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ADVANCING THE SCIENCE OF PLANT BIOLOGY
The Tig1 Histone Deacetylase Complex Regulates Infectious Growth in the Rice Blast Fungus *Magnaporthe oryzae*  

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*Magnaporthe oryzae* is the most damaging fungal pathogen of rice (*Oryza sativa*). In this study, we characterized the *TIG1* transducin β-like gene required for infectious growth and its interacting genes that are required for plant infection in this model phytopathogenic fungus. Tig1 homologs in yeast and mammalian cells are part of a conserved histone deacetylase (HDAC) transcriptional corepressor complex. The *tig1* deletion mutant was nonpathogenic and defective in conidiogenesis. It had an increased sensitivity to oxidative stress and failed to develop invasive hyphae in plant cells. Using affinity purification and coimmunoprecipitation assays, we identified several Tig1-associated proteins, including two HDACs that were core components of the Tig1 complex in *M. oryzae*. The *set3, snt1*, and *hos2* deletion mutants displayed similar defects as those observed in the *tig1* mutant, but deletion of *HST1* or *HOS4* had no detectable phenotypes. Deletion of any of these core components of the Tig1 complex resulted in a significant reduction in HDAC activities. Our results showed that *TIG1*, like its putative yeast and mammalian orthologs, is one component of a conserved HDAC complex that is required for infectious growth and conidiogenesis in *M. oryzae* and highlighted that chromatin modification is an essential regulatory mechanism during plant infection.

INTRODUCTION

The ascomycetous fungus *Magnaporthe oryzae* is the causal agent of rice blast, which is one of the most destructive fungal diseases of rice (*Oryza sativa*) throughout the world (Dean et al., 2005; Wilson and Talbot, 2009). It produces three-celled pyriform conidia for dispersal. The infection process is initiated with the attachment and germination of conidia on the plant surface. An appressorium, a highly specialized infection structure, forms at the tip of the germ tube and penetrates the plant cuticle and cell wall (Tucker and Talbot, 2001). After penetration, the fungus forms unbranched primary invasive hyphae, which in turn differentiate into bulbous invasive hyphae. *M. oryzae* is a hemibiotrophic fungus that does not kill infected plant cells during the early stages of infection. Invasive hyphae are enclosed within the host cell membrane (Kankanala et al., 2007). Although it is not clear when necrotrophic growth begins, plant cells eventually die due to infectious growth of *M. oryzae*. Abundant conidia are produced on lesions that develop on rice plants during the late stages of infection to reinitiate the infection cycle. Under suitable conditions, infected seedlings can be killed by infection with *M. oryzae*, and panicle blast can cause severe yield losses.

The *M. oryzae*–rice pathosystem is a model system for studying fungal–plant interactions. In the past decade, there have been extensive studies on the molecular mechanisms that regulate appressorium morphogenesis and penetration in *M. oryzae* (Zhao et al., 2007; Wilson and Talbot, 2009). In addition to *ALB1, BUF1*, and *RSY1*, which are required for melanin synthesis, many genes that are important for appressorium formation and penetration have been characterized (Ebbole, 2007; Xu et al., 2007), including *PTH12, MMT1, TPS1, CYP1, PLS1, MIG1*, and *MHP1* (Clergeot et al., 2001; Tucker et al., 2004; Wilson et al., 2007; Mehrabi et al., 2008; Kim et al., 2009). Among these genes found to be important for early plant infection processes are several components of cAMP signaling and two mitogen-activated protein (MAP) kinase pathways. In *M. oryzae*, the cAMP-PKA pathway regulates the recognition of hydrophobic surfaces and initiation of appressorium formation (Mitchell and Dean, 1995; Fang and Dean, 2000). The Pmk1 MAP kinase pathway is required for appressorium formation and maturation (Zhao et al., 2007). It is also essential for root infection (Sesma and Osbourn, 2004) and the proper regulation of the mobilization of...
storage carbohydrate and lipid reserves from conidia to appresoria (Thines et al., 2000). The other MAP kinase pathway essential for pathogenesis in M. oryzae is the MPS1 cascade, which is dispensable for appressorium formation but required for appressorial penetration (Xu et al., 1998; Jeon et al., 2008; Mehrabi et al., 2008). These two pathways also have been shown to be important for plant infection in other phytopathogenic fungi, including some species that do not form appressoria (Rispail et al., 2009).

Compared with our knowledge of appressorium formation and penetration, our knowledge of the molecular mechanisms involved in the differentiation and growth of invasive hyphae in infected plant cells is limited. Although several genes are known to be important for infectious growth in planta (for reviews, see Ebbole, 2007; Xu et al., 2007; Wilson and Talbot, 2009), most of them, such as the PMK1 MAP kinase and PDE1 P-type ATPase genes, also are involved in other developmental and infection processes. The corresponding mutants normally have pleiotropic defects. Only a few mutants, including the abc1, des1, and pth8 mutants, have no obvious defects in growth and appressorium-mediated penetration but are defective in plant infection. These genes must have cellular functions that are specific for invasive hyphae, such as the ABC1 transporter gene for avoiding toxic plant defense compounds (Urban et al., 1999) and DES1 for suppressing the plant defense response (Chi et al., 2009). Recently, microarray analysis has been used to identify genes specifically or highly expressed in invasive hyphae (Mosquera et al., 2009). Many of these genes expressed in planta have never been detected in vitro and encode biotrophy-associated secreted (BAS) proteins. Some, but not all, BAS proteins localize to biotrophic interfacial complexes (Mosquera et al., 2009). Because none of the BAS genes that have been functionally characterized are essential for pathogenicity (Mosquera et al., 2009), their functions in plant colonization and infectious growth are not clear.

In the wheat scab fungus Fusarium graminearum, the TBL1-like gene FTL1 was identified as a novel fungal pathogenicity factor by random insertional mutagenesis (Ding et al., 2009). FTL1 encodes a protein that is putatively orthologous to yeast SIF2 and mammalian TBL1. The ftl1 mutant was nonpathogenic. However, the molecular mechanism underlying its defects in plant infection is not clear. Because its infection processes, particularly fungal–plant interactions after plant penetration, are not well understood, F. graminearum is not suited for detailed characterization of this novel pathogenicity factor. In this study, we identified and characterized the TIG1 gene, an FTL1 ortholog, in the model plant pathogenic fungus M. oryzae. The tig1 mutant formed appressoria but was nonpathogenic. It was defective in the differentiation and growth of invasive hyphae in planta. The mutant had increased sensitivities to oxidative stress and other plant defensive compounds. Using affinity purification and mass spectrometry analyses, we identified several TIG1-associated proteins that are homologous to components of the yeast Set3 complex, including two histone deacetylases (HDACs). Coimmunoprecipitation assays were used to confirm the interactions among TIG1, Snt1, Set3, and Hos2. Mutants lacking any of these genes had similar defects in plant infection and conidiation. HDAC activities and histone acetylation levels were also affected in these mutants disrupted in the TIG1 complex. Our data indicate that TIG1 is a component of a well-conserved HDAC complex and that chromatin modification is an essential regulatory mechanism during plant infection.

RESULTS

Identification of the TIG1 Transducing β-Like Gene in M. oryzae

In the M. oryzae genome, MGG_03198.5 shares >50% amino acid identity with F. graminearum FTL1 and yeast SIF2. It has an N-terminal LisH domain (residues 6 to 38) and six WD40 repeats (residues 536 to 626) toward the C terminus. The cDNA of TIG1 (for TBL1-like gene required for invasive growth) was amplified and sequenced. Four predicted introns in the TIG1 coding region were confirmed by sequence analysis. Although no known protein domain was identified, the amino acid sequence between the LisH domain and WD40 repeats was conserved between TIG1 and its orthologs from Neurospora crassa and F. graminearum (see Supplemental Figure 1 online).

To determine the function of TIG1 in M. oryzae, a gene replacement construct (Figure 1A) was obtained by ligation-PCR and transformed into Guy11 (Chao and Ellingboe, 1991). The resulting hygromycin-resistant transformants were screened by PCR and analyzed by DNA gel blot hybridization. When hybridized with a TIG1 fragment, a 3.6-kb PstI band was observed in the wild type but not in the tig1 deletion mutant strains (Figure 1B). When hybridized with the hygromycin phosphotransferase gene (hph), the tig1 mutant but not the wild type had an 11.1-kb band that was characteristic of the gene replacement event (Figure 1B). The tig1 mutant KT-1 (Table 1) grew slightly slower than the wild type but...
Guy11, KT-1 produced was significantly reduced in conidiation. In comparison with results were obtained in infection assays with seedlings of barley dark brown spots were formed occasionally (Figure 2A). Similar mutant KT-1, no typical blast lesions were observed, but small lesions formed (Figure 2A). On leaves inoculated with the tig1 conidia from Guy11 and an ectopic transformant, abundant fungal growth and conidiation were observed over the necrotic areas or lesions (Figure 2C). Under the same conditions, abundant fungal growth and conidiation were observed over the necrotic areas or lesions caused by Guy11 (Figure 2C). These results indicate that the tig1 mutant was nonpathogenic and failed to colonize plant tissues through wounds. The rare, small, brown leaf spots caused by the tig1 mutant was not true blast lesions and were likely associated through wounds. The rare, small, brown leaf spots caused by the tig1 deletion mutant in strain 70-15 (Chao and Ellingboe (1991)) were directly related to deletion of TIG1. The resultant transformant CT-1 (Table 1) exhibited normal levels of appressorium formation. However, it was defective in the penetration of onion epidermal bic surfaces, the tig1 mutant exhibited normal levels of appressorium formation. How-ever, it was defective in appressorium formation on artificial hydrophobic surfaces. The tig1 mutant was responsible for defects of the TIG1 virulence on rice seedlings and produced abundant conidia These 70-15 tig1 null mutants displayed the same defects in growth and conidiation as mutant KT-1 (Table 2). On seedlings of rice cultivar Nipponbare, KT-2 and KT-4 failed to cause typical blast lesions in spray or injection infection assays (see Supplemental Figure 2 online).

To confirm that the phenotypes observed in these mutants were directly related to deletion of TIG1, mutant KT-4 (MAT1-1) was crossed with Guy11 (MAT1-2). A total of 19 ascospore progeny were isolated. Eight of these progeny were resistant to hygromycin and had similar defects as the tig1 mutant. The remaining 11 hygromycin-sensitive progeny had the wild-type phenotype, indicating that defects observed in the tig1 mutant cosegregated with the hygromycin resistance marker. In addition, we reintroduced the wild-type TIG1 allele into mutant KT-1. The resulting transformant CT-1 (Table 1) exhibited normal virulence on rice seedlings and produced abundant conidia (Table 2), demonstrating that the inactivation of TIG1 was responsible for defects of the tig1 mutant.

TIG1 Is Required for Infectious Growth after Penetration of Rice Cells

When assayed for appressorium formation on artificial hydrophobic surfaces, the tig1 mutant KT-1 produced abundant melanized appressoria (Figure 3A). No obvious defects in appressorium formation were observed (Table 2). On onion epidermal cells, the mutant exhibited normal levels of appressorium formation. However, it was defective in the penetration of onion epidermal cells and differentiation of invasive hyphae. By 48 h, Guy11

### Table 1. Wild-Type and Mutant Strains of M. oryzae Used in This Study

| Strain  | Genotype Description                  | Reference                  |
|---------|--------------------------------------|----------------------------|
| Guy11   | Wild-type (MAT1-2, avr-Pita)         | Chao and Ellingboe (1991)  |
| 70-15   | Wild-type (MAT1-1, AVR-Pita)         | Chao and Ellingboe (1991)  |
| Ku80    | MgKu80 deletion mutant of Guy11      | Villalta et al. (2008)     |
| KT-1    | tig1 deletion mutant of Guy11        | This study                 |
| KT-2    | tig1 deletion mutant of 70-15        | This study                 |
| KT-4    | tig1 deletion mutant of 70-15        | This study                 |
| ECT23   | Ectopic transformant of Guy11        | This study                 |
| CT-1    | KT-1 transformant complemented with TIG1 | This study                 |
| KT1NG1  | KT-1 transformant expressing the TIG1-GFP construct | This study |
| TgRG1   | 70-15 transformant expressing _P rp27-TIG1-GFP_ | This study |
| K1RG1   | KT-1 transformant expressing _P rp27-TIG1-GFP_ | This study |
| TFg1    | 70-15 transformant expressing _TIG1-3xFLAG_ fusion | This study |
| SetG1   | 70-15 transformant expressing _SET3-GFP_ fusion | This study |
| HosG1   | 70-15 transformant expressing _HOS2-GFP_ fusion | This study |
| TS-29   | _TIG1-3xFLAG_ and _SET3-GFP_ transformant | This study |
| HT-26   | _TIG1-3xFLAG_ and _HOS2-GFP_ transformant | This study |
| HS-9    | _SET3-3xFLAG_ and _HOS2-GFP_ transformant | This study |
| KH2-13  | _hos2_ deletion mutant of 70-15      | This study                 |
| CH-1    | _hos2/HOS2_ complementation strain of KH2-13 | This study |
| KH4-81  | _hos4_ deletion mutant of 70-15      | This study                 |
| KH1-23  | _has1_ deletion mutant of 70-15      | This study                 |
| ECT1    | _An ectopic transformant of 70-15_   | This study                 |
| KS1-1   | _snt1_ deletion mutant of Ku80       | This study                 |
| KS3-1   | _set3_ deletion mutant of Ku80       | This study                 |
| CS-1    | _set3/SET3_ complementation strain of KS3-1 | This study |
| ECT2    | _An ectopic transformant of Ku80_    | This study                 |

was significantly reduced in conidiation. In comparison with Guy11, KT-1 produced ~100-fold fewer conidia (Table 2).

The _tig1_ Deletion Mutant Is Defective in Plant Infection

When seedlings of rice cultivar CO-39 were spray inoculated with conidia from Guy11 and an ectopic transformant, abundant lesions formed (Figure 2A). On leaves inoculated with the _tig1_ mutant KT-1, no typical blast lesions were observed, but small dark brown spots were formed occasionally (Figure 2A). Similar results were obtained in infection assays with seedlings of barley (Hordeum vulgare) cultivar Golden Promise. The _tig1_ mutant failed to cause blast lesions and green islands on barley leaves. In injection infection assays, only limited necrosis was observed at the wound sites inoculated with KT-1 (Figure 2B). When the dark-brown spots and necrotic areas caused by the _tig1_ mutant were excised, surface sterilized, and incubated on water agar plates for 72 h, we failed to detect fungal growth or conidiation (Figure 2C). Under the same conditions, abundant fungal growth and conidiation were observed over the necrotic areas or lesions caused by Guy11 (Figure 2C). These results indicate that the _tig1_ mutant was nonpathogenic and failed to colonize plant tissues through wounds. The rare, small, brown leaf spots caused by the mutant were not true blast lesions and were likely associated with plant defense responses.

To determine whether the _TIG1_ function varies among strains, we also generated the _tig1_ deletion mutant in strain 70-15 (Chao and Ellingboe, 1991). Mutants KT-2 and KT-4 were identified and confirmed by DNA gel blot analysis to lack _TIG1_ (Figure 1B).
penetrated and formed invasive hyphae inside onion epidermal cells (Figure 3B). Under the same conditions, no invasive hyphae were observed in KT-1. Even up to 72 h, the unbranched primary invasive hyphae formed by KT-1 were blocked in the differentiated and growth of invasive hyphae.

