboB, pyroA4, nku70*	this study
RJW112.2	Δ*veA::argB*	Bayram *et al*., 2008
RJW41.A	*methG1;* Δ*laeA::methG; veA*	Bayram *et al*., 2008
RJW324.3	*veA::FLAG::AfriboB*	this study
RJW302.11	*laeA::FLAG::AfriboB, veA*	this study

Table S3-2. Oligonucleotides used in this study

Name	Sequence (5' → 3')	Purpose
laeAFlag5F	AGTCCATCACTGAACGAGAGCC	LaeA-FLAG tag
laeAFlag5R	CCAGCGCCTGCACCAGCTCCGGCACCTCTTAA TGGTTTCCTAGCCTGGTATATGTGC	LaeA-FLAG tag
FlagF		LaeA-FLAG tag
FlagjointR	AAGGGCGAATTCCAGCACACTGG	LaeA-FLAG tag
FlagriboF	CCAGTGTGCTGGAATTCGCCCTTTGAATCAAG GCGGACTGAGTTATGGATG	LaeA-FLAG tag
FlagriboR	TGCCACTCAACGCCATTGACTCAG	LaeA-FLAG tag
laeAFlag3F	GATCACTGAGTCAATGGCGTTGAGTGGCATAA GAGCAAAAGGCGACCACATCCAGGAACG	LaeA-FLAG tag
laeAFlag3R	TGGTGATGGTGAGAAGGATGGG	LaeA-FLAG tag
laeAFlagconfF	TTCCTTCCACTGTTCCACTCGG	LaeA-FLAG tag
flagconfR	TTTGTCAGGCCTGACGTGATCC	LaeA-FLAG tag
veAflag5F	ACATGGACCCGTACTCCTATCC	VeA-FLAG tag
veAflag5R	GGCTCCAGCGCCTGCACCAGCTCCGGCACCAC GCATGGTGGCAGGCTTTGAGACCATCCG	VeA-FLAG tag
veAflag3F	GATCACTGAGTCAATGGCGTTGAGTGGCATCA TAGTTCTTGGCGGGTTCTGGTATAGG	VeA-FLAG tag
veAflag3R	TCGTTTCGAAGTTGCGCAAGGG	VeA-FLAG tag
veAflag confF	GAATTCTTGGAGTTCCGGCTGG	VeA-FLAG tag

Table S3-3. Direct target genes of VeA in *A. nidulans* Vege

Gene ID	Gene Name	Description
AN0005		Protein of unknown function
AN0011		Possible pseudogene
AN0019		Protein of unknown function
AN0029		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN0031		Protein of unknown function
AN0045		Transcript induced by light in in developmentally competent mycelia
AN0057		Has domain(s) with predicted ATP binding, aminoacyl-tRNA ligase activity, tyrosine-tRNA ligase activity, role in tRNA aminoacylation for protein translation, tyrosyl-tRNA aminoacylation and cytoplasm localization
AN0066		Ortholog(s) have isopropylmalate transmembrane transporter activity, malonate(1-) transmembrane transporter activity, oxaloacetate transmembrane transporter activity, sulfate transmembrane transporter activity
AN0119	*dclA*	Putative Dicer protein
AN0131		Has domain(s) with predicted calcium ion binding activity
AN0133		Putative mRNA splicing factor ATP-dependent RNA helicase; ortholog of S. cerevisiae Prp43p; expression reduced after exposure to farnesol
AN0137		Ortholog(s) have glycerophosphocholine phosphodiesterase activity and role in cellular response to drug, glycerophospholipid catabolic process
AN0144	*nrc2*	Ribosomal S6 kinase (RSK); mutants have a moderate growth defect; reduced growth on AVICEL medium and reduced eglA and eglB expression
AN0148	*mdpE*	C6 zinc finger transcription factor similar to AflR; member of the monodictyphenone secondary metabolite biosynthesis gene cluster; required for synthesis of monodictyphenone and related compounds but not prenyl xanthones
AN0149	*mdpF*	Putative zinc-dependent hydrolase; member of the monodictyphenone (mdp) secondary metabolite biosynthesis gene cluster
AN0197		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN0208		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN0210		Ortholog(s) have ATPase activity, GTPase activity, adenosine-diphosphatase activity, cytidine diphosphatase activity, guanosine-diphosphatase activity and nucleoside-diphosphatase activity, more
AN0234		Has domain(s) with predicted hydrolase activity
AN0252		Putative F1F0-ATPase complex gamma subunit with a predicted role in energy metabolism; expression reduced after exposure to farnesol
AN0259		Putative adenylate kinase with a predicted role in nucleotide salvage pathways

AN0270		Ortholog(s) have role in pyridoxal phosphate biosynthetic process
AN0294		Protein of unknown function
AN0299		Putative chitinase; glycoside hydrolase family 18 (GH18) protein with a predicted role in chitin hydrolysis
AN0301		Protein of unknown function
AN0314		Putative aspartyl-tRNA synthetase with a predicted role in tRNA aminoacylation; expression upregulated after exposure to farnesol
AN0324		Protein of unknown function
AN0343		Protein of unknown function
AN0353		Putative F-box protein
AN0357		Putative ubiquinol-cytochrome-c reductase subunit with a predicted role in energy metabolism
AN0382		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN0403		Ortholog(s) have cytoplasm localization
AN0437		Has domain(s) with predicted hydrolase activity
AN0443		Putative zinc containing alcohol dehydrogenase; protein expressed at decreased levels in a hapX mutant versus wild-type
AN0461		Protein of unknown function
AN0465		Ortholog of S. cerevisiae RPS8A and RPS8B; palA-dependent expression independent of pH
AN0468		Protein of unknown function
AN0490		Putative CTP synthase with a predicted role in pyrimidine metabolism
AN0493		PalA-dependent expression independent of pH
AN0495		Has domain(s) with predicted amino acid binding, formyltetrahydrofolate deformylase activity, hydroxymethyl-, formyl- and related transferase activity and role in 'de novo' IMP biosynthetic process, biosynthetic process, metabolic process
AN0510		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN0521		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN0540		Protein of unknown function
AN0558	*gelB*	Putative 1,3-beta-transglycosidase with a predicted role in glucan processing; predicted glycosyl phosphatidylinositol (GPI)-anchor
AN0565	*pyrAB CN*	Multifunctional enzyme with carbamoyl-phosphate synthase (CPSase) and aspartate carbamoyltransferase (ATCase) activities that catalyze the first two steps in pyrimidine biosynthesis
AN0570		Ortholog(s) have RNA binding, structural constituent of ribosome activity, role in cytoplasmic translation and cytosolic large ribosomal subunit, yeast-form cell wall localization
AN0575		Protein of unknown function
AN0601		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN0649		Putative long-chain-fatty-acid-CoA ligase with a predicted role in fatty acid metabolism
AN0657		Ortholog(s) have mitochondrial outer membrane localization

AN0661		Ortholog(s) have nucleotidyltransferase activity
AN0677		Has domain(s) with predicted zinc ion binding activity
AN0723		Protein of unknown function
AN0726	*sunB*	Putative Sun-family protein; predicted glycosyl phosphatidylinositol (GPI)-anchor
AN0745		Putative nucleolar protein; ortholog of S. cerevisiae Nop1p; related to mammalian fibrillarin; expression reduced after exposure to farnesol
AN0750		Protein of unknown function
AN0751		Protein of unknown function
AN0756	*lacA*	Beta-galactosidase with a predicted role in lactose metabolism
AN0776		Ortholog(s) have structural constituent of ribosome activity and role in cellular response to drug, cleavage in ITS2 between 5.8S rRNA and LSU-rRNA of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA), cytoplasmic translation
AN0781		Protein of unknown function
AN0787	*mns1B*	Putative mannosyl-oligosaccharide 1,2-alpha-mannosidase with a predicted role in mannose polymer metabolism
AN0791		Protein of unknown function
AN0800		Protein of unknown function
AN0820		Protein of unknown function
AN0823		Protein of unknown function
AN0829	*pdeA*	Low-affinity cAMP phosphodiesterase
AN0843		Ortholog(s) have structural constituent of ribosome activity, role in rRNA export from nucleus and cytosolic small ribosomal subunit, membrane localization
AN0851		Has domain(s) with predicted cholestenol delta-isomerase activity, role in sterol metabolic process and endoplasmic reticulum, integral component of membrane localization
AN0857		Protein of unknown function
AN0860		Protein of unknown function
AN0887	*lamA*	Putative urea amidolyase with a predicted role in nitrogen metabolism; required for the utilization of lactams such as 2-pyrrolidinone
AN0899		Protein of unknown function
AN0903		Protein of unknown function
AN0904		Protein of unknown function
AN0910		Putative phosphatidylserine decarboxylase with a predicted role in phospholipid metabolism; expression reduced after exposure to farnesol
AN0912		Putative Beta-isopropylmalate dehydrogenase with a predicted role in valine, leucine, and isoleucine metabolism
AN0913		Putative phosphatidylinositol synthase with a predicted role in phospholipid metabolism
AN0914	*ptcD*	Putative serine/threonine phosphatase
AN0930		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN0933	*crhC*	Putative transglycosidase with a predicted role in glucan processing; predicted glycosyl phosphatidylinositol (GPI)-anchor

AN0936		Ortholog(s) have threonine-tRNA ligase activity
AN0943	*atp20*	Putative mitochondrial F1F0-ATP synthase subunit g; ortholog of S. cerevisiae Atp20p; expression reduced after exposure to farnesol
AN0948		Has domain(s) with predicted ATP binding, ATPase activity, nucleoside-triphosphatase activity, nucleotide binding activity
AN10005		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN10011		Has domain(s) with predicted oxidoreductase activity, oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor activity and role in oxidation-reduction process
AN10028	*CYP684A1*	Putative cytochrome P450
AN1003		Putative isocitrate dehydrogenase (NAD+) with a predicted role in the TCA cycle; intracellular, menadione stress-induced protein
AN10039		Putative histidine acid phosphatase
AN10044	*mdpK*	Putative oxidoreductase; member of the monodictyphenone (mdp) secondary metabolite biosynthesis gene cluster; required for monodictyphenone biosynthesis
AN10055		Protein of unknown function
AN10060		Putative alpha-amylase; glycogen debranching enzyme
AN10075		Putative permease of the major facilitator superfamily (MFS)
AN10078		Ortholog(s) have ATPase-coupled transmembrane transporter activity, role in fatty acid transport and integral component of peroxisomal membrane, peroxisome localization
AN10090		Protein of unknown function
AN10091		Has domain(s) with predicted structural constituent of ribosome activity and role in mitochondrial translation
AN10100		Protein of unknown function
AN10116		Protein of unknown function
AN10149		Protein of unknown function
AN1015		Putative phosphorylase with a predicted role in glycogen degradation
AN10168		Protein of unknown function
AN10199		Protein of unknown function
AN10242		Protein of unknown function
AN10243		Protein of unknown function
AN1026		Has domain(s) with predicted role in attachment of spindle microtubules to kinetochore and DASH complex, mitotic spindle localization
AN1028		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN10296		Ortholog(s) have fumarate reductase (NADH) activity, role in cellular response to anoxia and cytosol, intracellular localization
AN10299		Has domain(s) with predicted carboxy-lyase activity, catalytic activity, pyridoxal phosphate binding activity and role in carboxylic acid metabolic process, cellular amino acid metabolic process
AN10303		Protein of unknown function

AN10311	*mnpA*	Putative hyphal cell wall mannoprotein; expression is transcriptionally upregulated during sexual development; expression is flbA-, fadA- and veA-dependent; present in the hyphal cell wall, absent from the conidial cell wall
AN10318		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN1032	*afoC*	Putative oxidoreductase; required for asperfuranone biosynthesis
AN10321		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN10332		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN10344		CobW domain-containing protein; transcript repressed by nitrate
AN1035	*afoF*	Putative FAD/FMN-dependent oxygenase; required for asperfuranone biosynthesis
AN10356		Protein of unknown function
AN10384		Protein of unknown function
AN10391		Protein of unknown function
AN10398		Has domain(s) with predicted ADP binding activity and role in defense response
AN10399		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN10407		Has domain(s) with predicted zinc ion binding activity
AN10410		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN10420	*agdF*	Putative alpha-glucosidase with a predicted role in starch metabolism; transcriptionally induced by isomaltose in an amyR-dependent manner
AN10422		Protein of unknown function
AN1046	*chsG*	Putative class VII chitin synthase with a predicted role in chitin biosynthesis
AN1047		Putative heat shock protein
AN10487		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN10496		Putative regulator of mannosylphosphorylation
AN10499		Ortholog(s) have intracellular localization
AN1052	*veA*	Protein involved in light-sensitive control of differentiation and secondary metabolism; localizes to the nucleus in dark and to both nucleus and cytoplasm in the light; induced by light; AspGD sequence represents the veA1 mutant allele
AN10530		Protein of unknown function
AN10533		Putative trehalose-6-phosphate synthase; ortholog of S. cerevisiae Tps3p; expression upregulated after exposure to farnesol
AN10552		Protein of unknown function
AN10559		Has domain(s) with predicted oxidoreductase activity, acting on the CH-CH group of donors activity, role in lipid metabolic process and cytoplasm, integral component of membrane localization
AN10568		Has domain(s) with predicted role in mitochondrial pyruvate transmembrane transport and mitochondrial inner membrane localization

AN10578		Protein of unknown function
AN10581		Protein of unknown function
AN10592		Protein of unknown function
AN1061		Putative GABA permease; transcript is induced by nitrate
AN10614		Ortholog(s) have DNA binding, G-quadruplex DNA binding, ribosome binding, telomeric DNA binding, triplex DNA binding activity
AN10619		Has domain(s) with predicted carboxy-lyase activity, catalytic activity, pyridoxal phosphate binding activity and role in carboxylic acid metabolic process
AN10629		Has domain(s) with predicted role in lipid metabolic process
AN10648		Ortholog(s) have delta24(24-1) sterol reductase activity, role in ergosterol biosynthetic process and endoplasmic reticulum localization
AN10690		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN10694		Protein of unknown function
AN10726		Protein of unknown function
AN1074		Ortholog(s) have glycine dehydrogenase (decarboxylating) activity, role in glycine catabolic process, one-carbon metabolic process, protein lipoylation and mitochondrion localization
AN10740		Ortholog(s) have cell surface, cytosolic large ribosomal subunit localization
AN10745		Ortholog(s) have mitochondrion localization
AN10762		Has domain(s) with predicted phosphomethylpyrimidine kinase activity, thiaminase activity and role in thiamine biosynthetic process
AN10782		Putative mitochondrial phosphate carrier protein; expression upregulated after exposure to farnesol
AN10789		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN10805		Has domain(s) with predicted hydrolase activity and role in metabolic process
AN10819	*ffkB*	Has domain(s) with predicted ATP binding, protein kinase activity, transferase activity, transferring phosphorus-containing groups activity and role in protein phosphorylation
AN10827		Protein of unknown function
AN10828		Ortholog(s) have protein transmembrane transporter activity and role in protein import into mitochondrial matrix, protein insertion into mitochondrial outer membrane, protein targeting to mitochondrion
AN10846		Has domain(s) with predicted RNA binding, RNA-directed DNA polymerase activity, nucleic acid binding activity and role in DNA integration, RNA-dependent DNA biosynthetic process
AN10872		Has domain(s) with predicted role in ubiquitin-dependent protein catabolic process

AN10876		Has domain(s) with predicted cation transmembrane transporter activity, role in cation transport, transmembrane transport and integral component of membrane localization
AN10886		Protein of unknown function; predicted secondary metabolism gene cluster member
AN10893		Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN10896		Protein of unknown function
AN10906		Has domain(s) with predicted DNA binding, zinc ion binding activity, role in transcription, DNA-templated and nucleus localization
AN10907		Protein of unknown function
AN10915		Protein of unknown function
AN10964		Has domain(s) with predicted methyltransferase activity and role in metabolic process
AN10972		Has domain(s) with predicted amino acid transmembrane transporter activity, role in amino acid transmembrane transport, amino acid transport, transmembrane transport and integral component of membrane, membrane localization
AN10974		Has domain(s) with predicted methyltransferase activity
AN10982	*enaC*	Putative P-type ATPase sodium pump
AN11002		Protein of unknown function
AN11018		Protein of unknown function
AN11027		Has domain(s) with predicted acyl-CoA hydrolase activity and role in acyl-CoA metabolic process
AN11080		Putative dimethyl-allyl-tryptophan synthase (DMATS)-type aromatic prenyltransferase
AN1109		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN11098		Ortholog(s) have role in hyphal growth, regulation of glycogen metabolic process
AN11140		PASA transcript
AN11142	*CYP547C1*	Putative cytochrome P450
AN11158		Protein of unknown function
AN11159		Has domain(s) with predicted phosphatidylserine decarboxylase activity and role in phospholipid biosynthetic process
AN11177		Has domain(s) with predicted oxidoreductase activity, zinc ion binding activity and role in oxidation-reduction process
AN11209		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN11211	*furF*	Protein with homology to the Saccharomyces cerevisiae uracil transporter Fur4p; mutant is unaffected in uracil transport in A. nidulans
AN11222		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization

AN11280		Protein of unknown function
AN11281		Protein of unknown function
AN1129		Protein of unknown function
AN11292		Protein of unknown function
AN11313		Protein of unknown function
AN11337		Protein of unknown function
AN11347		Ortholog(s) have cytochrome-c oxidase activity, role in mitochondrial electron transport, cytochrome c to oxygen and mitochondrial respiratory chain complex IV, plasma membrane localization
AN1140	*qutC*	Dehydroshikimate dehydratase, involved in quinic acid utilization
AN11406		Ortholog(s) have DNA-directed 5'-3' RNA polymerase activity, RNA polymerase I activity, RNA polymerase II activity, RNA polymerase III activity, RNA-directed 5'-3' RNA polymerase activity, zinc ion binding activity
AN11411		Ortholog(s) have structural constituent of ribosome activity and cytosolic small ribosomal subunit, yeast-form cell wall localization
AN11511		Protein of unknown function
AN11543		Protein of unknown function
AN11551		Protein of unknown function
AN11568		Has domain(s) with predicted structural constituent of ribosome activity, role in translation and ribosome localization
AN11631		Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit, mitochondrial ribosome localization
AN1166		Ortholog(s) have RNA binding, structural constituent of ribosome activity
AN11670		Protein of unknown function
AN11693		Protein of unknown function
AN11732		Protein of unknown function
AN1174		Has domain(s) with predicted ATP binding, ATPase activity, ATPase-coupled transmembrane transporter activity, nucleoside-triphosphatase activity, nucleotide binding activity and role in transmembrane transport
AN11751		Protein of unknown function
AN11754		Protein of unknown function
AN11776		Ortholog(s) have mitochondrial inner membrane, mitochondrial large ribosomal subunit localization
AN11777		Protein of unknown function
AN11778		Putative exoinulinase
AN11801		Protein of unknown function
AN11874		Protein of unknown function
AN11907		Protein of unknown function
AN11931		Protein of unknown function
AN11934		Has domain(s) with predicted ATP binding, ATPase activity, ATPase-coupled transmembrane transporter activity, nucleoside-triphosphatase activity, nucleotide binding activity and role in transmembrane transport

AN1197	Has domain(s) with predicted carbohydrate binding, catalytic activity and role in carbohydrate metabolic process
AN11980	Protein of unknown function
AN11999	Protein of unknown function
AN12015	Protein of unknown function
AN12016	Protein of unknown function
AN12030	Protein of unknown function
AN12031	Protein of unknown function
AN12036	Protein of unknown function
AN12084	Protein of unknown function
AN12086	Has domain(s) with predicted carbon-sulfur lyase activity and role in metabolic process
AN12087	Protein of unknown function
AN12148	Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN12169	Protein of unknown function
AN12192	Protein of unknown function
AN12198	Protein of unknown function
AN1220	Protein of unknown function
AN12213	Ortholog(s) have ubiquinol-cytochrome-c reductase activity and role in aerobic respiration, mitochondrial electron transport, ubiquinol to cytochrome c
AN12222	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN12224	Protein of unknown function
AN1224	Protein of unknown function
AN12281	Protein of unknown function
AN12284	Protein of unknown function
AN12330	Protein of unknown function
AN12348	Protein of unknown function
AN12374	Protein of unknown function
AN12385	Protein of unknown function
AN12386	Protein of unknown function
AN12392	Protein of unknown function
AN12426	Has domain(s) with predicted integral component of membrane localization
AN12465	Ortholog(s) have role in cellular response to biotic stimulus, filamentous growth, filamentous growth of a population of unicellular organisms, filamentous growth of a population of unicellular organisms in response to biotic stimulus
AN12477	Has domain(s) with predicted GTP binding, GTPase activity
AN12483	Protein of unknown function
AN12487	Protein of unknown function
AN12488	Protein of unknown function

AN12492		Has domain(s) with predicted GTP binding, GTPase activity
AN1251		Has domain(s) with predicted nucleic acid binding, zinc ion binding activity
AN1257		Has domain(s) with predicted transferase activity, transferring glycosyl groups activity
AN1261		Protein of unknown function; transcript is induced by nitrate
AN1319		Ortholog(s) have RNA binding, U3 snoRNA binding, U4 snRNA binding activity
AN1333	*tctexA*	Dynein light chain
AN1356		Protein of unknown function
AN1404		Diacylglycerol kinase domain-containing protein; transcript upregulated by nitrate limitation
AN1412		Protein of unknown function
AN1414	*xprG*	p53-like transcription factor that contains a Ndt80-like DNA-binding domain; transcriptional regulator of extracellular proteases; putative acid phosphatase with a predicted role in gluconic acid and gluconate metabolism
AN1418		Ortholog(s) have glucosamine-6-phosphate deaminase activity
AN1425	*farB*	Putative transcription factor containing a Zn2-Cys6 binuclear cluster domain; required for transcriptional activation of genes involved in utilization of short-chain fatty acids; highly conserved in filamentous ascomycetes
AN1438		Protein of unknown function
AN1470		Protein of unknown function
AN1477		Putative beta-1,4-xylosidase
AN1505		Protein of unknown function
AN1509		Protein of unknown function
AN1517		Has domain(s) with predicted nucleic acid binding, nucleotide binding activity
AN1519	*rsdA*	Putative Argonaute protein involved in inverted repeat transgene (IRT)-induced RNA silencing
AN1528		Ortholog(s) have nucleus localization
AN1535		Protein of unknown function
AN1541		Has domain(s) with predicted oxidoreductase activity, oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor activity and role in oxidation-reduction process
AN1551	*btgE*	Putative beta-glucosidase with predicted role in degradation of glucans; covalently bound cell wall protein
AN1588		Protein of unknown function
AN1590		Protein of unknown function
AN1612		Ortholog(s) have inorganic phosphate transmembrane transporter activity, manganese ion transmembrane transporter activity, phosphate:proton symporter activity, selenite:proton symporter activity
AN1620		Protein of unknown function
AN1622		Protein of unknown function

AN1624	*oliC*	Subunit 9 of the mitochondrial inner membrane F1F0-ATPase complex; mutation confers oligomycin resistance; palA-dependent expression independent of pH
AN1664		Has domain(s) with predicted hydrolase activity
AN1686		Protein of unknown function
AN1693		Putative F-box protein
AN1713		Ortholog(s) have calcium-independent phospholipase A2 activity, lysophosphatidic acid acyltransferase activity, sterol esterase activity, triglyceride lipase activity
AN1714		Protein of unknown function
AN1715		Putative mannose-6-phosphate isomerase with a predicted role in mannose/mannitol, fructose, and sorbose/sorbitol metabolism
AN1726		Putative 3-methyl-2-oxobutanoate dehydrogenase
AN1747		Ortholog(s) have mitochondrial inner membrane localization
AN1755		Protein of unknown function
AN1787		Predicted UDP-N-acetylmuramate dehydrogenase; predicted secondary metabolism gene cluster member; coregulated with the PKS AN1784
AN1788		Has domain(s) with predicted metal ion transmembrane transporter activity, role in metal ion transport, transmembrane transport and membrane localization
AN1800	*tcsB*	Transmembrane histidine kinase, part of a two-component signal transducer involved in the HOG signaling pathway that regulates osmotic stress response' transcript upregulated by growth in glycerol
AN1803		Protein of unknown function
AN1806		Has domain(s) with predicted catalytic activity and role in nucleoside metabolic process
AN1812	*jlbA*	bZIP transcription factor; induced in response to amino acid starvation
AN1841		Protein of unknown function
AN1848	*nosA*	Zinc(II)2Cys6 putative transcription factor involved in the regulation of sexual development; mutant produces immature cleistothecia and reduced numbers of ascospores
AN1862		Protein of unknown function
AN1872		Has domain(s) with predicted hydrolase activity and role in metabolic process
AN1883		Putative argininosuccinate synthase with a predicted role in arginine metabolism; intracellular; protein abundance decreased by menadione stress
AN1885		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN1891		Putative asparaginase with a predicted role in asparagine metabolism
AN1894		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN1899	*hpdA*	Putative 4-hydroxyphenylpyruvate dioxygenase with a predicted role in aromatic amino acid biosynthesis; expression induced by phenylalanine and repressed by glucose; mutants unable to use phenylalanine as a sole carbon source

AN1915		Ortholog(s) have 2 iron, 2 sulfur cluster binding, electron transfer activity, iron-sulfur cluster binding, oxidoreductase activity, acting on NAD(P)H, oxidoreductase activity, acting on NAD(P)H, heme protein as acceptor activity
AN1923		Putative alanine transaminase with a predicted role in alanine and aspartate metabolism; intracellular, menadione stress-induced protein
AN1948	*SPA10*	Ortholog(s) have role in DNA methylation
AN1962		Protein of unknown function
AN1964		Ortholog of S. cerevisiae RPS6B and RPS6A; palA-dependent expression independent of pH
AN2000	*ubi4*	Polyubiquitin, contains four head to tail repeats of ubiqiutin; transcript upregulated in response to camptothecin
AN2002		Protein predicted to have a role in pheromone precursor processing
AN2004		Protein of unknown function
AN20054		Protein of unknown function
AN2019		Protein of unknown function
AN2024		Protein of unknown function
AN2029		Putative F-box protein
AN2036		Putative transcription factor; predicted role in secondary metabolite production; predicted secondary metabolism gene cluster member
AN2042		Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN2051	*cdc37*	Putative Hsp90p co-chaperone linked to a regulatory pathway that controls autophagy; protein repressed by and starvation- and rapamycin-induced autophagy
AN2057		Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit, mitochondrial nucleoid, mitochondrial ribosome localization
AN2059	*velC*	Velvet family protein with homology to VeA, involved in regulation of sexual development
AN2121		Has domain(s) with predicted proline racemase activity
AN2134		Protein of unknown function; transcript is induced by nitrate
AN2150		Prolyl-tRNA synthetase; protein induced by farnesol
AN2155		Ortholog(s) have 4 iron, 4 sulfur cluster binding, ATPase activity, iron ion binding activity, role in iron-sulfur cluster assembly, tRNA wobble uridine modification and Nbp35-Cfd1 ATPase complex, cytoplasm, nucleus localization
AN2157	*pepAa*	Putative aspartic endopeptidase
AN2189		Protein of unknown function
AN2194		Has domain(s) with predicted serine-type endopeptidase activity and role in proteolysis
AN2201		Predicted amino acid transmembrane transporter
AN2228		Protein of unknown function
AN2238		Has domain(s) with predicted unfolded protein binding activity and role in protein folding
AN2249		Protein of unknown function

AN2250		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN2268		Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit, mitochondrial ribosome localization
AN2277		Has domain(s) with predicted cytoplasm localization
AN2280		Ortholog(s) have role in autophagosome assembly, macroautophagy and Atg1/ULK1 kinase complex, phagophore assembly site localization
AN2290	*steA*	STE-like transcription factor with homeobox and zinc finger domains; null mutation blocks sexual cycle but not asexual development, forms Hulle cells but no ascogenous tissue nor cleistothecia
AN2305		Protein of unknown function
AN2311		Putative phosphomevalonate kinase with a predicted role in sterol metabolism
AN2312		Has domain(s) with predicted integral component of membrane localization
AN2314		Putative 1,4-alpha-glucan branching enzyme with a predicted role in starch metabolism
AN2315		Putative F1F0-ATPase complex subunit with a predicted role in energy metabolism; expression increased in salt-adapted strains
AN2325		Has domain(s) with predicted carbohydrate binding, catalytic activity and role in carbohydrate metabolic process
AN2335		Has domain(s) with predicted NADP binding, coenzyme binding, oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor, phosphogluconate dehydrogenase (decarboxylating) activity
AN2337		Protein of unknown function
AN2343		Putative nitroreductase; intracellular, menadione stress-induced protein
AN2349		Putative ATP-binding cassette (ABC) transporter of the P-glycoprotein cluster
AN2354		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN2360		Has domain(s) with predicted acid phosphatase activity, hydrolase activity, metal ion binding activity
AN2374		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN2386		Protein of unknown function
AN2390		Has domain(s) with predicted 2-oxoglutarate-dependent dioxygenase activity and role in oxidation-reduction process
AN2421	*flbC*	Putative C2H2 zinc finger transcription factor; involved in regulation of conidiophore development; required for light-dependent activation of brlA transcription
AN2426	*H4.2*	Histone H4.2, core histone protein; nearly identical to histone H4.1
AN2455		Has domain(s) with predicted zinc ion binding activity

AN2466		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN2473		Protein of unknown function
AN2475	*mstB*	Putative sugar transporter
AN2498		Protein of unknown function
AN2509		Putative tryptophan 2,3-dioxygenase with a predicted role in aromatic amino acid biosynthesis
AN2560		Protein of unknown function
AN2575		Protein of unknown function
AN2581	*hk-8-1*	Putative histidine-containing phosphotransfer protein
AN2623	*aatA*	Isopenicillin-N N-acyltransferase; null produces reduced levels of penicillin; partially redundant with aatB
AN2626		Protein of unknown function
AN2649		Protein of unknown function
AN2657		Protein of unknown function
AN2658		Protein of unknown function
AN2669		Has domain(s) with predicted role in response to stress and integral component of membrane localization
AN2674		Has domain(s) with predicted catalytic activity and role in metabolic process
AN2675		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN2682		Has domain(s) with predicted FMN binding, catalytic activity, oxidoreductase activity and role in oxidation-reduction process
AN2683		Protein of unknown function
AN2701		Ortholog(s) have role in conidiophore development, hyphal growth, sporocarp development involved in sexual reproduction, syncytium formation by plasma membrane fusion
AN2707		Ortholog(s) have amine transmembrane transporter activity, role in amine transport, ascospore wall assembly, transmembrane transport and prospore membrane localization
AN2718		Has domain(s) with predicted nucleic acid binding, zinc ion binding activity
AN2719		Has domain(s) with predicted lysozyme activity and role in cell wall macromolecule catabolic process, peptidoglycan catabolic process
AN2734		Ortholog(s) have large ribosomal subunit rRNA binding, structural constituent of ribosome activity, role in cytoplasmic translation, ribosomal large subunit assembly and cytoplasm, cytosolic large ribosomal subunit localization
AN2768		Has domain(s) with predicted role in attachment of spindle microtubules to kinetochore and DASH complex, spindle microtubule localization
AN2778		Has domain(s) with predicted heme binding activity
AN2809		Protein of unknown function
AN2822		Has domain(s) with predicted channel activity, transporter activity, role in transmembrane transport, transport and membrane localization

AN2826		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN2829	*gmdC*	Putative amidase with a predicted role in aromatic amino acid biosynthesis
AN2851		Has domain(s) with predicted L-ascorbic acid binding, iron ion binding, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen activity and role in oxidation-reduction process
AN2873	*lysA*	Putative saccharopine dehydrogenase (NAD+, L-lysine-forming) with a predicted role in lysine metabolism
AN2884		Protein of unknown function
AN2895		Has domain(s) with predicted ADP binding activity
AN2912		Protein of unknown function
AN2913		Protein of unknown function
AN2923		Protein of unknown function
AN2949		Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit, mitochondrial ribosome localization
AN2953		Protein of unknown function
AN2955		Has domain(s) with predicted role in biosynthetic process
AN2956		Protein of unknown function
AN2980		Ortholog(s) have structural constituent of ribosome activity, role in cytoplasmic translation and cytosolic large ribosomal subunit localization
AN2981	*gsdA*	Putative glucose 6-phosphate 1-dehydrogenase with a predicted role in the pentose-phosphate shunt; intracellular, menadione stress-induced protein; transcript downregulated by growth in ethanol
AN2999	*idpA*	Putative isocitrate dehydrogenase (NADP+) with a predicted role in the TCA cycle; regulated by carbon source; alternative transcription start sites specify mitochondrial or cytoplasmic and peroxisomal protein localization
AN3001	*isr1*	Has domain(s) with predicted ATP binding, protein kinase activity, protein tyrosine kinase activity and role in protein phosphorylation
AN3006		Protein of unknown function
AN3021		Protein of unknown function
AN3023		Protein of unknown function
AN3030		Alcohol dehydrogenase, class V; upregulated in A. oryzae and A. nidulans under hypoxic growth conditions
AN3047		Predicted DDE1 transposon-related ORF
AN3074		Protein of unknown function
AN3086		Protein of unknown function
AN3114		Protein of unknown function
AN3117		Ortholog(s) have copper transmembrane transporter activity, phosphorylative mechanism activity, role in cadmium ion transport, cellular copper ion homeostasis, copper ion transport, silver ion transport and plasma membrane localization