We also assayed appressorium penetration and infectious growth using rice leaf sheath epidermal cells. The tig1 mutant formed appressoria but failed to develop invasive hyphae in plant cells (Figure 4A). By 48 h, invasive hyphae formed by Guy11 began to invade nearby plant cells. Under the same conditions, unbranched primary invasive hyphae formed by the mutant had only limited growth in penetrated plant cells (Figure 4A). Infected plant cells often had discolored cell walls, and fungal hyphae appeared to be restricted and unhealthy in comparison with those formed by Guy11 (Figure 4A). When stained with 3,3'-diaminobenzidine (DAB), reactive oxygen species (ROS) accumulated around invasive hyphae in plant cells penetrated by the mutant (Figure 4B). By contrast, invasive hyphae formed by Guy11 did not trigger significant ROS accumulation in the host (Figure 4B). These results indicate that the tig1 deletion mutant is defective in its ability to overcome plant defense responses and maintain biotrophic growth after penetration. When stained with aniline blue, stronger fluorescence signals were observed in barley leaf epidermal cells penetrated by the tig1 mutant than the wild type (see Supplemental Figure 3 online), suggesting that primary infectious hyphae of the mutant face enhanced callose deposition from the host.

The tig1 Mutant Has an Increased Sensitivity to H2O2 and Plant Defense-Related Proteins

Because plant cells accumulated ROS in the region surrounding primary invasive hyphae of the tig1 mutant, we tested the sensitivity of the mutant to H2O2. When hydrogen peroxide was added to complete medium (CM) plates, fungal growth was decreased with increasing concentrations of H2O2. However, the reduction in the growth of the tig1 mutant was more severe than that in the wild type. Hyphal growth was completely inhibited by 5 mM H2O2 in the tig1 mutant KT-1 but not in Guy11 (Figure 5A). We also assayed the effects of two plant proteins, osmotin and MsDef1, that are toxic to fungal pathogens (Coca et al., 2000; Ramamoorthy et al., 2007). In the presence of 50 μg/mL osmotin, appressorium formation was normal in Guy11 but blocked in KT-1 on hydrophobic surfaces (Figure 5B). Osmotin treatment tended to stimulate the formation of multiple germ tubes and unmelanized apical or intercalary swollen bodies. The tig1 mutant also was more sensitive to MsDef1 than Guy11. In the presence of 20 μM MsDef1, melanized appressoria were formed by Guy11 but not by KT-1 (Figure 5B). Unlike osmotin, MsDef1 treatment did not cause KT-1 germ tubes to swell.

![Figure 2. Infection Assays with the tig1 Mutant.](image)

(A) Rice leaves sprayed with conidia from the wild-type strain Guy11, an ectopic transformant ECT23, and tig1 mutant KT-1.
(B) Injection assays with Guy11 and KT-1. Inoculation with 0.25% gelatin was the negative control. The tig1 mutant only caused limited necrosis at the wound site.
(C) Assays for fungal growth on surface-sterilized rice leaves inoculated by spray (top panels) or injection (bottom panels). Abundant hyphae and conidia were observed on leaves inoculated with Guy11. No fungal growth was observed on rare brown spots or limited necrotic regions at the wound sites caused by the tig1 mutant. [See online article for color version of this figure.]

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**Table 2. Vegetative Growth, Conidiation, and Appressorium Formation in the Wild Type and Transformants Generated in This Study**

| Strain       | Growth Rate on CM (mm/d) | Conidiation (×10^5 Spores/Plate) | Appressorium Formation (%)a |
|--------------|--------------------------|-----------------------------------|-----------------------------|
| Guy11 (wild type) | 6.3 ± 0.1               | 280.0 ± 6.2                      | 99.5 ± 0.1                  |
| KT-1 (tig1)   | 5.4 ± 0.2                | 3.2 ± 0.7                        | 98.7 ± 0.2                  |
| ECT23 (ectopic) | 6.1 ± 0.2               | 287.3 ± 5.6                      | 95.3 ± 0.1                  |
| CT-1 (tig1/TG1) | 6.1 ± 0.1               | 289.7 ± 4.6                      | 96.7 ± 0.1                  |
| 70-15 (wild type) | 6.2 ± 0.1               | 77.5 ± 2.5                       | 96.7 ± 0.1                  |
| KT-4 (tig1)   | 5.4 ± 0.1                | 0.9 ± 0.2                        | 90.6 ± 2.6                  |
| KHz-13 (hos2) | 4.1 ± 0.1                | 0.6 ± 0.1                        | 92.1 ± 0.1                  |
| KHz-81 (hos4) | 5.5 ± 0.1                | 7.5 ± 2.5                        | 94.0 ± 0.1                  |
| KHz-23 (hst1) | 6.2 ± 0.2                | 26.7 ± 2.9                       | 93.6 ± 0.1                  |
| ECT1 (ectopic) | 6.1 ± 0.1               | 49.2 ± 6.3                       | 96.9 ± 0.1                  |
| Ku80          | 6.3 ± 0.1                | 170.0 ± 10.0                     | No data                     |
| KS3-1 (set3)  | 3.4 ± 0.1                | 1.7 ± 0.3                        | No data                     |
| KS1-1 (snt1)  | 4.1 ± 0.1                | 0.4 ± 0.1                        | No data                     |
| ECT2 (ectopic) | 6.3 ± 0.2               | 150.0 ± 12.6                     | No data                     |

*Percentage of germ tubes that formed appressoria.*
Identification of TIG1-Interacting Proteins by Affinity Purification

TIG1 is putatively orthologous to yeast SIF2, which is a member of the Set3 complex involved in the late stages of ascospore formation (Pijnappel et al., 2001; Cerna and Wilson, 2005). To determine whether TIG1 functions in a similar complex in M. oryzae, we generated a TIG1-3xFLAG construct and transformed it into 70-15. In transformant TFg1 (Table 1), a 72-kD band of the expected size of Tig1-3xFLAG was detected with an anti-FLAG antibody in proteins isolated from vegetative hyphae grown in liquid CM medium (see Supplemental Figure 4 online).

For affinity purification, total proteins were isolated from transformant TFg1 and mixed with anti-FLAG M2 beads. Proteins bound to M2 beads were eluted and digested with trypsin and identified by mass spectrometry analysis (see Methods). Table 3 lists proteins that were copurified with the TIG1-3xFLAG fusion. MGG_09174.5, MGG_01663.5, MGG_02488.5, MGG_10447.5, and MGG_05727.5 (Table 3) are orthologous to yeast SNT1, HOS2, HST1, CPR1, and HOS4, respectively, which are members of the Set3 complex (Pijnappel et al., 2001; Mou et al., 2006). They were consistently identified as TIG1-interacting genes in four independent biological replicates. Other proteins that coimmunoprecipitated with Tig1 included MGG_09602.5 and MGG_01362.5 (Table 3). While MGG_09602.5 has no homologous sequence in Saccharomyces cerevisiae and appears to be unique to filamentous fungi, putative orthologs of other genes exist in yeast. MGG_01362.5 is homologous to yeast Cdc28, which interacts with Hos4 (Ubersax et al., 2003), but does not belong to the Set3 complex.

TIG1 Is a Member of a Conserved HDAC Protein Complex

The putative orthologs of all members of the yeast Set3 complex except Set3 itself were copurified with TIG1 (Table 3). We amplified the predicted open reading frame (ORF) of MGG_01558 (named SET3 in this study) and cloned it into the pAD-GAL4 prey vector. The TIG1 gene was cloned into the corresponding bait vector. Growth on SD-His plates and LacZ activity were detected in yeast cells expressing the SET3 prey and TIG1 bait constructs (Figure 6A), indicating that SET3 interacted with TIG1. However, their interaction was relatively weak in comparison with the positive control for yeast two-hybrid assays (Figure 6A).

To confirm the TIG1-SET3 interaction, we generated the SET3-green fluorescent protein (GFP) construct and cotransformed it into 70-15 with the TIG1-3xFLAG fusion. The resulting hygromycin-resistant transformant TS-29 (Table 1) was confirmed by PCR to contain both constructs, and successful transformation was further confirmed by immunoblot analysis with anti-FLAG and anti-GFP antibodies. Total proteins were isolated from transformant TS-29 and bound to anti-FLAG M2 beads. Proteins bound to the bead were eluted, separated on 12% SDS-PAGE gels, and transferred onto nitrocellulose membrane. As expected, the Tig1-3xFLAG protein was detected with an anti-FLAG antibody in both the eluted and total proteins (Figure 6B). A protein band of the expected size of the Set3-GFP fusion was also detected with the anti-GFP antibody in both the elution and total proteins (Figure 6B), indicating that Set3 coimmunoprecipitated with Tig1. The anti-actin antibody detected the actin band in total proteins isolated from the transformant, but not in the eluted proteins (Figure 6B).

Figure 3. Appressoria Formation and Penetration of Onion Epidermal Cells.

(A) Melanized appressoria formed by the wild type (Guy11) and tig1 mutant (KT-1) strains on plastic cover slips.
(B) Penetration assays with onion epidermal cells. By 48 h, invasive hyphae were observed in plant cells penetrated by Guy11 but not KT-1. A, appressorium; C, conidium; PI, primary invasive hyphae; SI, secondary invasive hyphae.
Bars = 10 μm.

Figure 4. Penetration Assays with Rice Leaf Sheath Epidermal Cells.

(A) Extensive invasive hyphae were developed by Guy11 inside plant cells by 72 h after inoculation. The tig1 mutant (KT-1) had only limited growth of primary invasive hyphae.
(B) When stained with DAB, rice cells penetrated by the tig1 mutant accumulated ROS around fungal hyphae. No obvious ROS accumulation was observed in plant cells penetrated by Guy11. A, appressorium; PI, primary invasive hyphae; SI, secondary invasive hyphae.
Bars = 10 μm.
SNT1, HOS2, and SET3 Are Required for Plant Infection

To determine the functions of Tig1-interacting proteins, we constructed deletion mutants of SNT1, HOS2, SET3, HST1, and HOS4 by gene replacement. The putative CPR1 ortholog, CYP1, was not included in this study because it is known to be involved in plant infection in M. oryzae (Viaud et al., 2002). The snt1, set3, hos4, hos2, and hst1 deletion mutants (Table 1) were identified by PCR and confirmed by DNA gel blot analysis (see Supplemental Figure 5 online). Like the tig1 mutant, snt1, set3, and hos2 mutants were nonpathogenic on seedlings of susceptible rice cultivars (Figure 8A) and barley (Figure 8B). These three mutants were also greatly impaired in conidiation (Table 2). By contrast, conidiation was not significantly impaired in the hst1 and hos4 mutants (Table 1), and these mutants were as virulent as the wild type (Figures 8A and 8B), indicating that HST1 and HOS4 are dispensable for plant infection and the function of the Tig1 complex.

In addition to reduced conidiation, conidia formed by the tig1, snt1, set3, and hos2 mutants varied significantly in size and morphology from those formed by the wild type (Figure 8C). In general, conidia from these mutants were still three celled, but most of them were narrower than wild-type conidia. Some conidia produced by the tig1, snt1, set3, and hos2 mutants no longer had the normal pyriform shape (Figure 8C). A reduction in conidiation and abnormal spore morphology indicate that TIG1, SNT1, SET3, and HOS2 play an important role in conidiogenesis in M. oryzae. We performed a complementation analysis, in which wild-type HOS2 and SET3 genes were reintroduced into the hos2 and set3 mutants, respectively. The resulting complemented transformants (CH-1 and CS-1; Table 1) exhibited normal virulence (Figure 8B), conidiation, and conidium morphology.

HDAC Activities and Histone Acetylation in the Tig1 Complex Mutants

Because the yeast Set3 complex is involved in histone deacetylation, it is likely that Tig1, Set3, Hos2, and Snt1 are components of a similar HDAC complex in M. oryzae. Therefore, we assayed HDAC activities in protein extracts isolated from protoplasts of the tig1, set3, hos2, and snt1 mutants. In comparison with the wild-type strains, the tig1, set3, snt1, and hos2 mutants were highly reduced in HDAC activities (Figure 9A). The three wild-type

| Table 3. Putative TIG1-Interacting Genes Identified by Affinity Purification |
|-----------------|-----------------|
| Gene ID         | Yeast Homolog   |
| MGG_01633.5     | HOS2            |
| MGG_09174.5     | SNT1            |
| MGG_02488.5     | HST1            |
| MGG_10447.5     | CPR1            |
| MGG_05727.5     | YIL112w (HOS4)  |
| MGG_06435.5     | YCR028C         |
| MGG_09602.5     | No homolog      |
| MGG_00446.5     | YGL019W (CKB1)  |
| MGG_01826.5     | YGL174W (BUD13)|
| MGG_01362.5     | YBR160W (CDC28) |
| MGG_03741.5     | YGR266W         |
strains, Guy11, 70-15, and Ku80, used for generating these mutants had similar HDAC activities in three biological replicates (Figure 9A). These results indicate that Tig1, Set3, Hos2, and Snt1 are required for the activity of the HDAC complex in M. oryzae. Because TIG1, SET3, and SNT1 have no predicted functions in histone modification, their effects on histone acetylation must be related to their participation in an HDAC complex.

Since reduced HDAC activities may lead to increased histone acetylation, we assayed the acetylation level of histone H3 in the hos2 mutant. In nuclear proteins isolated from Guy11, the expression level of histone 3 was similar to that of the mutant. However, when detected with an anti-H3K18Ac antibody that is specific for K18 acetylation of histone H3 (Figure 9B), Guy11 had weaker signals than the hos2 mutant, indicating that the hos2 mutant had higher levels of H3K18 acetylation than Guy11.

Disruption of the Tig1 Complex Affects the Activation of Mps1 MAP Kinase

Since the Set3 complex functions upstream of the Slt2 MAP kinase during secretory stress responses (Cohen et al., 2008), we assayed the activation of Mps1 in the mutants disrupted in the Tig1 complex. In vegetative hyphae, the expression of Mps1 was not affected by SNT1, TIG1, or SET3 deletion (Figure 9C). However, the phosphorylation level of Mps1 was lower in the snt1, tig1, and set3 mutants than in Ku80 (Figure 9C), indicating a reduction in Mps1 activation in these mutants disrupted in the Tig1 complex. By contrast, phosphorylation of Pmk1 was not affected or was slightly increased in the snt1, tig1, and set3 and mutants (Figure 9C).

Expression and Localization of TIG1-, SET3-, and HOS2-GFP

Although Tig1 has no predictable nuclear localization signal (NLS) sequences, it may localize to the nucleus as one component of a HDAC complex. To test this hypothesis, a TIG1-GFP fusion construct was transformed into the tig1 mutant KT-1. Transformant KT1NG1 (Table 1), which expresses the TIG1-GFP fusion, exhibited normal growth, conidiation, and virulence, indicating that the defects in the tig1 mutant were complemented. In KT1NG1, GFP signal was observed in the nucleus in conidia, appressoria, and invasive hyphae (Figure 10A). In vegetative hyphae, only a faint GFP signal was observed in the nuclei of some hyphal compartments (Figure 10A). When the TIG1-GFP fusion was overexpressed with the RP27 promoter in transformant KT1RP1 (Table 1), a stronger GFP signal was observed in the nuclei of vegetative hyphae (see Supplemental Figure 6 online), suggesting that lower expression of TIG1-GFP was responsible for weak GFP signals in vegetative hyphae in KT1NG1.

Figure 6. Yeast Two-Hybrid and Coimmunoprecipitation Assays for the TIG1-SET3 Interaction.

(A) Yeast transformants expressing the TIG1 bait and SET3 prey constructs were assayed for growth on SD-Leu-Trp-His plates (SD-His) and β-galactosidase (LacZ) activities. +, Positive control; −, negative control. (B) Immunoblot analysis of total proteins (TS-29) and proteins eluted from the anti-FLAG M2 beads (Elution) of transformant TS-29 that expressed the TIG1-3xFLAG and SET3-GFP constructs. Total proteins isolated from the wild-type strain (70-15) were included as the control. Top, middle, and bottom images represent detection with anti-FLAG, anti-GFP, and anti-actin antibodies, respectively. [See online article for color version of this figure.]

Figure 7. Co-IP Assays for Interactions between Components of the Tig1 Complex.

Co-IP assays of transformants HS-9 (A) and HT-26 (B) that expressed the SET3-FLAG/HOS2-GFP and TIG1-3xFLAG/HOS2-GFP constructs, respectively. Immunoblots of total proteins isolated from each transformant and proteins eluted from the anti-FLAG M2 beads (Elution) were detected with the anti-FLAG, anti-GFP, or anti-actin antibody. Total proteins isolated from the wild-type strain 70-15 were included as the control.
We also generated GFP fusion constructs with \( \text{SET3} \) and \( \text{HOS2} \). In transformants of 70-15 expressing these constructs (Table 1), the GFP signal was also observed in the nucleus in conidia, appressoria, and invasive hyphae (Figure 10B). Similar to the \( \text{TIG1-GFP} \) transformant, the expression of \( \text{SET3-GFP} \) was lower in vegetative hyphae than invasive hyphae, but a faint GFP signal could be observed in some nuclei. However, in transformants expressing the \( \text{HOS2-GFP} \) fusion, GFP signals were weak or not detectable in vegetative hyphae.

Expression Profiles of Selected Components of the \text{Tig1} Complex

We used quantitative RT-PCR (qRT-PCR) to quantify the transcripts of \( \text{TIG1}, \text{HOS2}, \text{HOS4}, \text{HST1}, \text{SET3}, \) and \( \text{SNT1} \) at different fungal developmental and infection stages. All of these genes were expressed at a relatively low level in vegetative hyphae and had their highest expression levels in conidia (Figure 11). In comparison with their expression levels in vegetative hyphae, transcription of \( \text{TIG1}, \text{HOS2}, \text{HOS4}, \text{HST1}, \text{SET3}, \) and \( \text{SNT1} \)
increased 23-, 6-, 25-, 46-, 16-, and 27-fold, respectively, in conidia. These results were in agreement with the weaker GFP signal observed in vegetative hyphae than in conidia in transformants expressing the TIG1-GFP or HOS2-GFP fusion. The expression of TIG1, HOS2, HOS4, HST1, SET3, and SNT1 was higher in appressoria than in vegetative hyphae (5- to 13-fold; Figure 11), but they all had the highest expression level in conidia (Figure 11). Transcripts of these six genes were not detectable by qRT-PCR during the early stages of infection (1 to 2 d after inoculation [DAI]), likely as a consequence of their low expression levels (<0.1-fold of the reference gene ILV5 encoding an acetohydroxyacid reductoisomerase) and a low percentage of fungal RNA in these infected rice leaves (<1% of total RNA). Among all of the genes assayed by qRT-PCR, only the expression of CYP1 was detectable during the early infection stages (1 to 2 DAI; see Supplemental Figure 7 online), which is likely related to its expression level being 2- to 4-fold higher than that of the reference gene ILV5. At later infection stages (3 to 4 DAI), transcripts of all of these genes were detectable by qRT-PCR. At 4 DAI, the expression level of TIG1, HOS2, HOS4, HST1, SET3, and SNT1 was 4-, 3-, 5-, 6-, 4-, and 6-fold higher in infected leaves than in vegetative hyphae, respectively (Figure 11). These data indicate that all of the proposed components of the Tig1 complex, except for CYP1, share the same expression pattern at the different fungal developmental and infection stages.

DISCUSSION

In this study, we identified and characterized the TIG1 gene and protein complex in M. oryzae. TIG1 and its orthologs are conserved from yeast to human (Pijnappel et al., 2001; Mou et al., 2006). TIG1 shares higher similarity with mammalian TBL1 than with yeast SIF2. It has an N-terminal Lsh domain and six WD domains in the C-terminal region. No other genes in M. oryzae have similar structural features. We also noticed that many of the TIG1 orthologs from filamentous fungi have an intron in the Lsh domain and another intron immediately before the stop codon. These two introns at the termini of TIG1 ORF may be evolutionarily conserved for regulating the expression or stability of TIG1 mRNA.

In budding yeast, SIF2 is involved in transcriptional repression of meiosis-specific genes (Pijnappel et al., 2001; Cerna and Wilson, 2005). In M. oryzae, the tig1 mutant was female sterile but male fertile. Among 30 ascospore progeny from a tig1 × TIG1 cross, 18 carried the tig1 null allele, suggesting that TIG1 deletion has no effect on ascospore formation and viability. However, conidiation was significantly reduced in the tig1 and other Tig1 complex-deficient mutants (Table 2). Conidium morphology was also abnormal in these mutants (Figure 8C). Because asexual reproduction plays a critical role in the infection cycle, the Tig1 complex may have been adapted to regulate conidiation in M. oryzae and other plant pathogens. In a number of phytopathogenic fungi, MAP kinases involved in the yeast pheromone response and filamentous growth pathways have evolved to regulate appressorium formation and other plant infection processes (Zhao et al., 2007; Rispail et al., 2009).

Figure 10. Expression and Localization of the GFP Fusion Proteins.
(A) In transformant KT1NG1, which expresses the TIG1-GFP fusion, GFP signals were observed in the nucleus in conidia (top), appressoria (middle), and invasive hyphae (bottom). A, appressoria; C, conidia; VH, vegetative hyphae; IH, invasive hyphae.
(B) Nuclear localization was also observed in the invasive hyphae of transformant CS-1, which expresses the SET3-GFP fusion. Bars = 10 μm.

Figure 11. Expression Patterns of Genes Associated with TIG1.
Expression levels of TIG1, HOS2, SNT1, HOS4, SET3, and HST1 in vegetative hyphae (Myc), conidia (Spo), 24-h-old appressoria (App), and infected rice leaves harvested at 3 or 4 DAI. qRT-PCR was used to quantify transcripts of these genes relative to that of the constitutive reference gene ILV5 using the 2−ΔΔCT method. Median and sd were calculated from three biological replicates.
Similar to ft1 mutants in *F. graminearum* (Ding et al., 2009), the *tig1* mutant was nonpathogenic. Close examination indicates that the differentiation of invasive hyphae was blocked in the *tig1* mutant. In plant cells penetrated by the *tig1* mutant, only limited growth of primary invasive hyphae was observed. DAB staining indicated that *tig1* primary invasive hyphae were surrounded by a high concentration of ROS. Plant cells appeared to mount strong defense responses and displayed HR-like cell death. The *tig1* mutant also had increased sensitivities to PR protein osmotin and plant defensin MsDef1 (Coca et al., 2000; Ramamoorthy et al., 2007). It is possible that attenuated invasive growth of the *tig1* mutant triggered strong defense responses in host cells. Rapid accumulation of high concentrations of ROS and other plant defense compounds at the penetration site may block the proliferation of the *tig1* mutant and subsequently result in the death of penetrated plant cells, which may be responsible for the rare, small brown spots formed on susceptible rice seedlings inoculated with the *tig1* mutant (Figure 2A).

In comparison with extensive studies on appressorium formation and function, the regulation of postpenetration infection processes is not well characterized in *M. oryzae* (Ebbole, 2007; Xu et al., 2007; Wilson and Talbot, 2009). Unlike other nonpathogenic mutants that are blocked in either appressorium formation or appressorium-mediated penetration, mutants disrupted in the Tig1 HDAC complex were defective in the differentiation and growth of bulbous secondary invasive hyphae. These results indicate that chromatin modification plays a critical role in regulating fungal invasive growth. Because histone deacetylation is normally associated with a suppression of gene expression (Pijnappel et al., 2001; Brosch et al., 2008), the Tig1 complex may be involved in the repression of genes that are detrimental to the differentiation and biotrophic growth of secondary invasive hyphae. The fact that *M. oryzae* uses a global regulatory mechanism such as histone modification for invasive growth indicates that a large number of genes may need to be repressed simultaneously in invasive hyphae. Further characterization of the Tig1 complex will be helpful in understanding the regulatory networks that regulate fungal–plant interactions after penetration and development of invasive hyphae.

In mammalian cells, **TBL1** and **TBLR1** are transducin β-like proteins associated with the N-CoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptors) complex (Guenther et al., 2001; Yoon et al., 2003; Zhang et al., 2006). SMRT proteins have an N-terminal domain that binds to nuclear receptors and a C-terminal region that interacts with HDACs. In yeast, **Sif2** is a component of the Set3 HDAC complex that consists of **Sif2**, **Hos2**, **Set3**, **Snt1**, **Hst1**, **Hos4**, and **Cpr1** (Pijnappel et al., 2001; Cerna and Wilson, 2005; Mou et al., 2008). Several of these proteins are conserved from yeast to human. The *M. oryzae* genome contains putative orthologs of every component of the yeast Set3 complex. All of these putative orthologs except **SET3** were coimmunoprecipitated with the Tig1-3xFLAG fusion. However, only a weak interaction was detectable between Tig1 and **Set3** in yeast two-hybrid assays. The Tig1-Set3 interaction was further confirmed by co-IP assays. We also confirmed the Tig1-Hos2 and Set3-Hos2 interactions by co-IP. **SNT1** was not included in the co-IP assays because of its size (~8 kb) and the difficulty of making fusion constructs. Overall, our results suggest that Tig1 functions in a protein complex that is similar to the yeast Set3 complex. The Tig1 complex of *M. oryzae* likely contains Tig1, Set3, Hos2, Snt1, Hst1, Hos4, and Cyp1.

Similar to the *tig1* mutant, the *set3*, *hos2*, and *snt1* mutants had altered conidium morphology and were defective in plant infection. By contrast, the *hst1* and *hos4* mutants had no obvious defects in conidio genesis and pathogenesis. These results indicate that **TIG1**, **SET3**, **HOS2**, and **SNT1**, but not **HST1** and **HOS4**, are involved in regulating similar biological processes in *M. oryzae*. In yeast, **SIF2**, **SET3**, **HOS3**, and **SNT1** are core components of the Set3 complex. Our results indicate that essential components of the *M. oryzae* Tig1 complex are Tig1, Set3, Hos2, and Snt1. Deletion of any one of these components may disrupt the integrity and function of this Tig1 complex. The putative Cpr1 ortholog, **CYP1**, is known to be involved in calcium signaling and to be required for virulence in *M. oryzae* (Viaud et al., 2002).

Based on the similarities in composition, the Tig1 complex appeared to function as a Set3-like HDAC complex in *M. oryzae*. The HDAC activities in the *tig1*, *set3*, *hos2*, and *snt1* mutants were significantly reduced in comparison with that of the wild type. HDACs catalyze the removal of acetyl groups from Lys residues in histones and nonhistone proteins (Ficner, 2009). The *tig1*, *set3*, *hos2*, and *snt1* mutants had increased levels of H3K18 acetylation. Further indirect evidence that these proteins are essential components of a Set3-like HDAC complex is that Tig1-, Set3-, and Hos2-GFP fusion proteins all localized to the nucleus. Tig1 has no predictable NLS sequence. Its association with other components of the complex may be responsible for nuclear localization of the Tig1-GFP fusion proteins. Unlike Tig1, the Set3 and Snt1 proteins have putative NLS sequences.

Recently, the core components of the Set3 complex, including Hos2, Set3, Sif2, and Snt1, have been shown to be important for efficient responses to secretory stress via the Slt2 MAP kinase pathway in yeast (Cohen et al., 2008). In *M. oryzae*, **MPS1** and **BCK1**, putative orthologs of yeast Slt2 and Bck1, are important for fungal cell wall integrity and pathogenesis (Xu et al., 1998; Jeon et al., 2008). In this study, we showed that phosphorylation of Mps1 was reduced in the *tig1*, *set3*, and *snt1* mutants (Figure 9). Similar to the *mps1* mutant (Xu et al., 1998), conidiation was significantly reduced in the *tig1* mutant. It is likely that the Tig1 HDAC complex plays a role in the activation of the Mps1 pathway in response to certain stresses or signals in *M. oryzae*.

As a key component of the Tig1 complex, **HOS2** had increased expression levels in conidia, appressorium, and infected rice leaves at 3 to 4 DAI (Figure 11), and expression was highest in conidia. This expression pattern is shared by all the genes encoding components of the Tig1 HDAC complex, except for **CYP1** (see Supplemental Table 5 online). **HOS2** is a class II HDAC gene. In yeast, **HDA1** and **HOS3** are two other class II HDAC genes (Trojer et al., 2003), but they are not part of the Set3 complex. The *M. oryzae* genome contains HDAC genes that are putatively orthologous to yeast **HDA1** and **HOS3**. While the expression of **HDA1** (MGG_01076.5) was higher in appressorium than in conidia, **HOS3** (MGG_06043.5) had an expression pattern similar to that of **HOS2** in vegetative hyphae, conidia, and appressorium (see Supplemental Figure 7 online). Deletion of **HDA1** and **HOS3** had no obvious effects on fungal growth,
conidiogenesis, and appressorium formation. Unlike the hos2 mutant, hda1 and hos3 null mutants were still pathogenic on rice and barley leaves (see Supplemental Figure 8 online). While the hda1 mutant was delayed in symptom development for about 1 d, the hos3 mutant had slightly reduced virulence. These results suggest that Hos2 is the main class II HDAC in M. oryzae. TIg1-interacting genes, including SET3, SNT1, and HO52, are also conserved in the genomes of other filamentous fungi that have been sequenced. However, none of the putative SET3 and SNT1 orthologs had previously been functionally characterized in plant pathogenic fungi. For HO52, its putative ortholog in Cochliobolus heterostrophus is an important pathogenicity factor, but its role in conidiogenesis is not clear (Baidyaroy et al., 2001). In F. graminearum, we also generated mutants that lack the putative orthologs of yeast SET3, HO52, and SNT1. Similar to the tfl1 mutant, the Fgset3, Fghos2, and Fgsnt1 mutants were defective in plant infection and conidiogenesis (J.-R. Xu, unpublished data), indicating that the TIg1-HDAC complex plays a similar role in M. oryzae and F. graminearum, and possibly also in other fungal pathogens, by regulating genes important for pathogenesis and conidiogenesis. To date, the molecular mechanisms that regulate the expression of genes that are specifically expressed or highly induced in invasive hyphae, such as BAS or other effector genes (Mosquera et al., 2009), are not clear in M. oryzae. Our results indicate that the TIg1 HDAC complex regulates the differentiation and growth of secondary invasive hyphae after penetration. The identification and characterization of downstream target genes of this well-conserved HDAC complex will enhance our understanding of the regulatory networks that regulate gene expression in invasive hyphae or during the early stages of plant colonization.