AN3147		Ortholog(s) have role in glycolipid transport, pathogenesis and intracellular localization
AN3172		Ortholog of S. cerevisiae RPS0A and RPS0B; expression reduced after exposure to farnesol
AN3180		Protein of unknown function
AN3182		Protein of unknown function
AN3193		Protein of unknown function
AN3195		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN3198		Protein of unknown function
AN3201	*lacD*	Putative beta-galactosidase with a predicted role in lactose metabolism
AN3206		Putative aryl-alcohol oxidase-related protein; protein expressed at decreased levels in a hapX mutant versus wild-type; transcript is induced by nitrate
AN3225	*CYP63 1B1*	Putative cytochrome P450; aspernidine A secondary metabolism gene cluster member
AN3226	*pkfC*	Has domain protein; aspernidine A secondary metabolism gene cluster member; protein levels decrease in response to farnesol
AN3249		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN3253	*CYP64 9A1*	Putative cytochrome P450; predicted secondary metabolism gene cluster member
AN3255		Predicted glutathione peroxidase; predicted secondary metabolism gene cluster member
AN3265	*apyA*	Arrestin domains and PY motif-containing protein with homology to Saccharomyces cerevisiae Rod1p and Rog3p proteins
AN3270		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN3279		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN3300		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN3318		Has domain(s) with predicted zinc ion binding activity and role in lipid metabolic process
AN3321		Has domain(s) with predicted aspartic-type endopeptidase activity and role in proteolysis
AN3322		Protein of unknown function
AN3344	*ngn27*	Putative GNAT-type acetyltransferase
AN3346		Protein of unknown function
AN3352	*furC*	Protein with homology to the Saccharomyces cerevisiae uracil transporter Fur4p; mutant is unaffected in uracil transport in A. nidulans
AN3356		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated

AN3380	*pkiB*	Putative fatty-acyl-CoA synthase with a predicted role in cytosolic fatty acid formation; involved in secondary metabolite production
AN3385		Putative transcription factor; predicted role in secondary metabolite production
AN3387	*gprD*	Putative G-protein coupled receptor
AN3479		Protein of unknown function
AN3490		Predicted acetyl CoA synthase; inp secondary metabolite gene cluster member
AN3498		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN3517		Protein of unknown function
AN3520		Protein of unknown function
AN3524		Putative galactose 1-dehydrogenase with a predicted role in galactonic acid and galactonate metabolism
AN3525		Protein of unknown function
AN3529		Protein of unknown function
AN3553		Protein of unknown function
AN3554		Protein of unknown function
AN3555		Small heat-shock protein; Hsp30p ortholog/paralog; expression upregulated after exposure to farnesol; palA-dependent expression independent of pH
AN3581	*trxR*	Thioredoxin reductase with a predicted role in pyrimidine metabolism; putative flavoprotein; intracellular, menadione stress-induced protein; transcripts of two different sizes have been detected
AN3582		Protein of unknown function
AN3606		Protein of unknown function
AN3632		Ortholog(s) have role in iron-sulfur cluster assembly and cytosol, membrane localization
AN3679		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN3681		Ortholog(s) have role in cellular iron ion homeostasis and fungal-type vacuole membrane localization
AN3702		Has domain(s) with predicted ATP binding, aminoacyl-tRNA editing activity, aminoacyl-tRNA ligase activity, leucine-tRNA ligase activity and role in leucyl-tRNA aminoacylation, tRNA aminoacylation for protein translation
AN3713		Protein of unknown function
AN3719	*mpkB*	MAP kinase, component of a signaling module SteD-SteC-MkkB-MpkB that controls coordination of development and secondary metabolism; phosphorylates VeA in vitro; mutant has moderate growth defect and arrested sexual development
AN3725	*awh11*	Developmentally regulated protein with similarity to Candida albicans Wh11p; transcription is repressed by StuA; expression upregulated after exposure to farnesol
AN3734		Possible pseudogene, similar to autophagy-related protein

AN3751	Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN3752	Has domain(s) with predicted intracellular localization
AN3763	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN3769	Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN3787	Ortholog(s) have metal ion binding, misfolded protein binding, peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase activity, structural constituent of cell wall activity
AN3796	Protein of unknown function
AN3823	Ortholog(s) have structural constituent of ribosome activity
AN3832	Ortholog(s) have role in mitochondrial translation and mitochondrion localization
AN3858	Possible pseudogene
AN3865	Has domain(s) with predicted ATP binding, aminoacyl-tRNA ligase activity, methionine-tRNA ligase activity, role in methionyl-tRNA aminoacylation, tRNA aminoacylation for protein translation and cytoplasm localization
AN3881	Has domain(s) with predicted ADP binding, catalytic activity and role in nucleoside metabolic process
AN3888	Ortholog(s) have basic amino acid transmembrane transporter activity
AN3891	Protein of unknown function
AN3894	Putative aconitate hydratase with a predicted role in the TCA cycle
AN3915	Has domain(s) with predicted channel activity, role in transmembrane transport and membrane localization
AN3925	Has domain(s) with predicted catalytic activity and role in carbohydrate metabolic process
AN3934	Has domain(s) with predicted GTP binding, GTPase activity, role in intracellular protein transport, protein transport, signal transduction, small GTPase mediated signal transduction and intracellular, membrane localization
AN3935	Protein of unknown function
AN3952	Has domain(s) with predicted ATP binding, ATPase activity, ATPase-coupled transmembrane transporter activity, nucleoside-triphosphatase activity, nucleotide binding activity and role in transmembrane transport
AN3958	Ortholog(s) have cytoplasm, nucleus localization
AN3960	Has domain(s) with predicted flavin adenine dinucleotide binding, oxidoreductase activity, acting on CH-OH group of donors activity and role in oxidation-reduction process
AN3979	Protein of unknown function
AN3998	Transcript induced in response to calcium dichloride in a CrzA-dependent manner

AN4051		Has domain(s) with predicted heme binding, iron ion binding, oxygen binding activity and role in oxygen transport
AN4054		Putative dehydrogenase with a predicted role in metabolism or penicillin biosynthesis
AN4061		Protein of unknown function
AN4077		Has domain(s) with predicted DNA binding activity
AN4078		Protein of unknown function
AN4082		Ortholog(s) have UDP-glucosyltransferase activity, glycogenin glucosyltransferase activity and role in glycogen metabolic process
AN4086		Ortholog(s) have phenylalanine-tRNA ligase activity, role in cellular response to drug, phenylalanyl-tRNA aminoacylation and phenylalanine-tRNA ligase complex localization
AN4087		Putative 40S ribosomal protein subunit; ortholog of S. cerevisiae Rps3p; expression reduced after exposure to farnesol
AN4088		Protein of unknown function
AN4110	*fmoB*	Has domain(s) with predicted N,N-dimethylaniline monooxygenase activity, NADP binding, flavin adenine dinucleotide binding activity and role in oxidation-reduction process
AN4113	*hk-8-2*	Histidine kinase, histidine-containing phosphotransfer protein; expression upregulated after exposure to farnesol; palA-dependent expression independent of pH
AN4131		Has domain(s) with predicted solute:proton antiporter activity, role in cation transport, transmembrane transport and integral component of membrane localization
AN4138		Protein of unknown function
AN4148	*xtrE*	Putative xylose transporter; transcriptionally induced by growth on xylose
AN4172		Protein of unknown function
AN4190		Predicted glycosylphosphatidylinositol (GPI)-anchored protein; palA-dependent expression independent of pH
AN4199		Protein of unknown function
AN4202	*rpl16a*	Predicted ribosomal protein of the large (60S) ribosomal subunit; differentially expressed during sexual development
AN4222		Ortholog(s) have cytosolic large ribosomal subunit, small-subunit processome localization
AN4250		Ortholog(s) have glycine transmembrane transporter activity, organic acid transmembrane transporter activity and role in glycine import into mitochondrion, heme biosynthetic process, mitochondrial transport
AN4264		Ortholog(s) have role in syncytium formation by plasma membrane fusion
AN4267		Protein of unknown function
AN4273		Protein of unknown function
AN4277		Ortholog(s) have glucose transmembrane transporter activity, role in cellular response to drug, glucose transmembrane transport and plasma membrane localization
AN4311		Protein of unknown function

AN4312		Protein of unknown function
AN4323		Putative branched chain amino acid aminotransferase with a predicted role in valine, leucine, and isoleucine metabolism
AN4373		Protein of unknown function
AN4376	*gdhA*	Putative NADP-linked glutamate dehydrogenase; predicted role in glutamate/glutamine metabolism; involved in nitrogen catabolite repression; induced by low nitrate; intracellular, menadione stress-induced protein; protein induced by farnesol
AN4386		Ortholog(s) have role in lipid homeostasis, mitochondrion organization and integral component of mitochondrial membrane, mitochondrial inner membrane localization
AN4402		Ortholog(s) have ubiquinone binding, voltage-gated anion channel activity
AN4408		Protein of unknown function
AN4423		Has domain(s) with predicted nucleic acid binding activity
AN4424		Has domain(s) with predicted catalytic activity, heme binding, oxidoreductase activity
AN4443	*metH*	Putative methionine synthase with a predicted role in methionine metabolism; protein expressed at increased levels in a hapX mutant versus wild-type
AN4469		Ortholog(s) have role in cellular response to biotic stimulus, cellular response to starvation, chromosome organization and filamentous growth, more
AN4475		Ortholog(s) have role in ribosomal large subunit assembly and cytosolic large ribosomal subunit localization
AN4483	*cmkD*	Predicted protein serine/threonine kinase, part of HogA mitogen-activated protein kinase (MAPK) signaling pathway, involved in regulation of stress response and development
AN4484	*SPA13*	Ortholog of A. fumigatus Af293 : Afu2g03480, A. niger CBS 513.88 : An07g07940, A. oryzae RIB40 : AO090120000236, Aspergillus wentii : Aspwe1_0109794 and Aspergillus sydowii : Aspsy1_0036785
AN4494		Ortholog(s) have cytosol, cytosolic large ribosomal subunit, hyphal cell wall, nucleus localization
AN4515	*crhB*	Putative transglycosidase with a predicted role in glucan processing; predicted glycosyl phosphatidylinositol (GPI)-anchor
AN4521	*fhpA*	Forkhead domain protein with a possible role in sexual development
AN4532		Putative catechol oxygenase
AN4544	*msgA*	Putative dual-specificity protein tyrosine/serine/threonine phosphatase
AN4582		Protein of unknown function
AN4590		Sugar transporter; transcriptionally induced by growth on xylose
AN4603		Putative allantoinase with a predicted role in purine metabolism
AN4627		Protein of unknown function
AN4645		Protein of unknown function
AN4652		Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit, mitochondrial membrane, mitochondrial ribosome localization

AN4654		Protein of unknown function
AN4688	*ivdA*	Putative acyl-coA dehydrogenase
AN4702		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN4711		Has domain(s) with predicted hydrolase activity
AN4734	*MAT2*	HMG domain mating-type protein; regulator of sexual development; acts with Mat1 alpha-domain protein; null mutant cleistothecia are sterile and Hulle cells show abnormal aggregation; gene expression is induced during sexual development
AN4787	*rpl37*	Putative ribosomal protein L37; palA-dependent expression independent of pH
AN4794		Putative ribosomal protein; expression increased in salt-adapted strains
AN4795		Has domain(s) with predicted guanyl-nucleotide exchange factor activity, role in small GTPase mediated signal transduction and intracellular localization
AN4807		Protein of unknown function
AN4810		Has domain(s) with predicted hydrolase activity and role in metabolic process
AN4812		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN4817		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN4825		Putative glucan 1,3-beta-glucosidase with a predicted role in glucan metabolism
AN4848		Protein of unknown function
AN4863		Ortholog(s) have copper chaperone activity, role in mitochondrial respiratory chain complex IV assembly, protein maturation by copper ion transfer and cytosol, mitochondrial intermembrane space localization
AN4872	*ubi1*	Fusion protein consisting of N-terminal ubiquitin and C-terminal extension protein (CEP) of the small ribosomal subunit; transcript upregulated in response to camptothecin
AN4877		Predicted DDE1 transposon-related ORF
AN4880		Protein of unknown function
AN4887	*bckA*	Putative mitogen-activated protein kinase kinase kinase (MAPKKK); inviable mutant arrests as branched germlings, remediated by NaCl or sucrose
AN4901		Putative glutaminase A with a predicted role in glutamate and glutamine metabolism
AN4909		Protein of unknown function
AN4912		Ortholog(s) have role in cellular response to drug, lipid translocation, sphingoid metabolic process and fungal-type vacuole, membrane raft, plasma membrane localization
AN4923		Putative 3-hydroxy-3-methylglutaryl coenzyme A synthase with a predicted role in sterol metabolism; protein expressed at decreased levels in a hapX mutant versus wild-type; expression reduced after exposure to farnesol
AN4926		Protein of unknown function

AN4927		Putative F1F0-ATPase complex subunit with a predicted role in energy metabolism
AN4941		Protein of unknown function
AN4953		Putative Rho-like GTPase
AN4956		Large subunit of acetolactate synthase involved in branched-chain amino acid biosynthesis under hypoxic conditions
AN4990		Ortholog(s) have ferrous iron transmembrane transporter activity, manganese ion transmembrane transporter activity
AN4998	*gapA*	Putative Ras GTPase-activating protein; required for normal cell polarity and conidiophore development
AN5008		Ortholog(s) have copper ion binding activity, role in cellular copper ion homeostasis, mitochondrial respiratory chain complex IV assembly and mitochondrial intermembrane space localization
AN5028	*ppoC*	Fatty acid oxygenase that plays a role in oxylipin biosynthesis; responsible for the formation of the psi-factor component psiB-beta; expression reduced after exposure to farnesol
AN5046		Anisin-1; secreted defensin-like peptide with a predicted role in defense response; high homology to the mosquito defensin AaDefA1
AN5067		Putative transporter of the major facilitator superfamily (MFS); expression reduced after exposure to farnesol
AN5074		Protein of unknown function
AN5076		Has domain(s) with predicted role in cell wall macromolecule catabolic process
AN5082		Has domain(s) with predicted GTP binding, GTPase activity, structural constituent of cytoskeleton activity, role in microtubule-based process, protein polymerization and microtubule localization
AN5083		Has domain(s) with predicted proton transmembrane transporter activity, role in proton transmembrane transport and proton-transporting V-type ATPase, V0 domain, vacuolar proton-transporting V-type ATPase, V0 domain localization
AN5111		Has domain(s) with predicted nucleic acid binding, zinc ion binding activity
AN5148		Ortholog(s) have structural constituent of ribosome activity, role in cellular response to drug and mitochondrial ribosome, mitochondrial small ribosomal subunit localization
AN5155		Ortholog(s) have tRNA-specific adenosine-34 deaminase activity, role in tRNA wobble adenosine to inosine editing and tRNA-specific adenosine-34 deaminase complex localization
AN5164		Ortholog(s) have structural constituent of ribosome activity and mitochondrial small ribosomal subunit localization
AN5169		Ortholog(s) have role in syncytium formation by plasma membrane fusion
AN5175		Ortholog(s) have mitochondrial outer membrane localization
AN5177		Ortholog(s) have role in ribosomal large subunit biogenesis
AN5181	*nudC*	Protein involved in nuclear migration; interacts directly with NudF at spindle-pole bodies; also localizes to hyphal cortex; null mutants inviable

		except at low temperatures or high osmolarity; null mutants display thickened cell walls
AN5199		Has domain(s) with predicted aminopeptidase activity, metalloexopeptidase activity, zinc ion binding activity and role in cellular process, proteolysis
AN5206	*lysB*	Putative homoisocitrate dehydrogenase with a predicted role in lysine metabolism
AN5213		Ortholog(s) have cell cortex of cell tip localization
AN5229		Protein of unknown function
AN5258		Ortholog(s) have role in fumiquinazoline C biosynthetic process, secondary metabolite biosynthetic process and fungal-type cell wall localization
AN5274		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN5283		Protein of unknown function
AN5298		Protein of unknown function
AN5302		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN5317		Protein of unknown function
AN5328		Has domain(s) with predicted catalytic activity, ferric iron binding, iron ion binding, oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen activity
AN5331		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN5347		Has domain(s) with predicted cation transmembrane transporter activity, role in cation transport, transmembrane transport and integral component of membrane localization
AN5354		Has domain(s) with predicted oxidoreductase activity, transferase activity, transferring acyl groups other than amino-acyl groups activity and role in oxidation-reduction process
AN5361		Putative beta-glucuronidase with a predicted role in carbohydrate catabolism
AN5394		Has domain(s) with predicted metal ion transmembrane transporter activity, role in metal ion transport, transmembrane transport and membrane localization
AN5396		Protein of unknown function
AN5402		Has domain(s) with predicted role in lipid metabolic process, metabolic process
AN5408		Has domain(s) with predicted RNA binding, ribonuclease III activity and role in RNA processing
AN5413		Protein of unknown function
AN5421		Protein of unknown function
AN5423		Protein of unknown function