METHODS

Culture Conditions and Genetic Manipulations

All the wild-type and mutant strains used in this study (Table 1) were cultured on oatmeal agar or CM plates as described (Talbot et al., 1993; Park et al., 2006; Villalba et al., 2008). Mycelia harvested from 2-d-old 5×YEG (0.5% yeast extract and 1% glucose) cultures shaken at 150 rpm were used for isolation of genomic DNA and protoplasts (Sweigard et al., 1996; Zhao et al., 2009). Hygromycin- or zeocin-resistant transformants were selected on media supplemented with 250 μg/mL hygromycin B or 150 μg/mL zeocin (Invitrogen). Protoplast transformation and genetic crosses were performed as described previously (Talbot et al., 1993; Zhao and Xu, 2007).

Appressorium Formation and Plant Infection Assays

Conidia harvested from 10-d-old oatmeal agar cultures were resuspended to 1×10^4 spores/mL in water for appressorium formation and penetration assays and to 1×10^5 spores/mL in 0.25% gelatin for plant infection assays (Li et al., 2007; Mosquera et al., 2009). Two-week-old seedlings of rice (Oryza sativa) cultivar CO-39, Nipponbare, or Sariceltik and 8-d-old protoplasts of Guy11 or 70-15. Hygromycin-resistant transformants were selected via PCR products were digested with FseI and Ascl, respectively, and ligated with the hph gene from pCX63. After ligation, a 3.1-kb TIg1 replacement construct was amplified with primers F1/R1 and F2/R2 (see Supplemental Table 1 online). The resulting PCR products were digested with FseI and Ascl, respectively, and ligated with the hph gene from pCX63. After ligation, a 3.1-kb TIg1 replacement construct was amplified with primers F1/R2 and transformed into protoplasts of Guy11 or 70-15. Hygromycin-resistant transformants were screened by PCR and confirmed by DNA gel blot analysis using the TIg1 fragment, which was amplified with primers NF1/NR2, and the hph gene as probes (Figure 1A). For complementation, a 4.1-kb fragment containing the entire TIg1 gene (with a 1.5-kb promoter region) was cloned into pYK11 (Park et al., 2006) and transformed into the tig1 mutant KT-1.
The same approach was used to generate gene replacement constructs for other components of the TIG1 complex. Primers used to amplify the flanking sequences of HOS2, SNT1, SET3, HST1, and HOS4 are listed in Supplemental Table 1 online. The ligation-PCR products (Zhao et al., 2004) were transformed into protoplasts of Guy11, 70-15, or Ku80 (Villalba et al., 2008) to generate the hos2, snt1, set3, hst1, and hos4 deletion mutants (Table 1). Putative knockout mutants were identified by PCR screens and confirmed by DNA gel blot analysis.

**Construction of TIG1-GFP and TIG1-3xFLAG Fusion Constructs**

To construct the TIG1-GFP fusion, the TIG1 gene was amplified with primers TNG-F/TGFP-R (see Supplemental Table 1 online) and cotransformed with Xhol-digested pKD04 into Saccharomyces cerevisiae strain XK1-25 as described (Bourett et al., 2002; Bruno et al., 2004). Plasmid pSD11 was rescued from the resulting Trp+ yeast transformants, confirmed by sequence analysis to contain the TIG1-GFP fusion, and transformed into the tig1 mutant KT-1. The resulting zeocin-resistant transformants, which exhibited wild-type colony morphology and growth rate, were analyzed by DNA gel blot hybridization. The expression of the TIG1-GFP fusion construct was observed under a Nikon E-800 epifluorescence microscope.

To generate the TIG1-3xFLAG fusion construct, the TIG1 coding region was amplified with primers RPF and RPR (Supplemental Table 1). Primer RPR contains three copies of the FLAG epitope sequence followed by a termination codon. The resulting PCR products were co-transformed with Xhol-digested pDL2 (Bourett et al., 2002) into XK1-25 (Bruno et al., 2004). The TIG1-3xFLAG fusion vector was recovered from yeast transformants and transformed into 70-15.

**Affinity Purification and Mass Spectrometry Analysis**

About 150 to 200 mg of freshly harvested mycelia were resuspended in 2 mL of extraction buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 50 mM NaF, 2 mM PMSF, 5 mM EDTA, 1 mM EGTA, 1% Triton X-100, and 10% glycerol) and 10 μL of protease inhibitor cocktail (Sigma-Aldrich). After homogenization with a Biospec mini bead beater (Bruno et al., 2004), the lysate was centrifuged at full speed in a microcentrifuge for 20 min at 4°C. The supernatants were further centrifuged at 45,000 rpm at 4°C for 1 h to remove cell debris. About 50 μL of anti-FLAG M2 beads (Sigma-Aldrich) were added to capture Tig1-interacting proteins, following the instructions of the manufacturer. After incubation at 4°C for 2 h, the beads were washed three times each with 500 μL of lysis buffer, 50 mM TMAB (1 M trimethylammonium bicarbonate, Fluka), and sterile distilled water. Proteins binding to the beads were eluted with 150 μL of 50 mM TMAB containing 0.1% Rapigest and digested with trypsin as described (Tao et al., 2005; Zhou et al., 2007). Tryptic peptides were analyzed by nanoflow liquid chromatography–tandem mass spectrometry on a high-resolution hybrid linear ion trap orbitrap mass spectrometer (LTQ-Orbitrap XL; ThermoFisher) coupled to an Agilent Nanoflow LC system. The tandem mass spectrometry data were queried against the National Center for Biotechnology Information nonredundant system. The tandem mass spectrometry data were queried against the SwissProt database using the SEQUEST algorithm (Tabb et al., 2001) on the Sorcerer IDA server (SageN). Putative TIG1-interacting genes were identified by MS analysis, and four biological replicates were performed. Proteins that bound nonspecifically to the anti-FLAG antibody had been identified in our preliminary studies (Supplemental Dataset 1) and were removed from the list of proteins copurified with TIG1-3xFLAG for further analysis (see Supplemental Table 2 online).

**Co-IP Assays**

The yeast gap repair approach (Bourett et al., 2002; Bruno et al., 2004) was used to generate the SET3-3xFLAG, HOS2-GFP, and SET3-GFP fusion constructs. Primers used to amplify the SET3 and HOS2 genes are listed in Supplemental Table 1 online. The resulting fusion constructs were cotransformed into protoplasts of 70-15 in pairs. Transformants containing the TIG1-3xFLAG/SET3-GFP, TIG1-3xFLAG/HOS2-GFP, and SET3-3xFLAG/HOS2-GFP constructs were identified by PCR and confirmed by DNA gel blot hybridization. The expression of the GFP and 3xFLAG fusion proteins was further confirmed by immunoblot analyses. For co-IP assays, total proteins were isolated and incubated with the anti-FLAG M2 beads as described above. Proteins eluted from M2 beads were analyzed by immunoblot detection with the anti-FLAG (Sigma-Aldrich) and anti-GFP (Roche) antibodies.

**Yeast Two-Hybrid Assays**

The HybridZap2.1 yeast two-hybrid system (Stratagene) was used to assay protein–protein interactions. The TIG1 ORF was cloned into pBD-GAL4 as the bait vector pSD17. The prey construct of SET3 was generated with pAD-GAL4-2.1. The resulting bait and prey vectors were transformed into yeast strain YRG-2 (Stratagene) with the Alkalication yeast transformation kit (MP Biomedical). The Lex+ and Trp+ transformants were isolated and assayed for growth on SD-Trp-Leu-His medium and the expression of the LacZ reporter gene as described (Zhao et al., 2005). The positive and negative controls were provided in the HybridZap2.1 XR library construction kit (Stratagene).

**HDAC Activity Assays**

Protoplasts of Guy11, 70-15, Ku80, and the tig1, hos2, set3, and snt1 mutants were resuspended to 4 × 10^9 protoplasts/mL in lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 15 mM MgCl₂, 250 mM sucrose, 0.5% Nonidet P-40, 0.1 mM EGTA, and 200 μM PMSF). Nuclei and crude nuclear extracts were prepared from disrupted protoplasts as described (Ding et al., 2009) and assayed for HDAC activities with the HDAC activity assay kit (Cayman Chemical Company) following the instructions provided by the manufacturer. Fluorescent signals in samples of different mutants were detected with a plate reader (Synergy HT; Bio-TEK). The concentration of deacetylated compounds was calculated with the deacetylation standard curve and used to estimate HDAC activity (nmol/min/mL) using the formula provided in the HDAC activity assay kit.

**Histone Acetylation Assays**

Vegetative hyphae harvested from 100 mL of 2-d-old 5×YEG cultures were resuspended in 400 μL lysis buffer (10 mM Tris-HCl, pH 7.4, 300 mM sorbitol, 600 mM NaCl, 5 mM MgCl₂, and 5 mM EDTA) with a mixture of protease and phosphatase inhibitors (1 μg/mL of aprotinin, leupeptin, and pepstatin A, 1 mM PMSF, 1 μM microcystin-LR, and 2 mM P-hloromercuriphenylsulfonic acid). Mycelia were homogenized with acid-washed glass beads in a Biospec minibead beater using three 40-s pulses applied at 1-min intervals on ice. The lysate was separated from the glass beads and centrifuged at 16,000g for 15 min at 4°C. The resulting supernatants containing whole-cell extracts were separated on 15% SDS-PAGE gels, transferred to a nitrocellulose membrane, and assayed for histone acetylation as described (Briggs et al., 2001). Monoclonal anti-H3 (Abcam) and anti-H3K18Ac (Abcam) antibodies were used for detection.

**Accession Numbers**

Sequence data for the M. oryzae genes from this article can be found in the GenBank/EMBL data libraries under the following accession numbers: TIG1, EDK00306; SET3, EDK04728; SNT1, EDJ98032; HOS2, EDK04649; HST1, EDJ98541; HOS4, EDK06449; CYP1, BAB59119; HD41, EDK02244; HOS3, EDJ96334; ACT1, EDJ99268; TUB1, EDK0278; and ILV5, EDK04462. Others are as follows: the putative
ortholog of TIG1 in Neurospora crassa, XP_963679; FT1 of Fusarium graminearum, XP_380508.

Supplemental Data
The following materials are available in the online version of this article.

Supplemental Figure 1. Alignment of Amino Acid Sequences of Tig1 and Its Putative Orthologs from Neurospora crassa, Fusarium graminearum, and Saccharomyces cerevisiae (Shif).

Supplemental Figure 2. KT-2 and KT-4 Failed to Cause Typical Blast Lesions on Rice Leaves.

Supplemental Figure 3. The Tig1 Mutant Triggered Enhanced Callose Deposition in Plant Cells.

Supplemental Figure 4. Immunoblot Analysis of the Expression and Immunoprecipitation of Tig1-3xFLAG Fusion Proteins with an anti-FLAG Antibody.

Supplemental Figure 5. Targeted Deletion of the SNT1, SET3, HOS4, HOS2, and HST1 Gene.

Supplemental Figure 6. GFP Signals in the pphp7-TIG1-GFP Transformant.

Supplemental Figure 7. Expression Patterns of HOS3, HDA1, and CYP1.

Supplemental Figure 8. Infection Assays with hda1 and hos3 Mutants.

Supplemental Table 1. PCR Primers Used in This Study.

Supplemental Data Set 1. Magnaporthe oryzae Proteins Nonspecifically Copurified with 3xFLAG.

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Supplemental Fig. 1. Alignment of amino acid sequences of Tig1 and its putative orthologs from *Neurospora crassa* and *Fusarium graminearum*. Identical and similar residues are shaded in black and gray, respectively.

|    | Mo Tig1 | Fg Ftl1 | Nc Tig1 |
|----|---------|---------|---------|
| 1  | MEKFLSDRLNFIVLWALKLEGVRETAQKOKREKVGKRPQGSPNFQPSKRRQKQ-RS | MEKFLSDRLNFIVLWALKLEGVRETAQKOKREKVGKRPQGSPNFQPSKRRQKQ-RS | MEKFLSDRLNFIVLWALKLEGVRETAQKOKREKVGKRPQGSPNFQPSKRRQKQ-RS |
| 117| TEAGGSAPTNNANVADTVTLALADADALGADDLADGDDPAALSGEEED | TEAGGSAPTNNANVADTVTLALADADALGADDLADGDDPAALSGEEED | TEAGGSAPTNNANVADTVTLALADADALGADDLADGDDPAALSGEEED |
| 177| PTCEGNNRKRQGAQVGSFRMARLARGYINAEGTVIIDDEAASNINNNHAYPSL | PTCEGNNRKRQGAQVGSFRMARLARGYINAEGTVIIDDEAASNINNNHAYPSL | PTCEGNNRKRQGAQVGSFRMARLARGYINAEGTVIIDDEAASNINNNHAYPSL |
| 237| EYQEAESEPPRTSSSEPQGTQVKTDLTPETLSATSQASTNACGAVPPAPASPTENT | EYQEAESEPPRTSSSEPQGTQVKTDLTPETLSATSQASTNACGAVPPAPASPTENT | EYQEAESEPPRTSSSEPQGTQVKTDLTPETLSATSQASTNACGAVPPAPASPTENT |
| 297| FEQAFEDPLVAYVTVTIAVGQETGTVTAVDADARLVRWVRVPVEQ-SQ---RATFPP | FEQAFEDPLVAYVTVTIAVGQETGTVTAVDADARLVRWVRVPVEQ-SQ---RATFPP | FEQAFEDPLVAYVTVTIAVGQETGTVTAVDADARLVRWVRVPVEQ-SQ---RATFPP |
| 357| HVSVDGNGLTEDDAAPDAVITAMAWNWDGSALALATDSGRNARISIWAADGTHLQRFDVA | HVSVDGNGLTEDDAAPDAVITAMAWNWDGSALALATDSGRNARISIWAADGTHLQRFDVA | HVSVDGNGLTEDDAAPDAVITAMAWNWDGSALALATDSGRNARISIWAADGTHLQRFDVA |
| 409| EFFINKAYPAVNTILALVAVWAKNLADTNSNARISIWRACGLHLQRFDVA | EFFINKAYPAVNTILALVAVWAKNLADTNSNARISIWRACGLHLQRFDVA | EFFINKAYPAVNTILALVAVWAKNLADTNSNARISIWRACGLHLQRFDVA |
| 469| TEFILCGGDLRESSTPMTYVYKPREK1R-CTACFTODSIAHSTAGSGVLDND | TEFILCGGDLRESSTPMTYVYKPREK1R-CTACFTODSIAHSTAGSGVLDND | TEFILCGGDLRESSTPMTYVYKPREK1R-CTACFTODSIAHSTAGSGVLDND |
| 529| ESQQRSIHASSHITSHSNQILOGNFP-DEERILASGEGCEDSWNARNEPNKPS | ESQQRSIHASSHITSHSNQILOGNFP-DEERILASGEGCEDSWNARNEPNKPS | ESQQRSIHASSHITSHSNQILOGNFP-DEERILASGEGCEDSWNARNEPNKPS |
| 587| WMNPKSAKIPDTPGAFIAGATIDRLINLKVQYSPFRESHBKANPNK | WMNPKSAKIPDTPGAFIAGATIDRLINLKVQYSPFRESHBKANPNK | WMNPKSAKIPDTPGAFIAGATIDRLINLKVQYSPFRESHBKANPNK |
| 647| ASDEEHLCLWDAGOKLAKAYCGNLSAVINFR | ASDEEHLCLWDAGOKLAKAYCGNLSAVINFR | ASDEEHLCLWDAGOKLAKAYCGNLSAVINFR |
| 611| Nc Tig1 | Fg Ftl1 | Mo Tig1 |
| 611| MEKFLSDRLNFIVLWALKLEGVRETAQKOKREKVGKRPQGSPNFQPSKRRQKQ-RS | MEKFLSDRLNFIVLWALKLEGVRETAQKOKREKVGKRPQGSPNFQPSKRRQKQ-RS | MEKFLSDRLNFIVLWALKLEGVRETAQKOKREKVGKRPQGSPNFQPSKRRQKQ-RS |
| 647| ASDEEHLCLWDAGOKLAKAYCGNLSAVINFR | ASDEEHLCLWDAGOKLAKAYCGNLSAVINFR | ASDEEHLCLWDAGOKLAKAYCGNLSAVINFR |
| 682| Nc Tig1 | Fg Ftl1 | Mo Tig1 |
| 682| MEKFLSDRLNFIVLWALKLEGVRETAQKOKREKVGKRPQGSPNFQPSKRRQKQ-RS | MEKFLSDRLNFIVLWALKLEGVRETAQKOKREKVGKRPQGSPNFQPSKRRQKQ-RS | MEKFLSDRLNFIVLWALKLEGVRETAQKOKREKVGKRPQGSPNFQPSKRRQKQ-RS |
Supplemental Fig. 2. KT-2 and KT-4 failed to cause typical blast lesions on rice leaves. Rice leaves were inoculated with conidia from the wild-type strain 70-15, ngl mutants (KT-2 and KT-4) and 0.25% gelatin solution (negative control). Typical blast lesions were observed only on leaves sprayed with the wild type.
**Supplemental Fig. 3.** The *Tig1* mutant triggered enhanced callose deposition in plant cells. Barley leaves inoculated with conidia from the wild type (Guy11) and the *tig1* mutant (KT-1) were sampled 72 hpi and stained with aniline blue. The same field was examined by epifluorescence (left) and DIC (right) microscopy. A, appressorium; IH, infectious hyphae. Bar = 10 μm.
**Supplemental Fig. 4.** Immunoblot analysis of the expression and immunoprecipitation of Tig1-3xFLAG fusion proteins with the anti-FLAG antibody. A 72-kD band was detected in total proteins and proteins eluted from anti-FLAG beads in transformant TFg1 expressing the TIG1-3xFLAG fusion. A non-specific band was detected in total proteins from TFg1 and wild-type strain 70-15.
**Supplemental Fig. 5.** Targeted deletion of the *SNT1*, *SET3*, *HOS4*, *HOS2*, and *HST1* genes. The upper panels are schematic diagrams of the genomic regions of these genes and corresponding gene replacement constructs. The lower panels are Southern blots hybridized with deleted gene fragments as the probes (marked in the schematic diagrams). B, *Bam*HI; H, *Hind*III; P, *Pst*I; Sm, *Sma*I; Sp, *Sph*I.