AN5429		Protein of unknown function
AN5435		Has domain(s) with predicted oxidoreductase activity, oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor activity and role in oxidation-reduction process
AN5441		Ortholog(s) have role in endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA and LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA), rRNA export from nucleus
AN5453	*artG*	Arrestin-like protein
AN5458		Ortholog(s) have role in syncytium formation by plasma membrane fusion
AN5464		Protein of unknown function
AN5487		Protein of unknown function
AN5490		Protein of unknown function
AN5492		Has domain(s) with predicted deaminase activity and role in purine ribonucleoside monophosphate biosynthetic process
AN5497		Ortholog(s) have 3,4-dihydroxy-2-butanone-4-phosphate synthase activity, role in aerobic respiration, riboflavin biosynthetic process and cytosol, fungal biofilm matrix, mitochondrial intermembrane space localization
AN5501		Ortholog(s) have lipase activity
AN5509		Putative F-box protein
AN5520		Ortholog(s) have role in maturation of LSU-rRNA and cytosolic large ribosomal subunit localization
AN5553	*CYP5080D1*	Putative cytochrome P450
AN5565		Ortholog(s) have lipase activity
AN5580		Protein of unknown function
AN5626	*facA*	Acetyl-CoA synthase, required for utilization of acetate as a carbon source; transcriptional induction by acetate is mediated by FacB; carbon catabolite repression is mediated by CreA
AN5647		Protein of unknown function
AN5665	*CYP531D2*	Putative cytochrome P450
AN5667		Protein of unknown function
AN5669		Putative succinyl-CoA:3-ketoacid-coenzyme A transferase
AN5678		Ortholog(s) have L-lysine transmembrane transporter activity, L-proline transmembrane transporter activity, amino acid transmembrane transporter activity and arginine transmembrane transporter activity, more
AN5715		Putative 40s ribosomal protein S26; ortholog of S. cerevisiae Rps26Bp which has role in rRNA export from nucleus; expression reduced after exposure to farnesol
AN5719		Ortholog(s) have protein kinase activator activity, structural constituent of ribosome activity, role in cytoplasmic translation, positive regulation

		of protein kinase activity and cytosolic large ribosomal subunit, ribosome localization
AN5741		Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit, mitochondrial membrane, mitochondrial ribosome localization
AN5759	*stk19*	Ortholog(s) have role in ascospore formation, sporocarp development involved in sexual reproduction
AN5764		Transcript induced by light in in developmentally competent mycelia
AN5768		Ortholog(s) have role in actin cytoskeleton organization, inositol lipid-mediated signaling, negative regulation of phospholipid translocation, vacuole organization and plasma membrane localization
AN5781		Putative 30 kilodalton heat shock protein; transcript levels increase during the unfolded-protein response (UPR); palA-dependent expression independent of pH
AN5818		Has domain(s) with predicted role in (1->6)-beta-D-glucan biosynthetic process, cell wall biogenesis and extracellular region localization
AN5821		Putative vacuolar H+/Ca++ exchanger; transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN5832	*rasB*	Putative Ras GTPase
AN5847		Protein of unknown function
AN5859		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN5867		Ortholog(s) have acetyl-CoA:L-glutamate N-acetyltransferase activity, role in ornithine biosynthetic process and mitochondrial matrix, mitochondrial membrane localization
AN5878		Has domain(s) with predicted catalytic activity, transferase activity, transferring acyl groups other than amino-acyl groups activity and role in metabolic process
AN5885	*agsA*	Catalytic subunit of alpha-1,3 glucan synthase complex; plays a minor role in alpha-1,3 glucan synthesis compared to AgsB; locus contains the conserved upstream open reading frame (uORF) AN5885-uORF
AN5917		Ortholog(s) have alpha-glucoside:proton symporter activity, maltose:proton symporter activity, trehalose transmembrane transporter activity and role in disaccharide catabolic process, maltose transport, trehalose transport
AN5927		Protein of unknown function
AN5937		Has domain(s) with predicted manganese ion binding, nutrient reservoir activity
AN5939		Putative 5'-nucleotidase with a predicted role in nucleotide salvage pathways; predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN5944		Protein of unknown function
AN5957		Putative branched chain amino acid aminotransferase with a predicted role in valine, leucine, and isoleucine metabolism

AN5960		Ortholog(s) have mRNA binding activity and role in maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA), ribosomal small subunit assembly
AN5962		Has domain(s) with predicted nucleic acid binding activity
AN5978		Ortholog(s) have mitochondrial ribosome localization
AN5979		Ortholog(s) have role in ribosomal small subunit assembly
AN5990		Putative long-chain-fatty-acid-CoA ligase with a predicted role in fatty acid metabolism
AN5996		Ortholog(s) have protein kinase activator activity, structural constituent of ribosome activity, role in cytoplasmic translation, positive regulation of protein kinase activity and cytosolic large ribosomal subunit, ribosome localization
AN5999		Carbamoyl-phosphate synthase, large subunit; predicted role in arginine or pyrimidine metabolism; protein induced by farnesol
AN6002	*aptC*	Putative monooxygenase; asperthecin (apt) gene cluster member required for asperthecin biosynthesis
AN6023		Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN6046		Putative p67phox regulatory subunit homolog with a predicted role in regulating hyphal reactive oxygen species (ROS) production; required for normal sexual and asexual development
AN6050		Protein of unknown function
AN6063		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN6082		Ortholog(s) have RNA binding, pre-mRNA 5'-splice site binding, structural constituent of ribosome activity and role in negative regulation of mRNA splicing, via spliceosome, rRNA processing
AN6089		Putative 60 kilodalton heat shock protein
AN6095	*jenA*	Short-chain carboxylic acid transporter involved in uptake of lactate, succinate, pyruvate and malate
AN6116		Protein of unknown function
AN6137		Protein of unknown function
AN6146		Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit localization
AN6159		Putative 1-acylglycerol-3-phosphate acyltransferase with a predicted role in glycerolipid metabolism
AN6167		Has domain(s) with predicted FMN binding, catalytic activity, oxidoreductase activity and role in oxidation-reduction process
AN6168	*maeA*	Putative malate dehydrogenase with a predicted role in oxidation of malate to pyruvate
AN6169		Protein of unknown function
AN6211	*plaA*	Putative phospholipase with a predicted role in phospholipid metabolism; calcium-dependent phospholipase A2 activity
AN6236	*sidD*	Nonribosomal peptide synthetase (NRPS); predicted backbone enzyme of a siderophore secondary metabolism biosynthetic gene cluster member

AN6272		Ortholog(s) have FAD binding, ferredoxin-NAD+ reductase activity, ferredoxin-NADP+ reductase activity
AN6277		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN6286		Has domain(s) with predicted mannosyl-oligosaccharide glucosidase activity and role in oligosaccharide metabolic process
AN6297		Ortholog(s) have copper ion binding activity and role in aerobic respiration, cellular protein-containing complex assembly
AN6315		Protein of unknown function
AN6320	*ppoB*	Fatty acid oxygenase involved in oxylipin biosynthesis; null mutant overproduces sterigmatocystin and penicillin, displays increased conidial production and decreased ascospore production, accumulation of psiBbeta decreased
AN6322		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN6327		Protein of unknown function
AN6367		Has domain(s) with predicted phosphatidylinositol phosphate kinase activity and role in phosphatidylinositol metabolic process
AN6369		Has domain(s) with predicted ATP binding, ATPase activity, nucleoside-triphosphatase activity, nucleotide binding activity and membrane localization
AN6370		Protein of unknown function
AN6376		Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit localization
AN6379		Protein of unknown function
AN6386		Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN6404		Has domain(s) with predicted zinc ion binding activity
AN6410		Protein of unknown function; transcript is induced by nitrate
AN6412	*xtrA*	Putative xylose transporter
AN6418		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN6419		Protein of unknown function
AN6426		Has domain(s) with predicted metallopeptidase activity
AN6428		Putative beta-1,4-endoglucanase
AN6431		Putative polyketide synthase; predicted backbone enzyme of a secondary metabolism gene cluster member; coregulated with AN6432
AN6432		Protein of unknown function; has domain(s) with predicted nucleotide binding activity; coregulated with the PKS AN6431; predicted secondary metabolism gene cluster member
AN6436		Has domain(s) with predicted ATP binding, ATPase activity, ATPase-coupled transmembrane transporter activity, nucleoside-triphosphatase activity, nucleotide binding activity and role in transmembrane transport
AN6437		Protein of unknown function

AN6446	*cicD*	Predicted transcription factor; coregulated with the NRPS/AN6444; encoded within the cichorine gene cluster; possible transcriptional regulator of cluster
AN6450		Tetrahydroxynaphthalene reductase; role in melanin biosynthesis;; coregulated with the PKS pkbA/AN6448; predicted secondary metabolism gene cluster member
AN6459		Has domain(s) with predicted UDP-N-acetylmuramate dehydrogenase activity, flavin adenine dinucleotide binding, oxidoreductase activity and role in oxidation-reduction process
AN6460		Protein of unknown function
AN6470		Protein with lysozyme activity, involved in carbohydrate catabolism
AN6471		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN6472	*dfgF*	Putative endo-mannanase GH76 family protein
AN6525	*aciA*	Putative formate dehydrogenase with a predicted role in oxalic acid metabolism; intracellular; protein abundance decreased by menadione stress; inducible by acetate; expression reduced after exposure to farnesol
AN6565		Protein of unknown function
AN6589		Ortholog(s) have mannose-ethanolamine phosphotransferase activity, transferase activity, transferring phosphorus-containing groups activity
AN6596		Protein of unknown function
AN6624		Protein of unknown function
AN6629		Putative ribosomal protein L14; ortholog of S. cerevisiae Rpl14Ap; expression reduced after exposure to farnesol
AN6632		Putative 40S ribosomal protein S28; ortholog of S. cerevisiae Rps28Bp; expression reduced after exposure to farnesol
AN6640		Has domain(s) with predicted hydrolase activity and role in metabolic process
AN6643	*bioB*	Putative biotin synthase with a predicted role in Coenzyme A and pantothenate biosynthesis
AN6645	*bioF*	Putative 8-amino-7-oxononanoate synthase with a predicted role in Coenzyme A and pantothenate biosynthesis
AN6658		Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN6659		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN6669	*mstC*	High-affinity glucose transporter active in germinating conidia
AN6693		Protein of unknown function; transcript repressed by nitrate
AN6697	*sunA*	Putative Sun-family protein
AN6703	*jenB*	Short-chain carboxylic acid transporter involved in uptake of lactate, succinate, pyruvate and malate
AN6723	*dhbD*	Putative 2,3-dihydroxybenzoate carboxylyase
AN6727		Protein of unknown function
AN6739		Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN6747		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and

		role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN6752	*aoxA*	Putative multifunctional enzyme with a predicted role in fatty acid degradation; required for beta-oxidation
AN6755		Putative acyl-coA dehydrogenase
AN6757		Has domain(s) with predicted role in transmembrane transport and membrane localization
AN6761		Putative acyl-coA dehydrogenase
AN6767		Has domain(s) with predicted catalytic activity and role in metabolic process
AN6772		Protein of unknown function
AN6774		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN6787	*CYP682C1*	Putative cytochrome P450
AN6794		Protein of unknown function
AN6796		Ortholog(s) have intracellular localization
AN6818		Protein of unknown function
AN6823		Protein of unknown function
AN6835	*CYP505A8*	Putative cytochrome P450; expression upregulated after exposure to farnesol
AN6836		Protein of unknown function
AN6843		Mitochondrial ribosomal protein L4; this locus is reported to contain an upstream open reading frame (uORF)
AN6846		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN6849		Ortholog(s) have role in cellular response to heat, cellular response to hydrogen peroxide
AN6857		Ortholog(s) have alpha-1,2-mannosyltransferase activity, role in protein glycosylation and Golgi apparatus localization
AN6866	*aroC*	Putative chorismate mutase with a predicted role in aromatic amino acid biosynthesis
AN6873		Protein of unknown function
AN6881		Protein of unknown function
AN6885		Protein of unknown function
AN6891		Protein of unknown function
AN6904		Ortholog(s) have structural constituent of ribosome activity and role in cellular respiration, regulation of mitochondrial DNA metabolic process, response to oxidative stress
AN6921		Ortholog(s) have chaperone binding activity and role in negative regulation of DNA binding, positive regulation of telomere maintenance via telomerase, protein folding, regulation of telomerase activity
AN6930		Has domain(s) with predicted catalytic activity, pyridoxal phosphate binding, transaminase activity

AN6934		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN6936		Putative 2-hydroxychromene-2-carboxylate isomerase
AN6941		Protein of unknown function
AN6948	*crhE*	Putative transglycosidase with a predicted role in glucan processing
AN6954		Protein of unknown function
AN6963		Ortholog(s) have flavin-linked sulfhydryl oxidase activity and role in oxidation-reduction process
AN7003		Ortholog(s) have cell surface, cytosolic large ribosomal subunit localization
AN7010		Has domain(s) with predicted catalytic activity and role in biosynthetic process
AN7018		Protein of unknown function
AN7029		Ortholog(s) have role in cellular response to oxidative stress, protein import into peroxisome matrix, protein quality control for misfolded or incompletely synthesized proteins and mitochondrial inner membrane localization
AN7033		Protein of unknown function
AN7053		Protein of unknown function
AN7071	*pkgA*	Putative polyketide synthase; involved in the production of alternariol and other secondary metabolites; predicted backbone enzyme of a secondary metabolism gene cluster
AN7098		Protein of unknown function
AN7101		Protein of unknown function
AN7131	*CYP52 H1*	Putative cytochrome P450
AN7149		Ortholog(s) have role in nucleobase-containing compound transport, regulation of fungal-type cell wall organization, regulation of phospholipid translocation and plasma membrane localization
AN7189		Ortholog(s) have DNA-binding transcription factor activity
AN7194		Has domain(s) with predicted oxidoreductase activity, zinc ion binding activity and role in oxidation-reduction process
AN7200		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN7201		Ortholog(s) have role in proteolysis
AN7223		Has domain(s) with predicted DNA-binding transcription factor activity, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated
AN7225		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN7228		Protein of unknown function
AN7232		Protein of unknown function
AN7233		Putative epoxide hydrolase; expression reduced after exposure to farnesol

AN7252	*steD*	Component of the MAP kinase signaling module that includes SteC, MkkB, MpkB, and controls coordination of development and secondary metabolism
AN7253		Protein of unknown function
AN7265		Has domain(s) with predicted role in mycotoxin biosynthetic process
AN7269		Ortholog(s) have role in fumiquinazoline C biosynthetic process, secondary metabolite biosynthetic process and fungal-type cell wall localization
AN7271		Protein of unknown function
AN7295		Putative transmembrane transporter; upregulated in A. oryzae and A. nidulans under hypoxic growth conditions
AN7329		Protein of unknown function
AN7330		Protein of unknown function
AN7343		Putative Zn(II)2Cys6-domain containing transcription factor; transcript is induced by nitrate
AN7351		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, plasma membrane localization
AN7352		Protein of unknown function
AN7354		Ortholog(s) have structural constituent of ribosome activity, role in translation and cytosolic large ribosomal subunit localization
AN7362		Has domain(s) with predicted nucleic acid binding, zinc ion binding activity
AN7364		Has domain(s) with predicted 2 iron, 2 sulfur cluster binding, oxidoreductase activity and role in oxidation-reduction process
AN7375		Has domain(s) with predicted role in lipid biosynthetic process
AN7389		Putative laccase related protein
AN7414		Protein of unknown function
AN7418		Has domain(s) with predicted FAD binding, oxidoreductase activity and role in metabolic process
AN7419		Protein of unknown function
AN7425		Has domain(s) with predicted lipid transporter activity, role in lipid transport and integral component of membrane localization
AN7437		Ortholog(s) have L-amino acid transmembrane transporter activity and role in L-alpha-amino acid transmembrane transport, amino acid transmembrane export from vacuole
AN7452		Protein of unknown function
AN7457		Protein of unknown function
AN7463	*meaA*	Major ammonium transporter of A. nidulans; transcript upregulated by nitrate limitation
AN7467		Protein of unknown function
AN7476		Protein of unknown function
AN7500	*ndeA*	Putative NADH dehydrogenase (ubiquinone) with a predicted role in energy metabolism; expression upregulated after exposure to farnesol
AN7502		Putative uridine kinase with a predicted role in pyrimidine metabolism; expression reduced after exposure to farnesol

AN7504		Protein of unknown function
AN7509		Protein of unknown function
AN7511	*gelE*	Putative 1,3-beta-transglycosidase with a predicted role in glucan processing; predicted glycosyl phosphatidylinositol (GPI)-anchor
AN7523		Protein of unknown function
AN7552		Protein of unknown function
AN7580		Protein of unknown function
AN7585		Has domain(s) with predicted transmembrane transporter activity and role in transmembrane transport
AN7590		Putative reductase with a predicted role in carbohydrate metabolism; mannitol 2-dehydrogenase; intracellular, menadione stress-induced protein; HapX-regulated; protein induced by farnesol
AN7594		DUF636 domain-containing protein; intracellular, menadione stress-induced protein; protein levels decrease in response to farnesol
AN7608		Has domain(s) with predicted DNA-directed 5'-3' RNA polymerase activity, role in transcription, DNA-templated and nucleus localization
AN7625		Putative myo-inositol-1-phosphate synthase with a predicted role in phospholipid metabolism; intracellular, menadione stress-induced protein; palA-dependent expression independent of pH
AN7644		Protein of unknown function
AN7657	*gelA*	Putative 1,3-beta-transglycosidase with a predicted role in glucan processing; predicted glycosyl phosphatidylinositol (GPI)-anchor; palA-dependent expression independent of pH
AN7670		Protein of unknown function
AN7672		Ortholog(s) have alpha-1,6-mannosyltransferase activity, mannosyltransferase activity
AN7687		Ortholog(s) have preprotein binding activity and role in conidium formation, hyphal growth, mitochondrion organization, protein targeting to mitochondrion
AN7690		Protein of unknown function
AN7697	*sskA*	Response regulator, part of a two-component signal transducer involved in the HOG signaling pathway that regulates osmotic stress response; transcript induced by hydrogen peroxide; null spores are heat labile and lose viability at 4 degrees
AN7710		Ortholog(s) have intracellular localization
AN7717		Protein of unknown function
AN7722		Putative N-acetyltransferase with a predicted role in arginine metabolism
AN7772	*CYP5080B1*	Putative cytochrome P450; transcript repressed by nitrate
AN7773	*CYP573A3*	Putative cytochrome P450
AN7779		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN7782		Protein of unknown function
AN7797		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization

AN7800	*mirA*	Siderophore iron transporter
AN7822	*stcS*	Ortholog of A. fumigatus Af293 : Afu5g00770, Aspergillus brasiliensis : Aspbr1_0058805, N. fischeri NRRL 181 : NFIA_041410, Aspergillus flavus NRRL 3357 : AFL2T_07218 and A. clavatus NRRL 1 : ACLA_004530
AN7832		Has domain(s) with predicted flavin adenine dinucleotide binding, oxidoreductase activity, acting on CH-OH group of donors activity and role in oxidation-reduction process
AN7834		Protein of unknown function
AN7847		Has domain(s) with predicted role in cell wall macromolecule catabolic process
AN7872		Predicted transcription factor; predicted secondary metabolism gene cluster member
AN7892		Small heat-shock protein; molecular chaperone; expression upregulated after exposure to farnesol
AN7895	*cipB*	Putative oxidoreductase; contains Zn-dependent alcohol dehydrogenase domain; protein expressed at increased levels during osmoadaptation
AN7912	*orsC*	Putative tyrosinase; member of the F9775 secondary metabolite gene cluster
AN7927		Protein of unknown function
AN7954		Ortholog(s) have intracellular localization
AN7959		Protein of unknown function
AN7986	*ffkA*	Has domain(s) with predicted ATP binding, protein kinase activity, protein tyrosine kinase activity and role in protein phosphorylation
AN7995		Putative ribokinase with a predicted role in ribose metabolism
AN8010		Putative glycogen (starch) synthase with a predicted role in glycogen biosynthesis
AN8026		Protein of unknown function
AN8041	*gpdA*	Glyceraldehyde-3-phosphate dehydrogenase with a predicted role in gluconeogenesis and glycolysis; the gpdA promoter is a commonly used regulatory sequence for driving constitutive heterologous gene expression
AN8078	*phacA*	Phenylacetate 2-hydroxylase; cytochrome P450 monooxygenase involved in phenylacetate utilization; transcript is induced by phenylacetate
AN8079		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, protein kinase regulator activity, zinc ion binding activity
AN8085		Protein of unknown function
AN8095		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN8101		Protein of unknown function
AN8103		Putative transcription factor; predicted role in secondary metabolite production
AN8118		Putative cytochrome c oxidase subunit with a predicted role in energy metabolism

AN8122		Ortholog(s) have role in cellular response to drug, hexose transmembrane transport, pathogenesis
AN8134		Has domain(s) with predicted transferase activity, transferring acyl groups other than amino-acyl groups activity
AN8154		Protein of unknown function
AN8166		Protein of unknown function
AN8167		Protein of unknown function
AN8174		Protein of unknown function
AN8176		Has domain(s) with predicted structural constituent of ribosome activity, role in translation and ribosome localization
AN8235		Protein of unknown function
AN8241	*chiA*	Endochitinase with a predicted role in chitin hydrolysis; glycosylphosphatidylinositol (GPI) anchored protein; modiﬁed by O-linked glycosylation; localized to germination sites, hyphal branch points and regions of polarized growth
AN8262	*gprH*	Secretin-like G-protein coupled receptor, involved in nutrient sensing and control of sexual development
AN8265		Protein of unknown function
AN8277	*cysD*	Putative bifunctional enzyme with a predicted role in methionine metabolism; O-acetlylhomoserine (homocysteine synthase)
AN8279		Ortholog of S. cerevisiae Can1p which has arginine transmembrane transporter activity; basic amino acid transporter; expression reduced after exposure to farnesol
AN8298		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN8311		Protein of unknown function
AN8321		Protein of unknown function
AN8346		Ortholog(s) have FAD binding, sulfide:quinone oxidoreductase activity
AN8361		Protein of unknown function
AN8362		Protein of unknown function
AN8365		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN8369		Has domain(s) with predicted role in biosynthetic process
AN8373	*ngn11*	Putative GNAT-type acetyltransferase
AN8374		Ortholog(s) have oligopeptide transmembrane transporter activity
AN8403		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN8421	*dfgB*	Putative endo-mannanase GH76 family protein; role in polysaccharide degradation
AN8428		Protein of unknown function
AN8433		Has domain(s) with predicted catalytic activity and role in metabolic process
AN8437	*CYP5125A*	Putative cytochrome P450

AN8441		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN8460		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN8478		Protein of unknown function
AN8495		Protein of unknown function
AN8503		Protein of unknown function
AN8507		Ortholog(s) have role in secondary metabolite biosynthetic process
AN8512		Protein of unknown function; adjacent to tdi (terrequinone biosynthesis) gene cluster. Not required for terrequinone biosynthesis
AN8514	*tdiB*	Asterriquinone prenyltransferase; member of the tdi gene cluster; required for terrequinone A production; catalyzes the reverse prenylation event during terrequinone A biosynthesis; lacks canonical prenyl diphosphate binding motif (D/N)DXXD
AN8518	*tdiC*	Similar to NADPH-dependent quinone reductases; member of the tdi (terrequinone A biosynthesis) gene cluster; transcriptionally regulated by LaeA
AN8524		Has domain(s) with predicted catalytic activity and role in nucleoside metabolic process
AN8525		Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN8529		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN8530		Ortholog(s) have catalytic activity and role in steroid metabolic process
AN8532		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN8533		Has domain(s) with predicted catalytic activity and role in nucleoside metabolic process
AN8539	*ngn26*	Ortholog of A. fumigatus SidG; triacetylfusarinine C (TAFC) siderophore biosynthetic transacetylase; transcript induced by light in developmentally competent mycelia
AN8548		Protein of unknown function
AN8549		Protein of unknown function
AN8556		Has domain(s) with predicted role in transmembrane transport and membrane localization
AN8565	*cysA*	Putative serine O-acetyltransferase with a predicted role in cysteine metabolism
AN8567		Protein of unknown function
AN8573		Protein of unknown function
AN8595		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN8601		Protein of unknown function

AN8602		Protein of unknown function
AN8606	*gudA*	Putative glucose 1-dehydrogenase
AN8611		Has domain(s) with predicted catalytic activity and role in nucleoside metabolic process
AN8612		Protein of unknown function
AN8615	*CYP677A1*	Putative cytochrome P450
AN8625		Has domain(s) with predicted role in mycotoxin biosynthetic process
AN8640	*conF*	Ortholog of N. crassa conF, light-induced transcript expressed during conidiation in N. crassa; double conF conJ deletion results in increased cellular glycerol or erythritol leading to delayed germination and desiccation resistance
AN8661		Protein of unknown function
AN8665		Protein of unknown function
AN8666		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN8683		Ortholog(s) have ferric-chelate reductase activity, role in copper ion import, iron ion transport and plasma membrane localization
AN8692	*prxA*	Thioredoxin-dependent peroxidase; intracellular; PRX5-like domain; highly similar to the allergen Aspf3 from related fungi; menadione stress-repressed protein; osmoadaptation-induced protein; repressed by starvation-induced autophagy
AN8694		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN8732		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN8734		Protein of unknown function
AN8774		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN8777	*amdS*	Acetamidase, produces ammonium and acetate from acetamide, allowing utilization of acetamide as sole carbon or nitrogen source; transcript induced under low nitrogen conditions
AN8778		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN8781		Protein of unknown function
AN8794		Ortholog(s) have structural molecule activity and mitochondrial large ribosomal subunit, mitochondrial ribosome localization
AN8819		Putative dehydrogenase with a predicted role in carbohydrate metabolism
AN8830	*halA*	Predicted protein kinase involved in halotolerance; suppressor of molybdate sensitivity of pacC mutant; mutants are sensitive to NaCl and have a moderate growth defect

AN8833	*llmI*	Putative LaeA-like methyltransferase
AN8842	*mid1*	Putative stretch-activated calcium channel; predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN8859		Putative aspartate kinase with a predicted role in glycine, serine, and threonine metabolism
AN8903		Putative peptide transporter; transcript upregulated by nitrate limitation
AN8918		Putative transcription factor; predicted role in secondary metabolite production
AN8933		Protein of unknown function
AN8938		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN8943		Protein of unknown function
AN8953	*agdB*	Putative alpha-glucosidase with a predicted role in maltose metabolism; transcriptionally induced by isomaltose; induced by rapamycin-induced autophagy
AN8955		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN8957		Has domain(s) with predicted ATP binding, ATPase activity, ATPase-coupled transmembrane transporter activity, nucleoside-triphosphatase activity, nucleotide binding activity and role in transmembrane transport
AN8970		Protein of unknown function
AN8971		Putative integral membrane protein
AN8972		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN8977	*alcP*	Putative gluconolactonase with a predicted role in gluconic acid and gluconate metabolism; transcript upregulated by exposure to ethanol
AN8983		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN8989		Protein of unknown function
AN8994		Protein of unknown function
AN9001		Protein of unknown function
AN9003		Has domain(s) with predicted zinc ion binding activity
AN9004		Predicted monooxygenase
AN9007	*CYP548D1*	Putative cytochrome P450; predicted secondary metabolism gene cluster member
AN9021		Protein of unknown function
AN9025		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, nucleic acid binding, zinc ion binding activity
AN9028		Protein of unknown function
AN9069		Protein of unknown function
AN9071		Protein of unknown function

AN9096		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN9103	*aifA*	Putative apoptosis-inducing factor (AIF)-like mitochondrial oxidoreductase; mutants display decreased survival in the presence of farnesol or menadione, decreased electron transport; expression upregulated after exposure to farnesol
AN9105	*artD*	Arrestin-like protein
AN9106		Ortholog(s) have triglyceride lipase activity, role in triglyceride catabolic process and mitochondrion localization
AN9108		Has domain(s) with predicted heme binding activity
AN9121	*esdC*	Protein with a glycogen binding domain involved in sexual development; regulated by VeA and FlbA
AN9141		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN9145		Protein of unknown function
AN9151		Ortholog(s) have role in Group I intron splicing and mitochondrion localization
AN9152		Has domain(s) with predicted catalytic activity, coenzyme binding activity and role in cellular metabolic process
AN9156		Protein of unknown function
AN9159		Protein of unknown function
AN9168		Ortholog(s) have solute:proton symporter activity, role in glycerol transport, transmembrane transport and plasma membrane localization
AN9174		Has domain(s) with predicted role in transmembrane transport and membrane localization
AN9183	*bglR*	Putative beta-glucosidase with a predicted role in polysaccharide degradation
AN9184		Ortholog(s) have plasma membrane localization
AN9204	*ngn6*	Putative GNAT-type acetyltransferase
AN9206		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN9240		Putative C2H2 transcription factor; transcript repressed by light in developmentally competent mycelia
AN9243		Has domain(s) with predicted catalytic activity and role in metabolic process
AN9244		Putative nonribosomal peptide synthase
AN9285	*ccgA*	Ortholog of A. fumigatus grg1; homologous to ccg-1 from N. crassa; transcript induced by light in developmentally competent mycelia
AN9288		Has domain(s) with predicted oxidoreductase activity, transferase activity, transferring acyl groups other than amino-acyl groups, zinc ion binding activity and role in oxidation-reduction process
AN9292		Ortholog(s) have role in positive regulation of secondary metabolite biosynthetic process, regulation of secondary metabolic process, secondary metabolite biosynthetic process

AN9304		Glutathione S-transferase; upregulated in A. oryzae and A. nidulans under hypoxic growth conditions
AN9355		Protein of unknown function
AN9364		Protein of unknown function
AN9389		Has domain(s) with predicted role in cell wall macromolecule catabolic process
AN9397	*hacA*	Putative basic leucine zipper (bZIP) transcription factor that regulates the unfolded protein response; hacA mRNA expression increased in the presence of farnesol
AN9425		Has domain(s) with predicted carbon-carbon lyase activity, catalytic activity and role in cellular aromatic compound metabolic process
AN9449		Has domain(s) with predicted catalytic activity, metal ion binding, phosphoric diester hydrolase activity
AN9465		Ortholog(s) have structural constituent of ribosome activity, role in cytoplasmic translation and cytosolic large ribosomal subunit localization
AN9529		Has domain(s) with predicted role in regulation of store-operated calcium entry and integral component of endoplasmic reticulum membrane localization

Table S3-4. Direct target genes of LaeA in *A. nidulans* Vege

Gene ID	Gene Name	Description
AN0034		Putative glycerone kinase with a predicted role in glycerol metabolism; transcript upregulated by growth in glycerol
AN0055	*tmpA*	Transmembrane protein involved in regulation of conidium development; required for expression of brlA; predicted oxidoreductase with FAD-binding and haem-binding domains
AN0078	*samB*	MYND zinc-finger protein; mutation causes polarity defects, abnormal hyphal branching and septation, abnormal germling morphology; mutation suppresses aconidial phenotype of apsA mutant; C-terminal zinc-fingers required for function
AN0170	*trxA*	Thioredoxin; predicted role in cell redox homeostasis; required for conidiation; expression upregulated after exposure to farnesol
AN0214		Protein of unknown function
AN0223		Has domain(s) with predicted DNA-binding transcription factor activity, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated
AN0259		Putative adenylate kinase with a predicted role in nucleotide salvage pathways
AN0283		Protein of unknown function
AN0356		Protein of unknown function
AN0364		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN0413		Protein of unknown function
AN0457		Protein of unknown function
AN0474		Has domain(s) with predicted role in nucleoside metabolic process
AN0481		Protein of unknown function
AN0493		PalA-dependent expression independent of pH
AN0495		Has domain(s) with predicted amino acid binding, formyltetrahydrofolate deformylase activity, hydroxymethyl-, formyl- and related transferase activity and role in 'de novo' IMP biosynthetic process, biosynthetic process, metabolic process
AN0546		Protein of unknown function
AN0610		Protein of unknown function
AN0655	*sepM*	Protein with homology to Schizosaccharomyces pombe Cdc14p, a component of the septation initiation network (SIN)-complex
AN0677		Has domain(s) with predicted zinc ion binding activity
AN0694		Ortholog(s) have intracellular localization
AN0756	*lacA*	Beta-galactosidase with a predicted role in lactose metabolism
AN0764		Has domain(s) with predicted catalytic activity, catechol 1,2-dioxygenase activity, ferric iron binding, iron ion binding and oxidoreductase activity, more

AN0771		Putative ABC multidrug transporter; confers resistance to azole antifungal drugs
AN0781		Protein of unknown function
AN0801		Has domain(s) with predicted catalytic activity and role in metabolic process
AN0807	*laeA*	Methyltransferase-domain protein; velvet complex component composed of VelB, VeA and LaeA; self-methylates; coordinates asexual development in response to light; regulates secondary metabolism and is required for Hulle cell formation
AN0820		Protein of unknown function
AN0859		Protein of unknown function
AN0902		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN0903		Protein of unknown function
AN0913		Putative phosphatidylinositol synthase with a predicted role in phospholipid metabolism
AN0964		Protein of unknown function
AN10060		Putative alpha-amylase; glycogen debranching enzyme
AN1007	*niiA*	Putative nitrite reductase with a predicted role in nitrogen metabolism; transcript stabilized by intracellular nitrate
AN10078		Ortholog(s) have ATPase-coupled transmembrane transporter activity, role in fatty acid transport and integral component of peroxisomal membrane, peroxisome localization
AN10095		Protein of unknown function
AN10099		Has domain(s) with predicted FMN binding, oxidoreductase activity and role in oxidation-reduction process
AN10135		Has domain(s) with predicted role in endosome transport via multivesicular body sorting pathway and ESCRT I complex localization
AN10197		Has domain(s) with predicted catalytic activity and role in coenzyme M biosynthetic process
AN10252		Protein of unknown function
AN10268		Protein of unknown function
AN10296		Ortholog(s) have fumarate reductase (NADH) activity, role in cellular response to anoxia and cytosol, intracellular localization
AN10311	*mnpA*	Putative hyphal cell wall mannoprotein; expression is transcriptionally upregulated during sexual development; expression is flbA-, fadA- and veA-dependent; present in the hyphal cell wall, absent from the conidial cell wall
AN10391		Protein of unknown function
AN1041		Putative beta-1,4-endoglucanase
AN10447		Has domain(s) with predicted transferase activity, transferring acyl groups activity
AN10487		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN10581		Protein of unknown function