A. **SNT1.** The wild-type 4.7 kb *Hind*III band was detected in the ectopic transformant (ECT7) but not in *snt1* deletion mutants (KS1-1, KS1-3).

B. **SET3.** The wild-type 4.8 kb *Pst*I band was detected in the ectopic transformant ECT8 but not in the *set3* deletion mutant KS3-1.

C. **HOS4.** A 9.1 kb *Sph*I band was detected in the wild type (70-15) and ectopic transformants (ECT1, ECT2, ECT3) but not in the *hos4* deletion mutant (KH4-81).

D. **HOS2.** A 4.7 kb *Sma*I band was detected in the wild type (70-15) and ectopic transformant (ECT4) but not in *hos2* deletion mutants (KH2-13, KH2-22, KH2-45).

E. **HST1.** A 1.5 kb *Bam*HI band was detected in the wild type (70-15) and ectopic transformant (ECT5, ECT6) but not in the *hst1* deletion mutant (KH1-23).
Supplemental Fig. 6. Expression and localization of GFP fusion proteins in vegetative hyphae of transformants expressing the P_{TIG1}-TIG1-GFP (KT1NG1) and P_{RP27}-TIG1-GFP (KT1RG1) constructs. Stronger GFP signals were observed in the nucleus in transformant KT1RG1 than in transformant KT1NG1. Bar = 10 μm.
Supplemental Fig. 7. Expression patterns of HOS3, HDA1, and CYP1. A. The relative expression levels of HOS3, HOS2, and HDA1 in vegetative hyphae, conidia, and 24-h-old appressoria (24 h). B. Expression of CYP1 in vegetative hyphae, conidia, 24-h-old appressoria (24 h), and infected rice leaves harvested at 1, 2, 3 or 4 dpi. qRT-PCR was used to quantify transcripts of these genes relative to that of the constitutive reference gene ILV5 using the $2^{-\Delta \Delta C_T}$ method.
Supplemental Fig. 8. Infection assays with *hda1* and *hos3* mutants. **A.** Barley leaves were inoculated with drops of conidium suspensions (1x10^4 spores/ml) from the Ku80 (Villalba et al., 2008) and *hda1* null mutant strains. Representative leaves were photographed 4 dpi. At 4 dpi, the mutant induced lesions typical of younger wild-type lesions (2-3 dpi). Overall, this mutant was as virulent as the wild type but had a delay in symptom development. **B.** Barley leaves spray inoculated with conidia (3x10^5 spores/ml) from Ku80 and the *hos3* null mutant. Typical leaves were examined at 7 dpi. Leaves sprayed with the *hos3* mutant had fewer lesions than those sprayed with Ku80.
## Supplemental Table 1. PCR primers used in this study

| Primer | Sequence (5’→3’) | Application |
|--------|------------------|-------------|
| F1     | ccatcgatgccccattgtcgtgctggcagc | TIG1 deletion |
| R1     | atagccggccgtgcaagataaggaatt | TIG1 deletion |
| F2     | attggcgcggccgctgatggaggcctg | TIG1 deletion |
| R2     | ggcagtcttgcactactgttgacagacaa | TIG1 deletion |
| NF1    | gtcacagctggccctg | TIG1, negative screen |
| NR2    | cggcggtccagtctcattag | TIG1, negative screen |
| CTH    | taagaagctcctgatgcgtgctgagct | TIG1 complementation |
| CTX    | tatactcgagcgcagggagaaagataatgg | TIG1 complementation |
| TGFP-R | gaacagctcctcgcccttgctcaccctaaagttgatcacagcg | TIG1-GFP fusion |
| TNG-F  | gcggtaagttggactcactaattggctgtgctgatgcgtgctgagct | TIG1-GFP fusion (native) |
| TBLRG-F| cgtgaagaacccatctcactgtaatcttctgacagcttgcctgttgacagct | TIG1-GFP fusion (RP27) |
| RPF    | ttgcgtagagaacccatcactaattggctggtactcattgcctgttgacagct | TIG1-3XFlag (RP27) |
| RPR    | gaacagctcctcgcccttgctcacttacttgtcatcgtcatccttgtaatcctttatataatcaccgtcatggtctttgtagtccctaaagttgatcacagcg | TIG1-3XFlag (RP27) |
| C-TBLNF1 | gtcacaccaacccctac | TIG1 cDNA |
| C-TBLNR1 | cttgctctagcaacacct | TIG1 cDNA |
| TBLXb  | gcgcggatcagcaagggactagaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | TIG1 bait construct |
| TBLXo  | actgctctagcaacacctgtagaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | TIG1 bait construct |
| SET3BF | gatagacagacacagctgtagaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSET3 prey construct |
| SET3BR | taatctgcagctgtagaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSET3 prey construct |
| H2NGF  | cgcacaccaacccctactggaaggggtactcaaattggaacgccttgaaacgatg | MgHOS2-GFP (native promoter) |
| H2RGF  | cgcacaccaacccctactggaaggggtactcaaattggaacgccttgaaacgatg | MgHOS2-GFP (RP27) |
| H2NGR  | cgcacaccaacccctactggaaggggtactcaaattggaacgccttgaaacgatg | MgHOS2-GFP (reverse) |
| H2UF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS2 deletion |
| H2UR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS2 deletion |
| H2DF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS2 deletion |
| H2DR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS2 deletion |
| H4UF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS4 deletion |
| H4UR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS4 deletion |
| H4DF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS4 deletion |
| H4DR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS4 deletion |
| H4NF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS4 deletion |
| H4NR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHOS4 deletion |
| H1UF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHST1 deletion |
| H1UR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHST1 deletion |
| H1DF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHST1 deletion |
| H1DR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHST1 deletion |
| H1NF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHST1 deletion |
| H1NR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgHST1 deletion, negative screen |
| S3UF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSET3 deletion |
| S3UR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSET3 deletion |
| S3DF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSET3 deletion |
| S3DR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSET3 deletion |
| S3NF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSET3, negative screen |
| S3NR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSET3, negative screen |
| S1UF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSNT1 deletion |
| S1UR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSNT1 deletion |
| S1DF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSNT1 deletion |
| S1DR   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSNT1 deletion |
| S1NF   | ctcgctgactcgagcatcgtgactctgtgactgagtaccgatgatgaagtgagaatttgatgacagctgggcttctattgcacttgacagctttgacctttca | MgSNT1, negative screen |
| Genbank ID | Sequence                        | Description                      |
|------------|---------------------------------|----------------------------------|
| S1NR       | ctgctcgtagctgaaga               | MgSNT1, negative screen         |
| S3RGF      | cagatctcgttctcagaggaaccgtttcttataatgagactaagcttgccgc | MgSET3-GFP (RP27)               |
| S3RGR      | caccacccccgtgaacagctcactegcctctgtgcactaactctgctgcataactg | MgSET3-GFP (reverse)            |
| S3NGF      | gactctactataggggcaatggtctactcataatggtgtcctgctgctgactgag | MgSET3-GFP (native)             |
| S3FLF      | ttctgtaggaaaccctttttcaccattagataagcttgccgc | MgSET3-3XFlag (RP27)            |
| S3FLR      | gaacagctcactegcctctgtgcactaactctgctgcataatcctgtgctggtctgctgactgagtctcttataatcaccgtctcatgtctttctgctgcataactgatctgctgtgctgctgactg | MgSET3-3XFlag (RP27)            |
| MgHOS2F    | caacaacgcccccaggtga              | MgHOS2 cDNA                      |
| MgHOS2R    | tccgaagttccctgacagactc          | MgHOS2 cDNA                      |
| MgHOS4F    | tgaaggccccagagggagaa             | MgHOS4 cDNA                      |
| MgHOS4R    | tttgagccagatctgctggtac           | MgHOS4 cDNA                      |
| MgHST1F    | gagggggactgcaacacttgc           | MgHST1 cDNA                      |
| MgHST1R    | gcacccatcagcaacatgta             | MgHST1 cDNA                      |
| MgSNT1F    | tccggagcctaataatagttg            | MgSNT1 cDNA                      |
| MgSNT1R    | gaatggagatcatctaccggtagga        | MgSNT1 cDNA                      |
| MgCPR1F    | gtcatggccgaggtcgg             | MgCPR1 cDNA                      |
| MgCPR1R    | aagagcctcataatgggctgag         | MgCPR1 cDNA                      |

Supplemental Data. Ding et al. (2010). Plant Cell 10.1105/tpc.110.074302
**Supplemental Table 2.** *Magnaporthe oryzae* proteins nonspecifically co-purified with 3xFLAG by the anti-FLAG antibody

| Proteins     | Putative functions                                      |
|--------------|---------------------------------------------------------|
| MGG_00153    | Sphingolipid long chain base-responsive protein LSP1   |
| MGG_00167    | Succinate dehydrogenase iron-sulfur subunit            |
| MGG_00168    | Succinate dehydrogenase flavoprotein subunit           |
| MGG_00175    | 6-phosphogluconate dehydrogenase                       |
| MGG_00195    | Clock-controlled gene-9 protein                        |
| MGG_00206    | DNA-dependent ATPase MGS1                              |
| MGG_00221    | Ribosomal protein S7                                   |
| MGG_00330    | Proteasome-activating nucleotidase                      |
| MGG_00338    | Helicase conserved C-terminal domain                    |
| MGG_00341    | Eukaryotic translation initiation factor 3 subunit D    |
| MGG_00349    | Ribosomal protein S13                                  |
| MGG_00383    | S-adenosylmethionine synthetase                        |
| MGG_00604    | Beta-tubulin                                            |
| MGG_00657    | Adenine phosphoribosyltransferase                      |
| MGG_00689    | Acetyl-coenzyme A synthetase                           |
| MGG_00748    | Myosin-5                                                |
| MGG_00865    | 1,3-beta-glucan synthase component GLS1                |
| MGG_00878    | Hypothetical protein                                   |
| MGG_00884    | 26S proteasome non-ATPase regulatory subunit 3          |
| MGG_00901    | 2,3-bisphosphoglycerate-independent phosphoglycerate mutase |
| MGG_00905    | Sec14                                                   |
| MGG_00908    | 26S protease regulatory subunit 6B                      |
| MGG_00923    | Serine hydroxymethyltransferase                        |
| MGG_00949    | Endoplasmic reticulum-Golgi intermediate compartment protein 2 |
| MGG_01014    | C-1-tetrahydrofolate synthase                          |
| MGG_01046    | Methionyl-tRNA synthetase                              |
| MGG_01049    | 3,4-dihydroxy-2-butanone 4-phosphate synthase          |
| MGG_01061    | Coproporphyrinogen III oxidase                        |
| MGG_01084    | Glyceraldehyde-3-phosphate dehydrogenase               |
| MGG_01092    | Homocitrate synthase                                   |
| MGG_01110    | Hypothetical protein                                   |
| MGG_01113    | 40S ribosomal protein S10-A                            |
| MGG_01135    | Cytochrome C oxidase polypeptide IV                    |
| MGG_01140    | Alpha-1,2 mannosyltransferase                          |
| MGG_01160    | Histone H4.1                                           |
| MGG_01165    | Isoleucyl-tRNA synthetase                              |
| MGG_01202    | D-lactate dehydrogenase                                |
| MGG_01268    | 28 kDa ribonucleoprotein                               |
| MGG_01282    | Ubiquitin                                               |
| MGG_01333    | NADH dehydrogenase iron-sulfur protein 3               |
| MGG_01380    | 26S protease regulatory subunit 8                       |
| MGG_01535    | Pyridoxamine 5'-phosphate oxidase                      |
| MGG_01566    | Homoisocitrate dehydrogenase                           |
| MGG_01581    | 26S protease regulatory subunit 7                       |
| MGG_01582    | T-complex protein 1 subunit delta                       |
| MGG_01587    | Diphthamide biosynthesis protein 4                     |
| MGG_01592    | Eukaryotic translation initiation factor 2 subunit gamma|
| MGG_01631    | UTP-glucose-1-phosphate uridylyltransferase            |
| MGG_01712    | Isocitrate dehydrogenase subunit 2                     |
| MGG_01728    | Methylene tetrahydrofolate reductase 1                 |
| MGG_01742    | Elongation factor 2                                     |
| MGG_01793    | Short-chain dehydrogenaseductase                       |
| MGG_01808    | Ketol-acid reductoisomerase                            |
| Protein ID     | Description                                      |
|---------------|--------------------------------------------------|
| MGG_01819     | Glycogen phosphorylase                           |
| MGG_01906     | Nicotianamine synthase 3                        |
| MGG_01925     | Bifunctional P-450:NADPH-P450 reductase          |
| MGG_01995     | Isocitrate dehydrogenase subunit 1              |
| MGG_02072     | Amino-acid permease                              |
| MGG_02311     | Predicted protein                                |
| MGG_02370     | Mitochondrial phosphate carrier protein         |
| MGG_02378     | Glutamate decarboxylase 1                       |
| MGG_02432     | Leucyl-tRNA synthetase                           |
| MGG_02439     | 2-nitropropane dioxygenase                      |
| MGG_02449     | Tyrosyl-tRNA synthetase                         |
| MGG_02511     | Ribosomal protein L3                             |
| MGG_02521     | Carboxylesterase 3                              |
| MGG_02526     | Exportin-1                                       |
| MGG_02564     | Predicted protein                                |
| MGG_02608     | 26S proteasome regulatory subunit RPN5           |
| MGG_02612     | 3-oxoacyl-reductase                              |
| MGG_02634     | Coatamer subunit gamma                           |
| MGG_02648     | Interferon-induced GTP-binding protein           |
| MGG_02653     | 6-phosphofructokinase                           |
| MGG_02659     | Ribosomal protein L14                           |
| MGG_02661     | ATP synthase D chain, mitochondrial              |
| MGG_02744     | Ribosomal protein S4-A                          |
| MGG_02784     | DNA-binding protein SMUBP-2                     |
| MGG_02806     | ATP-dependent RNA helicase SUB2                 |
| MGG_02903     | Carboxylesterase                                |
| MGG_02914     | 2-deoxy-D-gluconate 3-dehydrogenase             |
| MGG_02952     | Ribosomal protein S9                            |
| MGG_02953     | Ribosomal protein L21-B                         |
| MGG_02983     | Cytochrome C oxidase subunit via                |
| MGG_03039     | Hsp70-like protein                              |
| MGG_03086     | U2 snRNP component IST3                         |
| MGG_03135     | Ribosomal protein L30                           |
| MGG_03136     | Ribosomal protein L10-B                         |
| MGG_03147     | Glycerol-3-phosphate dehydrogenase              |
| MGG_03152     | ATP synthase subunit 5                          |
| MGG_03185     | ATP synthase subunit beta                       |
| MGG_03192     | RNA polymerase II transcription factor B subunit 1 |
| MGG_03193     | Ribosomal protein L3                            |
| MGG_03211     | 26S proteasome non-Atpase regulatory subunit 11 |
| MGG_03217     | Phenylalanyl-tRNA synthetase                    |
| MGG_03220     | Isoleucyl-tRNA synthetase                       |
| MGG_03226     | Cytochrome b-c1 complex subunit 2               |
| MGG_03236     | Ribosomal protein S6-B                         |
| MGG_03244     | Vacuolar ATP synthase subunit B                 |
| MGG_03251     | Ribosomal protein S8-B                         |
| MGG_03310     | T-complex protein 1 subunit eta                 |
| MGG_03372     | Ribosomal protein S20                          |
| MGG_03388     | ATP-dependent RNA helicase                      |
| MGG_03414     | Short chain dehydrogenasereductase              |
| MGG_03441     | Trehalose-phosphatase                           |
| MGG_03494     | Aminotransferase                                |
| MGG_03511     | Coatamer subunit alpha                          |
| MGG_03517     | Translation initiation factor eif-2B subunit epsilon |
| MGG_03537     | Importin subunit beta-3                        |
| MGG_03549     | DNA repair and recombination protein RAD54      |
| MGG_03554     | Ribosomal protein L36                          |
| Acronym      | Description                                               |
|-------------|-----------------------------------------------------------|
| MGG_03556   | HACA ribonucleoprotein complex subunit 1                  |
| MGG_03577   | Histone H2A                                               |
| MGG_03600   | Mitochondrial-processing peptidase subunit beta           |
| MGG_03641   | Elongation factor 1-alpha                                 |
| MGG_03653   | Ribosomal protein L20                                     |
| MGG_03663   | Hypothetical protein                                      |
| MGG_03668   | Importin subunit beta-1                                   |
| MGG_03680   | Ribosomal protein S11                                     |
| MGG_03684   | Mitochondrial distribution and morphology protein 38      |
| MGG_03708   | NADH-cytochrome b5 reductase                             |
| MGG_03727   | Ribosomal protein L31                                     |
| MGG_03736   | Multidrug resistance-associated protein 1                 |
| MGG_03823   | NADH-dependent flavin oxidoreductase                      |
| MGG_03857   | T-complex protein 1 subunit zeta                          |
| MGG_03880   | Alcohol dehydrogenase                                     |
| MGG_03895   | Small nuclear ribonucleoprotein F                         |
| MGG_03897   | Lariat debranching enzyme                                 |
| MGG_03900   | Aldehyde dehydrogenase                                   |
| MGG_03915   | Pyridoxamine phosphate oxidase family protein             |
| MGG_03982   | Actin                                                     |
| MGG_04013   | Small nuclear ribonucleoprotein Sm D2                     |
| MGG_04042   | Leucyl-tRNA synthetase, mitochondrial precursor            |
| MGG_04083   | Small nuclear ribonucleoprotein Sm D3                     |
| MGG_04104   | Ribosomal protein L22                                     |
| MGG_04114   | Ribosomal protein S17                                     |
| MGG_04400   | ATP-dependent RNA helicase                                |
| MGG_04425   | Phenylalanyl-tRNA synthetase alpha chain                  |
| MGG_04432   | Cytochrome P450                                           |
| MGG_04438   | ADP-ribosylation factor 1                                 |
| MGG_04444   | Ribosomal protein L6-B                                    |
| MGG_04462   | Chaperone protein Dnaj 2                                  |
| MGG_04467   | 60S acidic ribosomal protein P0                            |
| MGG_04476   | FK506-binding protein 4                                   |
| MGG_04484   | Ribosomal protein L18                                     |
| MGG_04503   | Carbamoyl-phosphate synthase arginine-specific large chain|
| MGG_04545   | Cytochrome c peroxidase                                   |
| MGG_04550   | Calcium-transporting ATPase                               |
| MGG_04556   | Alcohol dehydrogenase                                     |
| MGG_04602   | Pre-mRNA-processing protein PRP40                         |
| MGG_04612   | Ribosomal protein L8-B                                    |
| MGG_04652   | DNA-directed RNA polymerase II subunit RPB1               |
| MGG_04696   | Ribosomal protein ll0a                                    |
| MGG_04738   | Bacilysin biosynthesis oxidoreductase                     |
| MGG_04741   | Small nuclear ribonucleoprotein Sm D1                     |
| MGG_04752   | ATP synthase subunit 4                                    |
| MGG_04775   | Fatty acid synthase S-acetyltransferase                   |
| MGG_04829   | Ribosomal protein L9-A                                    |
| MGG_04901   | Methyltransferase                                          |
| MGG_04921   | Ribosomal protein L23                                     |
| MGG_04988   | Chromosome segregation protein suda                        |
| MGG_04994   | Plasma membrane ATPase 1                                  |
| MGG_05025   | Fatty acid transporter protein                             |
| MGG_05063   | Phosphoglycerate kinase                                   |
| MGG_05140   | 2OG-Fe(II) oxygenase superfamily                          |
| MGG_05159   | Dihydrolipicolinate synthase                              |
| MGG_05170   | Ribosomal protein L17                                     |
| MGG_05201   | Guanine nucleotide-binding protein subunit beta            |
| Gene ID     | Description                              |
|------------|------------------------------------------|
| MGG_05223  | 3-isopropylmalate dehydrogenase          |
| MGG_05237  | Ribosomal protein L7                     |
| MGG_05238  | Ribosomal protein S14                    |
| MGG_05296  | Ribosomal protein L34-B                  |
| MGG_05307  | Hyaluronan mRNA binding family           |
| MGG_05438  | Small glutamine-rich tetratricopeptide repeat-containing protein 2 |
| MGG_05449  | Ribosomal protein L16                    |
| MGG_05480  | Succinyl-CoA ligase subunit alpha-2      |
| MGG_05565  | Glutathione S-transferase                |