AN1068		Has domain(s) with predicted hydrolase activity, acting on ester bonds activity and role in GPI anchor metabolic process, intracellular protein transport
AN10742		Protein of unknown function
AN10761		Predicted PIN domain-containing RNA-binding protein; expression upregulated after exposure to farnesol
AN10789		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN10864		Has domain(s) with predicted phosphatase activity, protein tyrosine phosphatase activity and role in dephosphorylation
AN10884		Predicted oxidoreductase; predicted secondary metabolism gene cluster member
AN1089	*artB*	Ortholog(s) have ubiquitin protein ligase binding activity, role in endocytosis, positive regulation of ubiquitin-dependent endocytosis, regulation of intracellular transport and early endosome, late endosome localization
AN10896		Protein of unknown function
AN10906		Has domain(s) with predicted DNA binding, zinc ion binding activity, role in transcription, DNA-templated and nucleus localization
AN10911		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN10964		Has domain(s) with predicted methyltransferase activity and role in metabolic process
AN11018		Protein of unknown function
AN11065		Putative oxidosqualene-lanosterol cyclase with a predicted role in ergosterol metabolism
AN11080		Putative dimethyl-allyl-tryptophan synthase (DMATS)-type aromatic prenyltransferase
AN11085		Putative D-arabinitol 4-dehydrogenaset
AN11105		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN1112		Protein of unknown function
AN11153		Ortholog(s) have endoplasmic reticulum localization
AN11174		Protein of unknown function
AN11210		Protein of unknown function; transcript repressed by nitrate
AN11281		Protein of unknown function
AN11432		Protein of unknown function
AN11570		Protein of unknown function
AN11581		Protein of unknown function
AN11670		Protein of unknown function
AN1174		Has domain(s) with predicted ATP binding, ATPase activity, ATPase-coupled transmembrane transporter activity, nucleoside-triphosphatase activity, nucleotide binding activity and role in transmembrane transport

AN11754		Protein of unknown function
AN11776		Ortholog(s) have mitochondrial inner membrane, mitochondrial large ribosomal subunit localization
AN11800		Protein of unknown function
AN1183		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN11850		Protein of unknown function
AN11872		Protein of unknown function
AN11878		Protein of unknown function
AN11886		Has domain(s) with predicted transferase activity, transferring phosphorus-containing groups activity and membrane localization
AN11907		Protein of unknown function
AN11931		Protein of unknown function
AN11934		Has domain(s) with predicted ATP binding, ATPase activity, ATPase-coupled transmembrane transporter activity, nucleoside-triphosphatase activity, nucleotide binding activity and role in transmembrane transport
AN11948		Protein of unknown function
AN11989		Has domain(s) with predicted 2-oxoglutarate-dependent dioxygenase activity, iron ion binding, oxidoreductase activity and role in oxidation-reduction process
AN11999		Protein of unknown function
AN12013		Protein of unknown function
AN12015		Protein of unknown function
AN12030		Protein of unknown function
AN12084		Protein of unknown function
AN12104		Protein of unknown function
AN12135		Protein of unknown function
AN12192		Protein of unknown function
AN12224		Protein of unknown function
AN12339		Protein of unknown function
AN12353		Protein of unknown function
AN12481		Protein of unknown function
AN12487		Protein of unknown function
AN1262		Protein of unknown function
AN1279		Protein of unknown function
AN1356		Protein of unknown function
AN1427		Ortholog(s) have N-acetylglucosamine transmembrane transporter activity, role in N-acetylglucosamine transport and plasma membrane localization
AN1433		Protein of unknown function
AN1438		Protein of unknown function
AN1519	*rsdA*	Putative Argonaute protein involved in inverted repeat transgene (IRT)-induced RNA silencing

AN1577		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN1612		Ortholog(s) have inorganic phosphate transmembrane transporter activity, manganese ion transmembrane transporter activity, phosphate:proton symporter activity, selenite:proton symporter activity
AN1614		Has domain(s) with predicted methyltransferase activity and role in metabolic process
AN1624	*oliC*	Subunit 9 of the mitochondrial inner membrane F1F0-ATPase complex; mutation confers oligomycin resistance; palA-dependent expression independent of pH
AN1693		Putative F-box protein
AN1714		Protein of unknown function
AN1715		Putative mannose-6-phosphate isomerase with a predicted role in mannose/mannitol, fructose, and sorbose/sorbitol metabolism
AN1716		Protein of unknown function
AN1747		Ortholog(s) have mitochondrial inner membrane localization
AN1803		Protein of unknown function
AN1831		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN1854		Putative inositol pentakisphosphate 2-kinase; locus contains the conserved upstream open reading frame (uORF) AN1854-uORF
AN1865		Putative sugar transporter
AN1871		Protein of unknown function
AN1872		Has domain(s) with predicted hydrolase activity and role in metabolic process
AN1877		Protein of unknown function
AN1915		Ortholog(s) have 2 iron, 2 sulfur cluster binding, electron transfer activity, iron-sulfur cluster binding, oxidoreductase activity, acting on NAD(P)H, oxidoreductase activity, acting on NAD(P)H, heme protein as acceptor activity
AN1928		Protein of unknown function
AN1948	*SPA10*	Ortholog(s) have role in DNA methylation
AN2000	*ubi4*	Polyubiquitin, contains four head to tail repeats of ubiqiutin; transcript upregulated in response to camptothecin
AN2004		Protein of unknown function
AN20054		Protein of unknown function
AN2024		Protein of unknown function
AN2029		Putative F-box protein
AN2032	*pkhA*	Putative polyketide synthase; involved in secondary metabolite production
AN2042		Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN2059	*velC*	Velvet family protein with homology to VeA, involved in regulation of sexual development
AN2133		Putative uracil phosphoribosyltransferase

AN2157	*pepAa*	Putative aspartic endopeptidase
AN2194		Has domain(s) with predicted serine-type endopeptidase activity and role in proteolysis
AN2195		Has domain(s) with predicted zinc ion binding activity
AN2238		Has domain(s) with predicted unfolded protein binding activity and role in protein folding
AN2286	*alcC*	Alcohol dehydrogenase III with a predicted role in two-carbon compound metabolism; required for long-term survival under anaerobic conditions; regulated at both the transcriptional and translational levels
AN2311		Putative phosphomevalonate kinase with a predicted role in sterol metabolism
AN2314		Putative 1,4-alpha-glucan branching enzyme with a predicted role in starch metabolism
AN2334		Putative ketose-1,6-bisphosphate aldolase
AN2343		Putative nitroreductase; intracellular, menadione stress-induced protein
AN2347	*CYP504E4*	Putative cytochrome P450; possibly a pseudogene
AN2360		Has domain(s) with predicted acid phosphatase activity, hydrolase activity, metal ion binding activity
AN2374		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN2407		Has domain(s) with predicted role in isoprenoid biosynthetic process
AN2421	*flbC*	Putative C2H2 zinc finger transcription factor; involved in regulation of conidiophore development; required for light-dependent activation of brlA transcription
AN2466		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN2475	*mstB*	Putative sugar transporter
AN2505	*fbx15*	Putative F-box protein; NeddH-associated protein; required for asexual and for sexual development
AN2556		Protein of unknown function
AN2571		Protein of unknown function
AN2615		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN2623	*aatA*	Isopenicillin-N N-acyltransferase; null produces reduced levels of penicillin; partially redundant with aatB
AN2629		Protein of unknown function
AN2658		Protein of unknown function
AN2669		Has domain(s) with predicted role in response to stress and integral component of membrane localization
AN2675		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN2682		Has domain(s) with predicted FMN binding, catalytic activity, oxidoreductase activity and role in oxidation-reduction process

AN2683		Protein of unknown function
AN2701		Ortholog(s) have role in conidiophore development, hyphal growth, sporocarp development involved in sexual reproduction, syncytium formation by plasma membrane fusion
AN2716		Has domain(s) with predicted catalytic activity and role in metabolic process
AN2727	*CYP665A1*	Putative cytochrome P450
AN2768		Has domain(s) with predicted role in attachment of spindle microtubules to kinetochore and DASH complex, spindle microtubule localization
AN2792		Protein of unknown function
AN2810		Ortholog(s) have metallopeptidase activity
AN2811		Has domain(s) with predicted protein dimerization activity
AN2858		Has domain(s) with predicted oxidoreductase activity
AN2894		Protein of unknown function
AN2921		Protein of unknown function
AN2999	*idpA*	Putative isocitrate dehydrogenase (NADP+) with a predicted role in the TCA cycle; regulated by carbon source; alternative transcription start sites specify mitochondrial or cytoplasmic and peroxisomal protein localization
AN3021		Protein of unknown function
AN3084		Ortholog(s) have lysophospholipase activity, role in lipid homeostasis and lipid droplet localization
AN3086		Protein of unknown function
AN3087		Protein of unknown function
AN3100		Protein of unknown function
AN3117		Ortholog(s) have copper transmembrane transporter activity, phosphorylative mechanism activity, role in cadmium ion transport, cellular copper ion homeostasis, copper ion transport, silver ion transport and plasma membrane localization
AN3130		Protein of unknown function
AN3163	*stoA*	Putative stomatin ortholog, predicted to have scaffolding functions in maintenance of lipid microdomains in membranes; mutation affects hyphal morphology
AN3179		Protein of unknown function
AN3182		Protein of unknown function
AN3206		Putative aryl-alcohol oxidase-related protein; protein expressed at decreased levels in a hapX mutant versus wild-type; transcript is induced by nitrate
AN3223	*pfkA*	Putative 6-phosphofructokinase with a predicted role in gluconeogenesis and glycolysis; upregulated under hypoxic growth conditions
AN3239		Has domain(s) with predicted acyl-CoA dehydrogenase activity, oxidoreductase activity, oxidoreductase activity, acting on the CH-CH group of donors activity and role in oxidation-reduction process
AN3264	*xtrB*	Putative xylose transporter

AN3265	*apyA*	Arrestin domains and PY motif-containing protein with homology to Saccharomyces cerevisiae Rod1p and Rog3p proteins
AN3267		Protein of unknown function
AN3276		Putative Type II fatty acid synthase with a predicted role in mitochondrial fatty acid formation
AN3281	*CYP567D1*	Putative cytochrome P450
AN3286		Protein of unknown function
AN3287		Has domain(s) with predicted role in transmembrane transport and membrane localization
AN3299		Protein of unknown function
AN3344	*ngn27*	Putative GNAT-type acetyltransferase
AN3348		Protein of unknown function
AN3387	*gprD*	Putative G-protein coupled receptor
AN3472		Protein of unknown function
AN3524		Putative galactose 1-dehydrogenase with a predicted role in galactonic acid and galactonate metabolism
AN3551		Protein of unknown function
AN3582		Protein of unknown function
AN3585		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN3627		Ortholog(s) have intracellular localization
AN3661		Protein of unknown function
AN3681		Ortholog(s) have role in cellular iron ion homeostasis and fungal-type vacuole membrane localization
AN3741	*alcB*	Alcohol dehydrogenase II, has a predicted role in two-carbon compound metabolism
AN3751		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN3763		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN3776		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN3796		Protein of unknown function
AN3846		Putative ornithine decarboxylase with a predicted role in arginine metabolism; mutants are unable to synthesize polyamines such as spermidine
AN3880		Putative acyl-coA dehydrogenase
AN3960		Has domain(s) with predicted flavin adenine dinucleotide binding, oxidoreductase activity, acting on CH-OH group of donors activity and role in oxidation-reduction process
AN3998		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN4008		Has domain(s) with predicted O-methyltransferase activity
AN4052	*exgC*	Putative glucan 1,3-beta-glucosidase with a predicted role in glucan metabolism

AN4077		Has domain(s) with predicted DNA binding activity
AN4078		Protein of unknown function
AN4102	*bglA*	Putative beta-glucosidase; induced by carbon starvation-induced autophagy
AN4131		Has domain(s) with predicted solute:proton antiporter activity, role in cation transport, transmembrane transport and integral component of membrane localization
AN4138		Protein of unknown function
AN4144		Protein of unknown function
AN4148	*xtrE*	Putative xylose transporter; transcriptionally induced by growth on xylose
AN4172		Protein of unknown function
AN4210		Has domain(s) with predicted transcription coregulator activity, role in regulation of transcription by RNA polymerase II and mediator complex localization
AN4239		Ortholog(s) have RSC-type complex localization
AN4256		Protein of unknown function
AN4264		Ortholog(s) have role in syncytium formation by plasma membrane fusion
AN4277		Ortholog(s) have glucose transmembrane transporter activity, role in cellular response to drug, glucose transmembrane transport and plasma membrane localization
AN4295		Ortholog(s) have role in cardiolipin metabolic process, cell-abiotic substrate adhesion, cristae formation, negative regulation of phosphatidylcholine biosynthetic process, phosphatidylethanolamine metabolic process, phospholipid transport
AN4336	*ladB*	Putative L-arabinitol 4-dehydrogenase with a predicted role in L-arabinose/arabitol and D-xylose/D,L-xylulose/xylitol metabolism
AN4394		Ortholog(s) have role in asexual sporulation resulting in formation of a cellular spore, positive regulation of asexual sporulation resulting in formation of a cellular spore, regulation of transcription, DNA-templated
AN4422	*pepAd*	Putative aspartic-type endopeptidase; predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN4443	*metH*	Putative methionine synthase with a predicted role in methionine metabolism; protein expressed at increased levels in a hapX mutant versus wild-type
AN4444		Protein of unknown function
AN4481		Ortholog(s) have role in secondary metabolite biosynthetic process and fungal-type vacuole membrane localization
AN4515	*crhB*	Putative transglycosidase with a predicted role in glucan processing; predicted glycosyl phosphatidylinositol (GPI)-anchor
AN4521	*fhpA*	Forkhead domain protein with a possible role in sexual development
AN4544	*msgA*	Putative dual-specificity protein tyrosine/serine/threonine phosphatase
AN4575		Protein of unknown function
AN4645		Protein of unknown function
AN4647		Ortholog(s) have role in cellular response to drug

AN4659		Putative acyl-CoA synthetase/AMP-binding domain protein; has a predicted mitochondrial localization signal
AN4697		Ortholog(s) have palmitoyltransferase activity, role in protein palmitoylation, vacuole fusion, non-autophagic and fungal-type vacuole, fungal-type vacuole membrane localization
AN4702		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN4711		Has domain(s) with predicted hydrolase activity
AN4774		Ortholog(s) have uroporphyrin-III C-methyltransferase activity and role in cellular response to drug, methionine biosynthetic process, siroheme biosynthetic process
AN4812		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN4817		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN4920	*pmcB*	Putative calcium-transporting mitochondrial ATPase involved in calcium homeostasis
AN4922		Protein of unknown function
AN4963		Ortholog(s) have cytoskeletal protein membrane adaptor, phospholipid binding activity
AN4984		Putative cyclin-dependent protein kinase; locus contains the conserved upstream open reading frame (uORF) AN4984-uORF
AN4990		Ortholog(s) have ferrous iron transmembrane transporter activity, manganese ion transmembrane transporter activity
AN5040		Has domain(s) with predicted DNA binding, zinc ion binding activity, role in transcription, DNA-templated and nucleus localization
AN5076		Has domain(s) with predicted role in cell wall macromolecule catabolic process
AN5156	*pho80*	Pho80-like cyclin involved in regulation of development and phosphate homeostasis, interacts with the cyclin-dependent kinase PhoA
AN5169		Ortholog(s) have role in syncytium formation by plasma membrane fusion
AN5170	*rosA*	Putative Zn(II)2Cys6 transcription factor; negative regulator of sexual development
AN5210	*pkiA*	Putative pyruvate kinase with a predicted role in gluconeogenesis and glycolysis; intracellular, menadione stress-induced protein
AN5264		Protein of unknown function
AN5283		Protein of unknown function
AN5295		Has domain(s) with predicted carbon-carbon lyase activity, catalytic activity and role in cellular aromatic compound metabolic process
AN5324	*dlpA*	Dehydrin-like protein; protein induced by farnesol
AN5408		Has domain(s) with predicted RNA binding, ribonuclease III activity and role in RNA processing
AN5457	*noxA*	Putative oxidoreductase with a predicted role in energy metabolism; NADPH oxidase; involved in the generation of reactive oxygen species (ROS); mutation blocks cleistothecia formation; homology to p91phox
AN5458		Ortholog(s) have role in syncytium formation by plasma membrane fusion