| MGG_05602  | Glucosidase 2 subunit beta               |
| MGG_05630  | AMP deaminase 2                          |
| MGG_05661  | Ribosomal protein S18                    |
| MGG_05663  | Carboxypeptidase Y                       |
| MGG_05671  | Protein phosphatase PP2A regulatory subunit A |
| MGG_05673  | Ribosomal protein S3                     |
| MGG_05734  | Phospho-2-dehydro-3-deoxyheptonate aldolase |
| MGG_05858  | 26S proteasome regulatory subunit RPN11  |
| MGG_05936  | Mannose-1-phosphate guanylyltransferase  |
| MGG_05940  | Short chain dehydrogenase                |
| MGG_05949  | Acyl-coa dehydrogenase                   |
| MGG_05999  | HACA ribonucleoprotein complex subunit 2 |
| MGG_06003  | Ubiquinone-binding protein               |
| MGG_06005  | RNA exonuclease 3                        |
| MGG_06011  | S-(hydroxymethyl)glutathione dehydrogenase |
| MGG_06030  | NADH-ubiquinone oxidoreductase 29.9 kda subunit |
| MGG_06047  | Cytochrome P450                           |
| MGG_06192  | Cytochrome b-c1 complex subunit 7        |
| MGG_06241  | Vacuolar protein sorting-associated protein 21 |
| MGG_06269  | Ribosomal protein L2                     |
| MGG_06276  | External NADH-ubiquinone oxidoreductase 1 |
| MGG_06289  | NADH-cytochrome b5 reductase 1           |
| MGG_06321  | Glycyl-tRNA synthetase                   |
| MGG_06362  | Small COPII coat GTPase SAR1              |
| MGG_06392  | Ornithine aminotransferase               |
| MGG_06445  | Histone H3                               |
| MGG_06453  | Single-strand binding protein            |
| MGG_06459  | Heat shock protein HSP98                 |
| MGG_06479  | Ribosomal protein S22                    |
| MGG_06480  | Ribosomal protein S12                    |
| MGG_06505  | mRNA cap methyltransferase               |
| MGG_06512  | Glucan 1,3-beta-glucosidase 2            |
| MGG_06524  | Fructose-2,6-bisphosphatase              |
| MGG_06561  | Glutaryl-CoA dehydrogenase               |
| MGG_06571  | Ribosomal protein L37a-2                 |
| MGG_06587  | Leucyl aminopeptidase                    |
| MGG_06609  | Acetyl-CoA hydrolase                     |
| MGG_06629  | Ribosomal protein L10                    |
| MGG_06639  | T-complex protein 1 subunit gamma        |
| MGG_06648  | Hypoxia up-regulated protein 1 precursor |
| MGG_06650  | Alpha-tubulin                            |
| MGG_06656  | ADP,ATP carrier protein                  |
| MGG_06657  | Ribosomal protein S25                    |
| MGG_06658  | Ribosomal protein S5                     |
| MGG_06693  | Ribosomal protein L27-B                  |
| MGG_06712  | Cobalamin-independent synthase           |
| MGG_06716  | Histone acetylase complex subunit        |
| MGG_06719  | ATP-citrate synthase subunit 1           |
| Gene ID     | Protein Name                                      |
|------------|--------------------------------------------------|
| MGG_06720  | ATP citrate lyase                                |
| MGG_06759  | Heat shock protein 80                            |
| MGG_06860  | Coatamer subunit beta                            |
| MGG_06910  | Transport protein SEC23                          |
| MGG_06913  | Seryl-t-RNA synthetase                           |
| MGG_06915  | Uricil phosphoribosyltransferase                 |
| MGG_06919  | Ribosomal protein S3                             |
| MGG_06923  | Myosin-2                                         |
| MGG_06924  | Nucleosome assembly protein                       |
| MGG_06936  | Elongation factor 1-gamma                        |
| MGG_06950  | L-serine dehydratase                             |
| MGG_06958  | Hsp70-like protein                               |
| MGG_06759  | Succinyl-CoA ligase beta-chain                   |
| MGG_07033  | ATP-dependent RNA helicase ded-1                 |
| MGG_07037  | Phosphatidylserine decarboxylase                 |
| MGG_07048  | Ribosomal protein L5                             |
| MGG_07087  | Pre-mRNA-splicing factor SYF-1                   |
| MGG_07099  | ATP-dependent RNA helicase                       |
| MGG_07110  | Phosphatidylethanolamine N-methyltransferase     |
| MGG_07120  | 26S proteasome regulatory subunit Rpn-1           |
| MGG_07195  | O-acetylhomoserine (thiol)-lyase                 |
| MGG_07197  | Long-chain-fatty-acid-CoA ligase 1               |
| MGG_07200  | Plasma membrane ATPase                           |
| MGG_07252  | Polyadenylate-binding protein 2                  |
| MGG_07268  | Isocitrate dehydrogenase                         |
| MGG_07289  | Glycogen synthase                                |
| MGG_07487  | ATP-dependent DNA helicase                       |
| MGG_07511  | Predicted protein with a RNA recognition motif   |
| MGG_07518  | Centromere microtubule-binding protein Cbf5      |
| MGG_07606  | General amino-acid permease GAP1                 |
| MGG_07613  | Acetyl-CoA carboxylase                           |
| MGG_07752  | ATP synthase subunit alpha                       |
| MGG_07753  | Ribosomal protein L11                           |
| MGG_07756  | Pyruvate carboxylase                             |
| MGG_07846  | Endoglucanase family 5 glycoside hydrolase       |
| MGG_07953  | Bifunctional P-450:NADPH-P450 reductase          |
| MGG_08012  | UDP-glucose 4-epimerase                          |
| MGG_08063  | Pyruvate kinase                                  |
| MGG_08072  | Cholesterol oxidase                              |
| MGG_08087  | Vacuolar ATP synthase catalytic subunit A        |
| MGG_08116  | Thioesterase family protein                      |
| MGG_08162  | Elongation factor Tu                             |
| MGG_08163  | Hypothetical protein                             |
| MGG_08164  | Disulfide-isomerase Erp38                        |
| MGG_08190  | Ribosomal protein L4-A                           |
| MGG_08210  | Endoplasmic reticulum vesicle protein 25         |
| MGG_08309  | Multidrug resistance-associated protein 2        |
| MGG_08313  | Predicted protein                                |
| MGG_08323  | Mitochondrial large ribosomal subunit            |
| MGG_08354  | Hypothetical protein                             |
| MGG_08370  | 1,3-beta-glucanosyltransferase                   |
| MGG_08464  | Aldehyde reductase                               |
| MGG_08619  | Voltage-gated potassium channel beta-1 subunit   |
| MGG_08661  | Acyl-CoA dehydrogenase family member 11         |
| MGG_08692  | Alpha-1,2 mannosyltransferase KTR1               |
| MGG_08766  | Predicted protein                                |
| MGG_08810  | 2,5-diketo-D-gluconic acid reductase A           |
| ID          | Description                                           |
|-------------|-------------------------------------------------------|
| MGG_08835   | Malate dehydrogenase                                  |
| MGG_08877   | Sarcosine oxidase                                     |
| MGG_08895   | Fructose-1,6-bisphosphatase                           |
| MGG_08897   | Asparagine-tRNA synthetase                            |
| MGG_08907   | Hypothetical protein                                  |
| MGG_08947   | Cytochrome P450                                       |
| MGG_08949   | Hypothetical protein                                  |
| MGG_09007   | Reticulon-4-interacting protein 1                     |
| MGG_09080   | Arginyl-tRNA synthetase                               |
| MGG_09160   | Beta-glucosidase 1                                    |
| MGG_09194   | Ribosomal protein L17                                 |
| MGG_09212   | Nitroreductase                                        |
| MGG_09222   | Ribosomal protein S2                                  |
| MGG_09272   | Beta-glucosidase 1                                    |
| MGG_09285   | NADH-ubiquinone oxidoreductase 49 kDa subunit         |
| MGG_09287   | Oligosaccharyltransferase alpha subunit               |
| MGG_09367   | Malate dehydrogenase                                  |
| MGG_09372   | Fasciclin domain family protein                       |
| MGG_09470   | Myosin regulatory light chain                         |
| MGG_09471   | Neutral trehalase                                     |
| MGG_09481   | Leukotriene A-4 hydrolase                             |
| MGG_09505   | Polyadenylate-binding protein                         |
| MGG_09523   | Transcription-associated protein 1                    |
| MGG_09540   | Tryptophanyl-tRNA synthetase                          |
| MGG_09571   | Hypothetical protein                                  |
| MGG_09718   | Predicted protein                                     |
| MGG_09766   | Phospho-2-dehydro-3-deoxyheptonate aldolase           |
| MGG_09769   | Hypothetical protein                                  |
| MGG_09779   | Hypothetical protein                                  |
| MGG_09878   | Pyruvate dehydrogenase protein                        |
| MGG_09894   | Hypothetical protein                                  |
| MGG_09916   | ATP synthase gamma chain                              |
| MGG_09927   | Ribosomal protein l35a-2                              |
| MGG_10005   | Glycerol kinase                                       |
| MGG_10052   | Hypoxanthine guanine phosphoribosyltransferase        |
| MGG_10087   | Sulfitoquinone oxidoreductase                         |
| MGG_10185   | Ribosomal protein L23a                                |
| MGG_10195   | Serinethreonine-protein phosphatase PP1               |
| MGG_10251   | Sulfate permease                                      |
| MGG_10320   | Methylcrotonoyl-CoA carboxylase subunit alpha         |
| MGG_10334   | Mannitol 2-dehydrogenase                              |
| MGG_10357   | Prolyl-tRNA synthetase 2                              |
| MGG_10358   | T-complex protein 1 subunit alpha                     |
| MGG_10370   | Ribosomal protein S15                                 |
| MGG_10413   | NADP-dependent leukotriene b4 12-hydroxydehydrogenase |
| MGG_10492   | Mitochondrial carnitine carrier                       |
| MGG_10568   | Sterol 24-C-methyltransferase                         |
| MGG_10605   | Kelch repeat-containing protein 3                     |
| MGG_10607   | Enolase                                               |
| MGG_10638   | Predicted protein                                     |
| MGG_10680   | Ribosomal protein S24                                 |
| MGG_10740   | Sm snRNP core protein Smg1                           |
| MGG_10847   | Ankyrin repeat domain-containing protein 29           |
| MGG_11205   | Dynein heavy chain                                    |
| MGG_11278   | Mitochondrion protein                                 |
| MGG_11309   | ATP-dependent DNA helicase PIF1                       |
| MGG_11394   | Predicted protein                                     |
| MGG_11412 | Tubulin alpha chain |
| MGG_11513 | Heat shock protein SSB1 |
| MGG_11517 | Heat shock protein SSB1 |
| MGG_11597 | Glucosamine-fructose-6-phosphate aminotransferase |
| MGG_11776 | Ribosomal protein S0 |
| MGG_11785 | Ribosomal protein L15 |
| MGG_11862 | ATP-binding cassette sub-family F member 2 |
| MGG_11889 | T-complex protein 1 subunit epsilon |
| MGG_11916 | Cap20 |
| MGG_12095 | NADPH-dependent methylglyoxal reductase GRE2 |
| MGG_12154 | Fatty acid synthase subunit alpha reductase |
| MGG_12244 | Carnitiny-CoA dehydratase |
| MGG_12300 | Hydroxycylglutathione hydrolase |
| MGG_12336 | Pattern formation protein EMB30 |
| MGG_12574 | Aspartyl-tRNA synthetase |
| MGG_12589 | 4-coumarate-CoA ligase 1 |
| MGG_12775 | Voltage-gated potassium channel subunit beta-3 channel subunit |
| MGG_12793 | DNA repair and recombination protein RAD5C |
| MGG_12797 | Hypothetical protein |
| MGG_12805 | Tryptophan synthase |
| MGG_12849 | Triacylglycerol lipase |
| MGG_12892 | Ribosomal protein S16 |
| MGG_12894 | ATP-dependent RNA helicase Dbp-2 |
| MGG_12911 | tRNA 2'-phosphotransferase 1 |
| MGG_12996 | Gamma-glutamyl phosphate reductase |
| MGG_13170 | Cytochrome c1 |
| MGG_13188 | Voltage-gated potassium channel subunit beta-2 |
| MGG_13200 | Eukaryotic translation initiation factor 2 subunit alpha |
| MGG_13230 | Oxidoreductase |
| MGG_13239 | Linoleate diol synthase |
| MGG_13410 | NADH:ubiquinone oxidoreductase 11.5kd subunit |
| MGG_13526 | RNP domain-containing protein |
| MGG_13645 | Ribosomal protein L13-A |
| MGG_13781 | Serine hydroxymethyltransferase |
| MGG_13782 | Ribosomal protein L19 |
| MGG_13797 | Predicted protein |
| MGG_13799 | Hypothetical protein |
| MGG_13806 | DNA damage checkpoint protein Rad24 |
| MGG_13917 | Sorbitol dehydrogenase |
| MGG_14097 | Predicted protein |
| MGG_14472 | Methylthioribose-1-phosphate isomerase |
| MGG_14547 | Hypothetical protein |
| MGG_14858 | Ribosomal protein L24 |
| MGG_14886 | Cyclopropane-fatty-acyl-phospholipid synthase |
| MGG_14947 | Arsenical pump-driving ATPase |
| MGG_14971 | Elongation factor 3 |
| MGG_14972 | Predicted protein |
| MGG_15088 | Cytochrome c oxidase polypeptide VI, variant 2 |
| MGG_15202 | 3-hydroxybutyryl-CoA dehydrogenase |
**Supplemental Dataset 1.** *Magnaporthe oryzae* proteins nonspecifically co-purified with 3xFLAG by the anti-FLAG antibody