AN5497		Ortholog(s) have 3,4-dihydroxy-2-butanone-4-phosphate synthase activity, role in aerobic respiration, riboflavin biosynthetic process and cytosol, fungal biofilm matrix, mitochondrial intermembrane space localization
AN5501		Ortholog(s) have lipase activity
AN5568		Putative F-box protein
AN5589		Putative glycerol kinase with a predicted role in glycerol metabolism; required for growth on glycerol; transcript upregulated by growth in glycerol
AN5591		Putative aminotransferase; protein expressed at decreased levels in a hapX mutant versus wild-type
AN5634	*acuD*	Isocitrate lyase, required for utilization of acetate and fatty acids as carbon sources; transcriptional induction in response to acetate is mediated by FacB; transcriptional induction in response to long-chain fatty acids mediated by FarA
AN5667		Protein of unknown function
AN5672		Ortholog(s) have mannonate dehydratase activity and role in cellular carbohydrate catabolic process
AN5691		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN5763		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN5768		Ortholog(s) have role in actin cytoskeleton organization, inositol lipid-mediated signaling, negative regulation of phospholipid translocation, vacuole organization and plasma membrane localization
AN5781		Putative 30 kilodalton heat shock protein; transcript levels increase during the unfolded-protein response (UPR); palA-dependent expression independent of pH
AN5836	*stuA*	APSES domain transcription factor involved in regulation of conidiophore development; represses abaA and other developmentally regulated genes; locus consists of stuA-alpha and stuA-beta transcriptional units; stuA-alpha contains a uORF
AN5841		Protein of unknown function
AN6024	*gstB*	Protein with glutathione S-transferase and glutathione peroxidase activities; intracellular, menadione stress-induced protein
AN6084		Protein of unknown function
AN6137		Protein of unknown function
AN6163		Protein of unknown function
AN6167		Has domain(s) with predicted FMN binding, catalytic activity, oxidoreductase activity and role in oxidation-reduction process
AN6277		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN6321	*CYP656A1*	Putative cytochrome P450
AN6327		Protein of unknown function

AN6367		Has domain(s) with predicted phosphatidylinositol phosphate kinase activity and role in phosphatidylinositol metabolic process
AN6379		Protein of unknown function
AN6380		Protein of unknown function
AN6382		Has domain(s) with predicted phosphoric diester hydrolase activity and role in lipid metabolic process
AN6384		Has domain(s) with predicted catalytic activity and role in nucleoside metabolic process
AN6404		Has domain(s) with predicted zinc ion binding activity
AN6461		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN6472	*dfgF*	Putative endo-mannanase GH76 family protein
AN6478		Protein of unknown function
AN6494		Ortholog(s) have RNA binding activity
AN6504		Protein of unknown function
AN6535		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN6543		Ortholog(s) have adenyl-nucleotide exchange factor activity, role in cytoplasm protein quality control by the ubiquitin-proteasome system and cytosol localization
AN6565		Protein of unknown function
AN6624		Protein of unknown function
AN6644	*biA*	Putative bifunctional dethiobiotin synthetase/adenosylmethionine-8-amino-7-oxononanoate aminotransferase, enzyme of the biotin biosynthesis pathway; common mutation in laboratory strains
AN6653	*acuE*	Malate synthase, required for utilization of acetate as carbon source; transcription induction by acetate mediated by FacB; carbon catabolite repression mediated by CreA; transcription induction by long-chain fatty acids mediated by FarA
AN6669	*mstC*	High-affinity glucose transporter active in germinating conidia
AN6701		Protein of unknown function
AN6703	*jenB*	Short-chain carboxylic acid transporter involved in uptake of lactate, succinate, pyruvate and malate
AN6754		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN6769		Has domain(s) with predicted metal ion transmembrane transporter activity, role in metal ion transport, transmembrane transport and membrane localization
AN6787	*CYP682C1*	Putative cytochrome P450
AN6794		Protein of unknown function
AN6805		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN6818		Protein of unknown function
AN6885		Protein of unknown function
AN6946		Protein of unknown function

AN6950		Protein of unknown function
AN6954		Protein of unknown function
AN7018		Protein of unknown function
AN7071	*pkgA*	Putative polyketide synthase; involved in the production of alternariol and other secondary metabolites; predicted backbone enzyme of a secondary metabolism gene cluster
AN7177		Protein of unknown function
AN7203		Protein of unknown function
AN7265		Has domain(s) with predicted role in mycotoxin biosynthetic process
AN7269		Ortholog(s) have role in fumiquinazoline C biosynthetic process, secondary metabolite biosynthetic process and fungal-type cell wall localization
AN7273		Protein of unknown function
AN7278		Putative glutamate decarboxylase with a predicted role in the 4-aminobutyrate (GABA) shunt
AN7334		Has domain(s) with predicted catalytic activity and role in metabolic process
AN7457		Protein of unknown function
AN7463	*meaA*	Major ammonium transporter of A. nidulans; transcript upregulated by nitrate limitation
AN7476		Protein of unknown function
AN7482		Protein of unknown function
AN7484		Protein expressed at increased levels during osmoadaptation; contains a DUF1349 domain
AN7511	*gelE*	Putative 1,3-beta-transglycosidase with a predicted role in glucan processing; predicted glycosyl phosphatidylinositol (GPI)-anchor
AN7552		Protein of unknown function
AN7580		Protein of unknown function
AN7596		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN7607		Protein of unknown function
AN7710		Ortholog(s) have intracellular localization
AN7727		Protein of unknown function
AN7779		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN7785		Protein of unknown function
AN7860		Protein of unknown function
AN7892		Small heat-shock protein; molecular chaperone; expression upregulated after exposure to farnesol
AN7895	*cipB*	Putative oxidoreductase; contains Zn-dependent alcohol dehydrogenase domain; protein expressed at increased levels during osmoadaptation
AN7937	*cipC*	Protein responsive to Concanamycin A
AN7964		Protein of unknown function
AN8007	*abnC*	Protein with arabinan endo-1,5-alpha-L-arabinosidase activity, involved in degradation of pectin

AN8041	*gpdA*	Glyceraldehyde-3-phosphate dehydrogenase with a predicted role in gluconeogenesis and glycolysis; the gpdA promoter is a commonly used regulatory sequence for driving constitutive heterologous gene expression
AN8060		Has domain(s) with predicted NAD binding, oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor activity and role in cellular amino acid metabolic process, oxidation-reduction process
AN8086		Putative protein of unknown function; expression upregulated after exposure to farnesol
AN8092		Protein of unknown function
AN8147		Protein of unknown function
AN8262	*gprH*	Secretin-like G-protein coupled receptor, involved in nutrient sensing and control of sexual development
AN8274		Ortholog(s) have DNA binding, tricarboxylate secondary active transmembrane transporter activity
AN8278	*mrvA*	Ortholog of Neosartorya fischeri NRRL 181 : NFIA_037840, Aspergillus wentii : Aspwe1_0605622, Aspergillus versicolor : Aspve1_0047287 and Aspergillus clavatus NRRL 1 : ACLA_000900
AN8321		Protein of unknown function
AN8326		Protein of unknown function
AN8348	*gprI*	Putative G-protein coupled receptor
AN8362		Protein of unknown function
AN8374		Ortholog(s) have oligopeptide transmembrane transporter activity
AN8383	*ausA*	Polyketide synthase (PKS); produces 3,5- dimethyl orsellinic acid, the first intermediate in the biosynthesis of austinol and dehydroaustinol; aus secondary metabolism gene cluster member
AN8400		Sugar transporter; transcriptionally induced by growth on xylose
AN8412	*apdA*	Putative hybrid polyketide synthase-nonribosomal peptide synthase (PKS-NRPS); aspyridone synthetase; member of the aspyridone (apd) gene cluster
AN8439		Protein of unknown function; transcript is induced by nitrate; predicted NirA binding site
AN8457		Has domain(s) with predicted catalytic activity, microtubule motor activity, role in nucleoside metabolic process and kinesin complex localization
AN8485		Has domain(s) with predicted iron-sulfur cluster binding activity, role in apoptotic process, iron-sulfur cluster assembly and cytoplasm localization
AN8492		Protein of unknown function
AN8502		Ortholog(s) have role in cellular response to drug
AN8512		Protein of unknown function; adjacent to tdi (terrequinone biosynthesis) gene cluster. Not required for terrequinone biosynthesis
AN8514	*tdiB*	Asterriquinone prenyltransferase; member of the tdi gene cluster; required for terrequinone A production; catalyzes the reverse prenylation

		event during terrequinone A biosynthesis; lacks canonical prenyl diphosphate binding motif (D/N)DXXD
AN8539	*ngn26*	Ortholog of A. fumigatus SidG; triacetylfusarinine C (TAFC) siderophore biosynthetic transacetylase; transcript induced by light in developmentally competent mycelia
AN8557		Protein of unknown function; transcript is induced by nitrate
AN8573		Protein of unknown function
AN8583		Has domain(s) with predicted catalytic activity, coenzyme binding activity and role in cellular metabolic process
AN8601		Protein of unknown function
AN8602		Protein of unknown function
AN8612		Protein of unknown function
AN8621		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN8627		Has domain(s) with predicted transferase activity, transferring glycosyl groups activity and membrane localization
AN8640	*conF*	Ortholog of N. crassa conF, light-induced transcript expressed during conidiation in N. crassa; double conF conJ deletion results in increased cellular glycerol or erythritol leading to delayed germination and desiccation resistance
AN8683		Ortholog(s) have ferric-chelate reductase activity, role in copper ion import, iron ion transport and plasma membrane localization
AN8692	*prxA*	Thioredoxin-dependent peroxidase; intracellular; PRX5-like domain; highly similar to the allergen Aspf3 from related fungi; menadione stress-repressed protein; osmoadaptation-induced protein; repressed by starvation-induced autophagy
AN8694		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN8739		Protein of unknown function
AN8741	*mtfA*	Putative C2H2 transcription factor involved in regulation of secondary metabolism and morphogenesis
AN8890		Has domain(s) with predicted carbohydrate binding, catalytic activity and role in carbohydrate catabolic process
AN8891	*pgxB*	Putative exopolygalacturonase
AN8905	*CYP537B1*	Putative cytochrome P450
AN8907		Putative C-4 sterol methyl oxidase with a predicted role in sterol metabolism
AN8989		Protein of unknown function
AN9021		Protein of unknown function
AN9025		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, nucleic acid binding, zinc ion binding activity
AN9035	*aglG*	Putative alpha-galactosidase with a predicted role in galactose and galactitol metabolism and in degradation of mannans

AN9069		Protein of unknown function
AN9103	*aifA*	Putative apoptosis-inducing factor (AIF)-like mitochondrial oxidoreductase; mutants display decreased survival in the presence of farnesol or menadione, decreased electron transport; expression upregulated after exposure to farnesol
AN9121	*esdC*	Protein with a glycogen binding domain involved in sexual development; regulated by VeA and FlbA
AN9142		Protein of unknown function
AN9143	*cnxG*	Putative molybdopterin synthase small subunit involved in molybdenum cofactor biosynthesis; molybdopterin cofactor required for the activity of nitrate reductase
AN9184		Ortholog(s) have plasma membrane localization
AN9195		Protein of unknown function
AN9197		Protein of unknown function
AN9202		Protein of unknown function
AN9274		Protein of unknown function
AN9288		Has domain(s) with predicted oxidoreductase activity, transferase activity, transferring acyl groups other than amino-acyl groups, zinc ion binding activity and role in oxidation-reduction process
AN9298	*gmtB*	Putative GDP-mannose transporter
AN9314		Protein with homology to entkaurene synthases; prediction backbone enzyme of a secondary metabolite biosynthesis gene cluster
AN9323		Protein of unknown function
AN9347		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN9361		Protein of unknown function
AN9364		Protein of unknown function
AN9369		Has domain(s) with predicted catalytic activity
AN9387		Protein of unknown function
AN9449		Has domain(s) with predicted catalytic activity, metal ion binding, phosphoric diester hydrolase activity
AN9456		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN9500		Has domain(s) with predicted protein kinase binding activity and role in regulation of cyclin-dependent protein serine/threonine kinase activity
AN9501		Protein of unknown function

Table S3-5. Common direct targets of VeA and LaeA in *A. nidulans* Vege

Gene ID	Gene Name	Description
AN0259		Putative adenylate kinase with a predicted role in nucleotide salvage pathways
AN0493		PalA-dependent expression independent of pH
AN0495		Has domain(s) with predicted amino acid binding, formyltetrahydrofolate deformylase activity, hydroxymethyl-, formyl- and related transferase activity and role in 'de novo' IMP biosynthetic process, biosynthetic process, metabolic process
AN0677		Has domain(s) with predicted zinc ion binding activity
AN0756	*lacA*	Beta-galactosidase with a predicted role in lactose metabolism
AN0781		Protein of unknown function
AN0820		Protein of unknown function
AN0903		Protein of unknown function
AN0913		Putative phosphatidylinositol synthase with a predicted role in phospholipid metabolism
AN10060		Putative alpha-amylase; glycogen debranching enzyme
AN10078		Ortholog(s) have ATPase-coupled transmembrane transporter activity, role in fatty acid transport and integral component of peroxisomal membrane, peroxisome localization
AN10296		Ortholog(s) have fumarate reductase (NADH) activity, role in cellular response to anoxia and cytosol, intracellular localization
AN10311	*mnpA*	Putative hyphal cell wall mannoprotein; expression is transcriptionally upregulated during sexual development; expression is flbA-, fadA- and veA-dependent; present in the hyphal cell wall, absent from the conidial cell wall
AN10391		Protein of unknown function
AN10487		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN10581		Protein of unknown function
AN10789		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated
AN10896		Protein of unknown function
AN10906		Has domain(s) with predicted DNA binding, zinc ion binding activity, role in transcription, DNA-templated and nucleus localization
AN10964		Has domain(s) with predicted methyltransferase activity and role in metabolic process
AN11018		Protein of unknown function
AN11080		Putative dimethyl-allyl-tryptophan synthase (DMATS)-type aromatic prenyltransferase
AN11281		Protein of unknown function
AN11670		Protein of unknown function

AN1174		Has domain(s) with predicted ATP binding, ATPase activity, ATPase-coupled transmembrane transporter activity, nucleoside-triphosphatase activity, nucleotide binding activity and role in transmembrane transport
AN11754		Protein of unknown function
AN11776		Ortholog(s) have mitochondrial inner membrane, mitochondrial large ribosomal subunit localization
AN11907		Protein of unknown function
AN11931		Protein of unknown function
AN11934		Has domain(s) with predicted ATP binding, ATPase activity, ATPase-coupled transmembrane transporter activity, nucleoside-triphosphatase activity, nucleotide binding activity and role in transmembrane transport
AN11999		Protein of unknown function
AN12015		Protein of unknown function
AN12030		Protein of unknown function
AN12084		Protein of unknown function
AN12192		Protein of unknown function
AN12224		Protein of unknown function
AN12487		Protein of unknown function
AN1356		Protein of unknown function
AN1438		Protein of unknown function
AN1519	*rsdA*	Putative Argonaute protein involved in inverted repeat transgene (IRT)-induced RNA silencing
AN1612		Ortholog(s) have inorganic phosphate transmembrane transporter activity, manganese ion transmembrane transporter activity, phosphate:proton symporter activity, selenite:proton symporter activity
AN1624	*oliC*	Subunit 9 of the mitochondrial inner membrane F1F0-ATPase complex; mutation confers oligomycin resistance; palA-dependent expression independent of pH
AN1693		Putative F-box protein
AN1714		Protein of unknown function
AN1715		Putative mannose-6-phosphate isomerase with a predicted role in mannose/mannitol, fructose, and sorbose/sorbitol metabolism
AN1747		Ortholog(s) have mitochondrial inner membrane localization
AN1803		Protein of unknown function
AN1872		Has domain(s) with predicted hydrolase activity and role in metabolic process
AN1915		Ortholog(s) have 2 iron, 2 sulfur cluster binding, electron transfer activity, iron-sulfur cluster binding, oxidoreductase activity, acting on NAD(P)H, oxidoreductase activity, acting on NAD(P)H, heme protein as acceptor activity
AN1948	*SPA10*	Ortholog(s) have role in DNA methylation
AN2000	*ubi4*	Polyubiquitin, contains four head to tail repeats of ubiqiutin; transcript upregulated in response to camptothecin
AN2004		Protein of unknown function
AN20054		Protein of unknown function

AN2024		Protein of unknown function
AN2029		Putative F-box protein
AN2042		Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN2059	*velC*	Velvet family protein with homology to VeA, involved in regulation of sexual development
AN2157	*pepAa*	Putative aspartic endopeptidase
AN2194		Has domain(s) with predicted serine-type endopeptidase activity and role in proteolysis
AN2238		Has domain(s) with predicted unfolded protein binding activity and role in protein folding
AN2311		Putative phosphomevalonate kinase with a predicted role in sterol metabolism
AN2314		Putative 1,4-alpha-glucan branching enzyme with a predicted role in starch metabolism
AN2343		Putative nitroreductase; intracellular, menadione stress-induced protein
AN2360		Has domain(s) with predicted acid phosphatase activity, hydrolase activity, metal ion binding activity
AN2374		Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN2421	*flbC*	Putative C2H2 zinc finger transcription factor; involved in regulation of conidiophore development; required for light-dependent activation of brlA transcription
AN2466		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization
AN2475	*mstB*	Putative sugar transporter
AN2623	*aatA*	Isopenicillin-N N-acyltransferase; null produces reduced levels of penicillin; partially redundant with aatB
AN2658		Protein of unknown function
AN2669		Has domain(s) with predicted role in response to stress and integral component of membrane localization
AN2675		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN2682		Has domain(s) with predicted FMN binding, catalytic activity, oxidoreductase activity and role in oxidation-reduction process
AN2683		Protein of unknown function
AN2701		Ortholog(s) have role in conidiophore development, hyphal growth, sporocarp development involved in sexual reproduction, syncytium formation by plasma membrane fusion
AN2768		Has domain(s) with predicted role in attachment of spindle microtubules to kinetochore and DASH complex, spindle microtubule localization
AN2999	*idpA*	Putative isocitrate dehydrogenase (NADP+) with a predicted role in the TCA cycle; regulated by carbon source; alternative transcription start sites specify mitochondrial or cytoplasmic and peroxisomal protein localization

AN3021		Protein of unknown function
AN3086		Protein of unknown function
AN3117		Ortholog(s) have copper transmembrane transporter activity, phosphorylative mechanism activity, role in cadmium ion transport, cellular copper ion homeostasis, copper ion transport, silver ion transport and plasma membrane localization
AN3182		Protein of unknown function
AN3206		Putative aryl-alcohol oxidase-related protein; protein expressed at decreased levels in a hapX mutant versus wild-type; transcript is induced by nitrate
AN3265	*apyA*	Arrestin domains and PY motif-containing protein with homology to Saccharomyces cerevisiae Rod1p and Rog3p proteins
AN3344	*ngn27*	Putative GNAT-type acetyltransferase
AN3387	*gprD*	Putative G-protein coupled receptor
AN3524		Putative galactose 1-dehydrogenase with a predicted role in galactonic acid and galactonate metabolism
AN3582		Protein of unknown function
AN3681		Ortholog(s) have role in cellular iron ion homeostasis and fungal-type vacuole membrane localization
AN3751		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN3763		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN3796		Protein of unknown function
AN3960		Has domain(s) with predicted flavin adenine dinucleotide binding, oxidoreductase activity, acting on CH-OH group of donors activity and role in oxidation-reduction process
AN3998		Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN4077		Has domain(s) with predicted DNA binding activity
AN4078		Protein of unknown function
AN4131		Has domain(s) with predicted solute:proton antiporter activity, role in cation transport, transmembrane transport and integral component of membrane localization
AN4138		Protein of unknown function
AN4148	*xtrE*	Putative xylose transporter; transcriptionally induced by growth on xylose
AN4172		Protein of unknown function
AN4264		Ortholog(s) have role in syncytium formation by plasma membrane fusion
AN4277		Ortholog(s) have glucose transmembrane transporter activity, role in cellular response to drug, glucose transmembrane transport and plasma membrane localization
AN4443	*metH*	Putative methionine synthase with a predicted role in methionine metabolism; protein expressed at increased levels in a hapX mutant versus wild-type
AN4515	*crhB*	Putative transglycosidase with a predicted role in glucan processing; predicted glycosyl phosphatidylinositol (GPI)-anchor

AN4521	*fhpA*	Forkhead domain protein with a possible role in sexual development
AN4544	*msgA*	Putative dual-specificity protein tyrosine/serine/threonine phosphatase
AN4645		Protein of unknown function
AN4702		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN4711		Has domain(s) with predicted hydrolase activity
AN4812		Predicted glycosylphosphatidylinositol (GPI)-anchored protein
AN4817		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN4990		Ortholog(s) have ferrous iron transmembrane transporter activity, manganese ion transmembrane transporter activity
AN5076		Has domain(s) with predicted role in cell wall macromolecule catabolic process
AN5169		Ortholog(s) have role in syncytium formation by plasma membrane fusion
AN5283		Protein of unknown function
AN5408		Has domain(s) with predicted RNA binding, ribonuclease III activity and role in RNA processing
AN5458		Ortholog(s) have role in syncytium formation by plasma membrane fusion
AN5497		Ortholog(s) have 3,4-dihydroxy-2-butanone-4-phosphate synthase activity, role in aerobic respiration, riboflavin biosynthetic process and cytosol, fungal biofilm matrix, mitochondrial intermembrane space localization
AN5501		Ortholog(s) have lipase activity
AN5667		Protein of unknown function
AN5768		Ortholog(s) have role in actin cytoskeleton organization, inositol lipid-mediated signaling, negative regulation of phospholipid translocation, vacuole organization and plasma membrane localization
AN5781		Putative 30 kilodalton heat shock protein; transcript levels increase during the unfolded-protein response (UPR); palA-dependent expression independent of pH
AN6137		Protein of unknown function
AN6167		Has domain(s) with predicted FMN binding, catalytic activity, oxidoreductase activity and role in oxidation-reduction process
AN6277		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN6327		Protein of unknown function
AN6367		Has domain(s) with predicted phosphatidylinositol phosphate kinase activity and role in phosphatidylinositol metabolic process
AN6379		Protein of unknown function
AN6404		Has domain(s) with predicted zinc ion binding activity
AN6472	*dfgF*	Putative endo-mannanase GH76 family protein
AN6565		Protein of unknown function
AN6624		Protein of unknown function
AN6669	*mstC*	High-affinity glucose transporter active in germinating conidia
AN6703	*jenB*	Short-chain carboxylic acid transporter involved in uptake of lactate, succinate, pyruvate and malate

AN6787	*CYP682C1*	Putative cytochrome P450
AN6794		Protein of unknown function
AN6818		Protein of unknown function
AN6885		Protein of unknown function
AN6954		Protein of unknown function
AN7018		Protein of unknown function
AN7071	*pkgA*	Putative polyketide synthase; involved in the production of alternariol and other secondary metabolites; predicted backbone enzyme of a secondary metabolism gene cluster
AN7265		Has domain(s) with predicted role in mycotoxin biosynthetic process
AN7269		Ortholog(s) have role in fumiquinazoline C biosynthetic process, secondary metabolite biosynthetic process and fungal-type cell wall localization
AN7457		Protein of unknown function
AN7463	*meaA*	Major ammonium transporter of A. nidulans; transcript upregulated by nitrate limitation
AN7476		Protein of unknown function
AN7511	*gelE*	Putative 1,3-beta-transglycosidase with a predicted role in glucan processing; predicted glycosyl phosphatidylinositol (GPI)-anchor
AN7552		Protein of unknown function
AN7580		Protein of unknown function
AN7710		Ortholog(s) have intracellular localization
AN7779		Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN7892		Small heat-shock protein; molecular chaperone; expression upregulated after exposure to farnesol
AN7895	*cipB*	Putative oxidoreductase; contains Zn-dependent alcohol dehydrogenase domain; protein expressed at increased levels during osmoadaptation
AN8041	*gpdA*	Glyceraldehyde-3-phosphate dehydrogenase with a predicted role in gluconeogenesis and glycolysis; the gpdA promoter is a commonly used regulatory sequence for driving constitutive heterologous gene expression
AN8262	*gprH*	Secretin-like G-protein coupled receptor, involved in nutrient sensing and control of sexual development
AN8321		Protein of unknown function
AN8362		Protein of unknown function
AN8374		Ortholog(s) have oligopeptide transmembrane transporter activity
AN8512		Protein of unknown function; adjacent to tdi (terrequinone biosynthesis) gene cluster. Not required for terrequinone biosynthesis
AN8514	*tdiB*	Asterriquinone prenyltransferase; member of the tdi gene cluster; required for terrequinone A production; catalyzes the reverse prenylation event during terrequinone A biosynthesis; lacks canonical prenyl diphosphate binding motif (D/N)DXXD
AN8539	*ngn26*	Ortholog of A. fumigatus SidG; triacetylfusarinine C (TAFC) siderophore biosynthetic transacetylase; transcript induced by light in developmentally competent mycelia

AN8573		Protein of unknown function
AN8601		Protein of unknown function
AN8602		Protein of unknown function
AN8612		Protein of unknown function
AN8640	*conF*	Ortholog of N. crassa conF, light-induced transcript expressed during conidiation in N. crassa; double conF conJ deletion results in increased cellular glycerol or erythritol leading to delayed germination and desiccation resistance
AN8683		Ortholog(s) have ferric-chelate reductase activity, role in copper ion import, iron ion transport and plasma membrane localization
AN8692	*prxA*	Thioredoxin-dependent peroxidase; intracellular; PRX5-like domain; highly similar to the allergen Aspf3 from related fungi; menadione stress-repressed protein; osmoadaptation-induced protein; repressed by starvation-induced autophagy
AN8694		Has domain(s) with predicted DNA-binding transcription factor activity, RNA polymerase II-specific, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AN8989		Protein of unknown function
AN9021		Protein of unknown function
AN9025		Has domain(s) with predicted DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, nucleic acid binding, zinc ion binding activity
AN9069		Protein of unknown function
AN9103	*aifA*	Putative apoptosis-inducing factor (AIF)-like mitochondrial oxidoreductase; mutants display decreased survival in the presence of farnesol or menadione, decreased electron transport; expression upregulated after exposure to farnesol
AN9121	*esdC*	Protein with a glycogen binding domain involved in sexual development; regulated by VeA and FlbA
AN9184		Ortholog(s) have plasma membrane localization
AN9288		Has domain(s) with predicted oxidoreductase activity, transferase activity, transferring acyl groups other than amino-acyl groups, zinc ion binding activity and role in oxidation-reduction process
AN9364		Protein of unknown function
AN9449		Has domain(s) with predicted catalytic activity, metal ion binding, phosphoric diester hydrolase activity

CHAPTER 4: Concluding Remarks and Future Directions

4.1 Concluding Remarks

The research featured in this dissertation is specifically aimed to unravel the gene regulatory networks of the developmental and metabolic global regulators NsdD, VeA, and LaeA in *Aspergillus* fungi. The previous studies in *Aspergillus* species have suggested that developmental processes are tightly coupled with secondary metabolite production; developmental mutants defective in sexual and/or asexual development(s) coincidentally lost the ability to produce some secondary metabolites including mycotoxins (Calvo *et al*., 2002; Bennett and Klich, 2003; Yu and Keller, 2005). Although this type of connection between development and metabolism has been observed in a variety of fungal species (Calvo *et al*., 2002), the detailed mechanisms at the genetic level have not been thoroughly comprehended yet due to the difficulty of performing ChIP sequencing analysis and the complexity of gene regulatory networks. In this study, we investigated the genome-wide direct target genes of NsdD, VeA, and LaeA, constructed gene regulatory networks of these regulators based on the ChIP- and RNA-seq analyses, and demonstrated the core sections of each GRN. Furthermore, the novel regulatory roles of NsdD were identified, suggesting NsdD functions as a novel master regulator of development and metabolism in *Aspergillus*. This valuable information will fill gaps in previous studies and provide insights into researchers and industries utilizing *Aspergillus* species.

4.2 Future Directions

The recent studies of our group have shed light on the significant roles of NsdD in development and secondary metabolism of *Aspergillus* species (Han *et al*. 2001; Lee *et al*. 2014; Lee *et al*. 2016). Chapter 2 further dissects the detailed regulatory roles and mechanisms of

NsdD and found that NsdD governs fungal development and metabolism via a species-specific NsdD-mediated gene regulatory network in *Aspergillus* species. In addition, the RNA-seq analyses demonstrated that NsdD-mediated gene regulation is cell type-dependent within a species, but we only performed ChIP-seq in conidia samples in this study. Thus, ChIP-seq analyses in Vege and Asex will greatly improve the understanding of how NsdD forms distinct GRNs depending on the cell type. Moreover, comparing all NsdD-mediated GRNs from different cell types will enable us to determine the central and cell type-specific regulatory mechanisms of NsdD in development and metabolism. Furthermore, a comparative analysis between *A. nidulans* and *A. flavus* NsdD networks will provide a vital clue to understanding how the evolutionary transition has reshaped the role of NsdD in the two distantly related species. Along with the network analysis, we conducted a cross-complementation experiment by generating *A. flavus* Δ*nsdD* strains expressing the *A. nidulans nsdD* gene to investigate the functional conservation of NsdD (data not shown). Although their NsdDs' polypeptides share 68% identity, partial or complete complementation of normal phenotypes was observed in the cross-complemented strains, however, some different gene regulation patterns of two NsdDs were recognized in *A. flavus* from RNA-seq analyses, suggesting the conserved and distinct roles of NsdD between *A. nidulans* and *A. flavus*. This cross-complementation analysis only provides preliminary data about the functional conservation of NsdD since the experiment needs to be thoroughly redesigned and optimized. Further studies on this will shed light on the fundamental function of NsdD in *Aspergillus* species, which is likely conserved throughout its evolution. In the *A. flavus* NsdD core network, three genes predicted to encode forkhead proteins appeared as core components of developmental and metabolic regulations. We speculate that these forkhead genes might affect the morphogenesis of conidiophore in *A. flavus* in that forkhead genes are involved in cell cycle control and morphogenesis in fungi (Ribár *et al*., 1999; Bulmer *et al*., 2004; Golson and Kaestner, 2016). Characterization of these genes is necessary to prove their roles in morphogenesis and development of *A. flavus*.

In Chapter 3, we performed RNA- and ChIP-seq analyses only in Vege samples. As vegetatively growing cells (24 hr) are in the initial stage of growth, it's suitable to observe the global nascent effects of VeA and LaeA in development and metabolism. However, VeA and LaeA also play vital roles in the later phase of growth and developmental stages (Bayram *et al*., 2008; Bayram *et al*., 2010). Thus, further systematic dissection of the regulatory mechanisms of VeA and LaeA in other life stages such as asexual development needs to be elucidated for providing clearer blueprints of the VeA- and LaeA-mediated gene regulatory networks governing development and metabolism in *Aspergillus*. In addition, our multi-omics analyses suggested that VeA and LaeA directly and indirectly regulate the expression of a large array of genes involved in primary metabolism, which has not been reported yet. To shed light on the net effect of VeA and LaeA in primary metabolism, a comprehensive metabolome analysis, conducted in Chapter 2, needs to be performed. Within the core sections of VeA and LaeA networks, AN5055 and AN5199, encoding putative methionine aminopeptidases, were not identified yet unlike other genes. As methionine aminopeptidases play a crucial role in a posttranslational modification from eubacteria to higher eukaryotes by removing the N-terminal methionine from newly synthesized proteins (reviewed in Upadhya *et al*., 2006), we speculated that AN5055 and AN5199 may function in a similar way like their orthologs in *A. nidulans*. Understanding the roles of these genes in development and metabolism will provide an advance in the knowledge of *A. nidulans* biology.

4.3 References

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APPENDIX I: List of Publications

Gibbons, J., **Moon, H.**, Zhao, S., Fortwendel, J., Yu, J.-H. Genomic approaches for the molecular characterization of WetB in the human pathogenic fungus *Aspergillus fumigatus*. (In preparation)

Moon, H., Lee, MK., Bok, I., Bok, J., Keller N, Yu, J.-H. Unraveling the Gene Regulatory Network of VeA and LaeA in *Aspergillus nidulans*. (In preparation)

Moon, H., Lee, MK., Shin, J., Park, SC, Vazquez, J., Amador-Noguez, D., Han, KH., Keller, N., Yu, J.-H. The Master Regulator NsdD Governs Development and Metabolism in *Aspergillus*: Network-based Multi-omics Studies. (In preparation)

Moon, H. and Yu, J.-H. Main upstream regulators of development and secondary metabolism in *Aspergillus fungi*. (In preparation)

Zhao, Y., Lee, MK., Lim, J., **Moon, H.**, Park, HS., Zheng, W., Yu, J.-H. The velvet-activated putative C6 transcription factor VadZ regulates development and sterigmatocystin production in *Aspergillus nidulans*. Fungal Biology. 2022, 1878-6146. https://doi.org/10.1016/j.funbio.2022.05.001.

Ajmal, M., Alshannaq, A.F., **Moon, H.**, Choi, D., Akram, A., Nayyar, B.G., Gibbons, J.G., Yu, J.-H. Characterization of 260 Isolates of *Aspergillus* Section *Flavi* Obtained from Sesame Seeds in Punjab, Pakistan. Toxins 2022, 14, 117. https://doi.org/10.3390/toxins14020117

Zhao, Y., Lee, MK., Lim, J. **Moon, H.**, Park, HS., Zheng, W., Yu, J.-H. The putative sensor histidine kinase VadJ coordinates development and sterigmatocystin production in

Aspergillus nidulans. J Microbiol. 59, 746–752 (2021). https://doi.org/10.1007/s12275-021-1055-2

Emri, T., Gila, B., Antal, K., Fekete, F., **Moon, H.**, Yu, J.-H., Pócsi, I. AtfA-independent adaptation to the toxic heavy metal cadmium in *Aspergillus nidulans*. Microorganisms. 2021; 9(7):1433. https://doi.org/10.3390/microorganisms9071433

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Hatmaker, A., Zhou, X., Mead, M., **Moon, H.**, Yu, J.-H., Rokas, A.. Revised Transcriptome-Based Gene Annotation for *Aspergillus flavus* strain NRRL 3357. Microbiol Resour Announc. 2020 Dec, 9 (49) e01155-20; DOI: 10.1128/MRA.01155-20

Eom, T., **Moon, H.**, Yu, J.-H., Park, HS.. Characterization of the velvet regulators in *Aspergillus flavus*. J Microbiol. 2018 Dec;56(12):893-901. doi: 10.1007/s12275-018-8417-4