| Proteins   | Putative functions                                                                 |
|------------|-----------------------------------------------------------------------------------|
| MGG_00153  | Sphingolipid long chain base-responsive protein LSP1                               |
| MGG_00167  | Succinate dehydrogenase iron-sulfur subunit                                       |
| MGG_00168  | Succinate dehydrogenase flavoprotein subunit                                       |
| MGG_00175  | 6-phosphogluconate dehydrogenase                                                   |
| MGG_00195  | Clock-controlled gene-9 protein                                                    |
| MGG_00206  | DNA-dependent ATPase MGS1                                                           |
| MGG_00221  | Ribosomal protein S7                                                                |
| MGG_00330  | Proteasome-activating nucleotidase                                                  |
| MGG_00338  | Helicase conserved C-terminal domain                                                |
| MGG_00341  | Eukaryotic translation initiation factor 3 subunit D                                |
| MGG_00349  | Ribosomal protein S13                                                               |
| MGG_00383  | S-adenosylmethionine synthetase                                                    |
| MGG_00604  | Beta-tubulin                                                                       |
| MGG_00657  | Adenine phosphoribosyltransferase                                                   |
| MGG_00689  | Acetyl-coenzyme A synthetase                                                        |
| MGG_00748  | Myosin-5                                                                           |
| MGG_00865  | 1,3-beta-glucan synthase component GLS1                                              |
| MGG_00878  | Hypothetical protein                                                                |
| MGG_00884  | 26S proteasome non-ATPase regulatory subunit 3                                      |
| MGG_00901  | 2,3-bisphosphoglycerate-independent phosphoglycerate mutase                        |
| MGG_00905  | Sec14                                                                              |
| MGG_00908  | 26S protease regulatory subunit 6B                                                   |
| MGG_00923  | Serine hydroxymethyltransferase                                                     |
| MGG_00949  | Endoplasmic reticulum-Golgi intermediate compartment protein 2                      |
| MGG_01014  | C-1-tetrahydrofolate synthase                                                       |
| MGG_01046  | Methionyl-tRNA synthetase                                                           |
| MGG_01049  | 3,4-dihydroxy-2-butanone 4-phosphate synthase                                       |
| MGG_01061  | Coproporphyrinogen III oxidase                                                      |
| MGG_01084  | Glyceraldehyde-3-phosphate dehydrogenase                                            |
| MGG_01092  | Homocitrate synthase                                                                |
| Gene ID    | Description                                    |
|-----------|------------------------------------------------|
| MGG_0110  | Hypothetical protein                           |
| MGG_01113 | 40S ribosomal protein S10-A                    |
| MGG_01135 | Cytochrome C oxidase polypeptide IV            |
| MGG_01140 | Alpha-1,2 mannosyltransferase                   |
| MGG_01160 | Histone H4.1                                   |
| MGG_01165 | Isoleucyl-tRNA synthetase                      |
| MGG_01202 | D-lactate dehydrogenase                        |
| MGG_01268 | 28 kDa ribonucleoprotein                       |
| MGG_01282 | Ubiquitin                                      |
| MGG_01333 | NADH dehydrogenase iron-sulfur protein 3       |
| MGG_01380 | 26S protease regulatory subunit 8              |
| MGG_01535 | Pyridoxamine 5′-phosphate oxidase              |
| MGG_01566 | Homoisocitrate dehydrogenase                   |
| MGG_01580 | 26S protease regulatory subunit 7              |
| MGG_01581 | T-complex protein 1 subunit delta              |
| MGG_01582 | Diphthamide biosynthesis protein 4             |
| MGG_01587 | Eukaryotic translation initiation factor 2 subunit gamma |
| MGG_01631 | UTP-glucose-1-phosphate uridylyltransferase    |
| MGG_01712 | Isocitrate dehydrogenase subunit 2            |
| MGG_01728 | Methylene tetrahydrofolate reductase 1         |
| MGG_01742 | Elongation factor 2                            |
| MGG_01793 | Short-chain dehydrogenase reductase            |
| MGG_01808 | Ketol-acid reductoisomerase                    |
| MGG_01819 | Glycogen phosphorylase                         |
| MGG_01906 | Nicotianamine synthase 3                      |
| MGG_01925 | Bifunctional P-450:NADPH-P450 reductase        |
| MGG_01995 | Isocitrate dehydrogenase subunit 1             |
| MGG_02072 | Amino-acid permease                            |
| MGG_02331 | Predicted protein                              |
| MGG_02370 | Mitochondrial phosphate carrier protein        |
| MGG_02378 | Glutamate decarboxylase 1                      |
| MGG_02432 | Leucyl-tRNA synthetase                         |
| MGG_02439 | 2-nitropropane dioxygenase                     |
| Accession   | Description                                      |
|------------|--------------------------------------------------|
| MGG_02449  | Tyrosyl-tRNA synthetase                          |
| MGG_02511  | Ribosomal protein L3                             |
| MGG_02521  | Carboxylesterase 3                               |
| MGG_02526  | Exportin-1                                       |
| MGG_02564  | Predicted protein                                |
| MGG_02608  | 26S proteasome regulatory subunit RPN5           |
| MGG_02612  | 3-oxoacyl-reductase                              |
| MGG_02634  | Coatamer subunit gamma                            |
| MGG_02648  | Interferon-induced GTP-binding protein           |
| MGG_02653  | 6-phosphofructokinase                            |
| MGG_02659  | Ribosomal protein L14                            |
| MGG_02661  | ATP synthase D chain, mitochondrial              |
| MGG_02747  | Ribosomal protein S4-A                           |
| MGG_02784  | DNA-binding protein SMUBP-2                      |
| MGG_02806  | ATP-dependent RNA helicase SUB2                  |
| MGG_02903  | Carboxylesterase                                 |
| MGG_02914  | 2-deoxy-D-gluconate 3-dehydrogenase              |
| MGG_02952  | Ribosomal protein S9                             |
| MGG_02953  | Ribosomal protein L21-B                          |
| MGG_02983  | Cytochrome C oxidase subunit via                 |
| MGG_03039  | Hsp70-like protein                               |
| MGG_03086  | U2 snRNP component IST3                          |
| MGG_03135  | Ribosomal protein L30                            |
| MGG_03136  | Ribosomal protein L10-B                          |
| MGG_03147  | Glycerol-3-phosphate dehydrogenase               |
| MGG_03152  | ATP synthase subunit 5                           |
| MGG_03185  | ATP synthase subunit beta                        |
| MGG_03192  | RNA polymerase II transcription factor B subunit 1|
| MGG_03193  | Ribosomal protein L3                             |
| MGG_03211  | 26S proteasome non-atpase regulatory subunit 11  |
| MGG_03217  | Phenylalanyl-tRNA synthetase                     |
| MGG_03220  | Isoleucyl-tRNA synthetase                        |
| MGG_03226  | Cytochrome b-c1 complex subunit 2                |
| Protein ID  | Description                                |
|------------|--------------------------------------------|
| MGG_03236  | Ribosomal protein S6-B                     |
| MGG_03244  | Vacuolar ATP synthase subunit B            |
| MGG_03251  | Ribosomal protein S8-B                     |
| MGG_03310  | T-complex protein 1 subunit eta            |
| MGG_03372  | Ribosomal protein S20                      |
| MGG_03388  | ATP-dependent RNA helicase                 |
| MGG_03414  | Short chain dehydrogenase-reductase        |
| MGG_03441  | Trehalose-phosphatase                      |
| MGG_03494  | Aminotransferase                           |
| MGG_03511  | Coatamer subunit alpha                     |
| MGG_03517  | Translation initiation factor eif-2B subunit epsilon |
| MGG_03537  | Importin subunit beta-3                    |
| MGG_03549  | DNA repair and recombination protein RAD54  |
| MGG_03554  | Ribosomal protein L36                      |
| MGG_03556  | HACA ribonucleoprotein complex subunit 1    |
| MGG_03577  | Histone H2A                                 |
| MGG_03600  | Mitochondrial-processing peptidase subunit beta |
| MGG_03641  | Elongation factor 1-alpha                  |
| MGG_03653  | Ribosomal protein L20                      |
| MGG_03663  | Hypothetical protein                        |
| MGG_03668  | Importin subunit beta-1                    |
| MGG_03680  | Ribosomal protein S11                      |
| MGG_03684  | Mitochondrial distribution and morphology protein 38 |
| MGG_03708  | NADH-cytochrome b5 reductase               |
| MGG_03727  | Ribosomal protein L31                      |
| MGG_03736  | Multidrug resistance-associated protein 1   |
| MGG_03823  | NADH-dependent flavin oxidoreductase       |
| MGG_03857  | T-complex protein 1 subunit zeta            |
| MGG_03880  | Alcohol dehydrogenase                      |
| MGG_03895  | Small nuclear ribonucleoprotein F           |
| MGG_03897  | Lariat debranching enzyme                   |
| MGG_03900  | Aldehyde dehydrogenase                     |
| MGG_03915  | Pyridoxamine phosphate oxidase family protein |
| Protein ID   | Description                                              |
|-------------|----------------------------------------------------------|
| MGG_03982   | Actin                                                    |
| MGG_04013   | Small nuclear ribonucleoprotein Sm D2                   |
| MGG_04042   | Leucyl-tRNA synthetase, mitochondrial precursor          |
| MGG_04083   | Small nuclear ribonucleoprotein Sm D3                   |
| MGG_04104   | Ribosomal protein L22                                   |
| MGG_04114   | Ribosomal protein S17                                   |
| MGG_04400   | ATP-dependent RNA helicase                               |
| MGG_04425   | Phenylalanyl-tRNA synthetase alpha chain                 |
| MGG_04432   | Cytochrome P450                                          |
| MGG_04438   | ADP-ribosylation factor 1                                |
| MGG_04444   | Ribosomal protein L6-B                                   |
| MGG_04462   | Chaperone protein Dnaj 2                                |
| MGG_04467   | 60S acidic ribosomal protein P0                          |
| MGG_04476   | FK506-binding protein 4                                  |
| MGG_04484   | Ribosomal protein L18                                   |
| MGG_04503   | Carbamoyl-phosphate synthase arginine-specific large chain |
| MGG_04545   | Cytochrome c peroxidase                                  |
| MGG_04550   | Calcium-transporting ATPase                              |
| MGG_04556   | Alcohol dehydrogenase                                    |
| MGG_04602   | Pre-mRNA-processing protein PRP40                        |
| MGG_04612   | Ribosomal protein L8-B                                   |
| MGG_04652   | DNA-directed RNA polymerase II subunit RPB1              |
| MGG_04696   | Ribosomal protein l10a                                   |
| MGG_04738   | Bacilysin biosynthesis oxidoreductase                    |
| MGG_04741   | Small nuclear ribonucleoprotein Sm D1                   |
| MGG_04752   | ATP synthase subunit 4                                   |
| MGG_04775   | Fatty acid synthase S-acetyltransferase                  |
| MGG_04829   | Ribosomal protein L9-A                                   |
| MGG_04901   | Methyltransferase                                         |
| MGG_04921   | Ribosomal protein L23                                    |
| MGG_04988   | Chromosome segregation protein suda                       |
| MGG_04994   | Plasma membrane ATPase 1                                 |
| MGG_05025   | Fatty acid transporter protein                           |
MGG_05063  Phosphoglycerate kinase
MGG_05140  2OG-Fe(II) oxygenase superfamily
MGG_05159  Dihydrodipicolinate synthase
MGG_05170  Ribosomal protein L17
MGG_05201  Guanine nucleotide-binding protein subunit beta
MGG_05223  3-isopropylmalate dehydrogenase
MGG_05237  Ribosomal protein L7
MGG_05238  Ribosomal protein S14
MGG_05296  Ribosomal protein L34-B
MGG_05307  Hyaluronan mRNA binding family
MGG_05438  Small glutamine-rich tetratricopeptide repeat-containing protein 2
MGG_05449  Ribosomal protein L16
MGG_05480  Succinyl-CoA ligase subunit alpha-2
MGG_05565  Glutathione S-transferase
MGG_05602  Glucosidase 2 subunit beta
MGG_05630  AMP deaminase 2
MGG_05661  Ribosomal protein S18
MGG_05663  Carboxypeptidase Y
MGG_05671  Protein phosphatase PP2A regulatory subunit A
MGG_05673  Ribosomal protein S3
MGG_05734  Phospho-2-dehydro-3-deoxyheptonate aldolase
MGG_05858  26S proteasome regulatory subunit RPN11
MGG_05936  Mannose-1-phosphate guanylyltransferase
MGG_05940  Short chain dehydrogenase
MGG_05949  Acyl-coa dehydrogenase
MGG_05999  HACA ribonucleoprotein complex subunit 2
MGG_06003  Ubiquinone-binding protein
MGG_06005  RNA exonuclease 3
MGG_06011  S-(hydroxymethyl)glutathione dehydrogenase
MGG_06030  NADH-ubiquinone oxidoreductase 29.9 kda subunit
MGG_06047  Cytochrome P450
MGG_06192  Cytochrome b-c1 complex subunit 7
MGG_06241  Vacuolar protein sorting-associated protein 21
MGG_06269  Ribosomal protein L2
MGG_06276  External NADH-ubiquinone oxidoreductase 1
MGG_06289  NADH-cytochrome b5 reductase 1
MGG_06321  Glycyl-tRNA synthetase 1
MGG_06362  Small COPII coat GTPase SAR1
MGG_06392  Ornithine aminotransferase
MGG_06445  Histone H3
MGG_06453  Single-strand binding protein
MGG_06459  Heat shock protein HSP98
MGG_06479  Ribosomal protein S22
MGG_06480  Ribosomal protein S12
MGG_06505  mRNA cap methyltransferase
MGG_06512  Glucan 1,3-beta-glucosidase 2
MGG_06524  Fructose-2,6-bisphosphatase
MGG_06561  Glutaryl-CoA dehydrogenase
MGG_06571  Ribosomal protein l37a-2
MGG_06587  Leucyl aminopeptidase
MGG_06609  Acetyl-CoA hydrolase
MGG_06629  Ribosomal protein L10
MGG_06639  T-complex protein 1 subunit gamma
MGG_06648  Hypoxia up-regulated protein 1 precursor
MGG_06650  Alpha-tubulin
MGG_06656  ADP,ATP carrier protein
MGG_06657  Ribosomal protein S25
MGG_06658  Ribosomal protein S5
MGG_06693  Ribosomal protein L27-B
MGG_06712  Cobalamin-independent synthase
MGG_06716  Histone acetylase complex subunit
MGG_06719  ATP-citrate synthase subunit 1
MGG_06720  ATP citrate lyase
MGG_06759  Heat shock protein 80
MGG_06860  Coatomer subunit beta
MGG_06910  Transport protein SEC23
| Gene ID   | Protein Name                                      |
|----------|--------------------------------------------------|
| MGG_06913 | Seryl-tRNA synthetase                             |
| MGG_06915 | Uracil phosphoribosyltransferase                  |
| MGG_06919 | Ribosomal protein S3                              |
| MGG_06923 | Myosin-2                                         |
| MGG_06924 | Nucleosome assembly protein                       |
| MGG_06936 | Elongation factor 1-gamma                         |
| MGG_06950 | L-serine dehydratase                              |
| MGG_06958 | Hsp70-like protein                                |
| MGG_06977 | Succinyl-CoA ligase beta-chain                    |
| MGG_07033 | ATP-dependent RNA helicase ded-1                  |
| MGG_07037 | Phosphatidylserine decarboxylase                  |
| MGG_07048 | Ribosomal protein L5                              |
| MGG_07087 | Pre-mRNA-splicing factor SYF-1                    |
| MGG_07099 | ATP-dependent RNA helicase                        |
| MGG_07110 | Phosphatidylethanolamine N-methyltransferase      |
| MGG_07120 | 26S proteasome regulatory subunit Rpn-1           |
| MGG_07195 | O-acetylhomoserine (thiol)-lyase                  |
| MGG_07197 | Long-chain-fatty-acid-CoA ligase 1                |
| MGG_07200 | Plasma membrane ATPase                            |
| MGG_07252 | Polyadenylate-binding protein 2                   |
| MGG_07268 | Isocitrate dehydrogenase                          |
| MGG_07289 | Glycogen synthase                                 |
| MGG_07487 | ATP-dependent DNA helicase                        |
| MGG_07511 | Predicted protein with a RNA recognition motif    |
| MGG_07518 | Centromere microtubule-binding protein Cbf5       |
| MGG_07606 | General amino-acid permease GAP1                  |
| MGG_07613 | Acetyl-CoA carboxylase                            |
| MGG_07752 | ATP synthase subunit alpha                        |
| MGG_07753 | Ribosomal protein L11                            |
| MGG_07756 | Pyruvate carboxylase                              |
| MGG_07846 | Endoglucanase family 5 glycoside hydrolase        |
| MGG_07953 | Bifunctional P-450:NADPH-P450 reductase           |
| MGG_08012 | UDP-glucose 4-epimerase                           |
| Gene ID       | Protein Name                                      |
|--------------|--------------------------------------------------|
| MGG_08063    | Pyruvate kinase                                  |
| MGG_08072    | Cholesterol oxidase                              |
| MGG_08087    | Vacuolar ATP synthase catalytic subunit A        |
| MGG_08116    | Thioesterase family protein                      |
| MGG_08162    | Elongation factor Tu                             |
| MGG_08163    | Hypothetical protein                             |
| MGG_08164    | Disulfide-isomerase Erp38                        |
| MGG_08190    | Ribosomal protein L4-A                           |
| MGG_08210    | Endoplasmic reticulum vesicle protein 25         |
| MGG_08309    | Multidrug resistance-associated protein 2        |
| MGG_08313    | Predicted protein                                |
| MGG_08323    | Mitochondrial large ribosomal subunit            |
| MGG_08354    | Hypothetical protein                             |
| MGG_08370    | 1,3-beta-glcanosyltransferase                    |
| MGG_08464    | Aldehyde reductase                               |
| MGG_08619    | Voltage-gated potassium channel beta-1 subunit   |
| MGG_08661    | Acyl-CoA dehydrogenase family member 11          |
| MGG_08692    | Alpha-1,2 mannosyltransferase KTR1               |
| MGG_08766    | Predicted protein                                |
| MGG_08810    | 2,5-diketo-D-gluconic acid reductase A           |
| MGG_08835    | Malate dehydrogenase                             |
| MGG_08877    | Sarcosine oxidase                                |
| MGG_08895    | Fructose-1,6-bisphosphatase                      |
| MGG_08897    | Asparaginyl-tRNA synthetase                      |
| MGG_08907    | Hypothetical protein                             |
| MGG_08947    | Cytochrome P450                                   |
| MGG_08949    | Hypothetical protein                             |
| MGG_09007    | Reticulon-4-interacting protein 1                |
| MGG_09080    | Arginyl-tRNA synthetase                          |
| MGG_09160    | Beta-glucosidase 1                               |
| MGG_09194    | Ribosomal protein L17                            |
| MGG_09212    | Nitroreductase                                   |
| MGG_09222    | Ribosomal protein S2                             |
| Gene ID    | Description                                      |
|-----------|--------------------------------------------------|
| MGG_09272 | Beta-glucosidase 1                               |
| MGG_09285 | NADH-ubiquinone oxidoreductase 49 kDa subunit    |
| MGG_09287 | Oligosaccharyltransferase alpha subunit          |
| MGG_09367 | Malate dehydrogenase                             |
| MGG_09372 | Fasciclin domain family protein                  |
| MGG_09470 | Myosin regulatory light chain                    |
| MGG_09471 | Neutral trehalase                                |
| MGG_09481 | Leukotriene A-4 hydrolase                        |
| MGG_09505 | Polyadenylate-binding protein                    |
| MGG_09523 | Transcription-associated protein 1               |
| MGG_09540 | Tryptophanyl-tRNA synthetase                     |
| MGG_09571 | Hypothetical protein                             |
| MGG_09718 | Predicted protein                                |
| MGG_09766 | Phospho-2-dehydro-3-deoxyheptonate aldolase      |
| MGG_09769 | Hypothetical protein                             |
| MGG_09779 | Hypothetical protein                             |
| MGG_09878 | Pyruvate dehydrogenase protein                   |
| MGG_09894 | Hypothetical protein                             |
| MGG_09916 | ATP synthase gamma chain                         |
| MGG_09927 | Ribosomal protein l35a-2                         |
| MGG_10005 | Glycerol kinase                                  |
| MGG_10052 | Hypoxanthine guanine phosphoribosyltransferase   |
| MGG_10087 | Sulfide:quinone oxidoreductase                   |
| MGG_10185 | Ribosomal protein L23a                           |
| MGG_10195 | Serinethreonine-protein phosphatase PP1          |
| MGG_10251 | Sulfate permease                                 |
| MGG_10320 | Methylcrotonoyl-COA carboxylase subunit alpha    |
| MGG_10334 | Mannitol 2-dehydrogenase                         |
| MGG_10357 | Prolyl-tRNA synthetase 2                         |
| MGG_10358 | T-complex protein 1 subunit alpha                |
| MGG_10370 | Ribosomal protein S15                            |
| MGG_10413 | NADP-dependent leukotriene b4 12-hydroxydehydrogenase |
| MGG_10492 | Mitochondrial carnitine carrier                  |
MGG_10568  Sterol 24-C-methyltransferase
MGG_10605  Kelch repeat-containing protein 3
MGG_10607  Enolase
MGG_10638  Predicted protein
MGG_10680  Ribosomal protein S24
MGG_10740  Sm snRNP core protein Smg1
MGG_10847  Ankyrin repeat domain-containing protein 29
MGG_11205  Dynein heavy chain
MGG_11278  Mitochondrion protein
MGG_11309  ATP-dependent DNA helicase PIF1
MGG_11394  Predicted protein
MGG_11412  Tubulin alpha chain
MGG_11513  Heat shock protein SSB1
MGG_11517  Heat shock protein SSB1
MGG_11597  Glucosamine-fructose-6-phosphate aminotransferase
MGG_11776  Ribosomal protein S0
MGG_11785  Ribosomal protein L15
MGG_11862  ATP-binding cassette sub-family F member 2
MGG_11889  T-complex protein 1 subunit epsilon
MGG_11916  Cap20
MGG_12095  NADPH-dependent methylglyoxal reductase GRE2
MGG_12154  Fatty acid synthase subunit alpha reductase
MGG_12244  Carnitiny-CoA dehydratase
MGG_12300  Hydroxyacylglutathione hydrolase
MGG_12336  Pattern formation protein EMB30
MGG_12574  Aspartyl-tRNA synthetase
MGG_12589  4-coumarate-CoA ligase 1
MGG_12775  Voltage-gated potassium channel subunit beta-3 channel subunit
MGG_12793  DNA repair and recombination protein RAD5C
MGG_12797  Hypothetical protein
MGG_12805  Tryptophan synthase
MGG_12849  Triacylglycerol lipase
MGG_12892  Ribosomal protein S16
MGG_12894 ATP-dependent RNA helicase Dbp-2
MGG_12911 tRNA 2'-phosphotransferase 1
MGG_12996 Gamma-glutamyl phosphate reductase
MGG_13170 Cytochrome c1
MGG_13188 Voltage-gated potassium channel subunit beta-2
MGG_13200 Eukaryotic translation initiation factor 2 subunit alpha
MGG_13230 Oxidoreductase
MGG_13239 Linoleate diol synthase
MGG_13410 NADH:ubiquinone oxidoreductase 11.5kd subunit
MGG_13526 RNP domain-containing protein
MGG_13645 Ribosomal protein L13-A
MGG_13781 Serine hydroxymethyltransferase
MGG_13782 Ribosomal protein L19
MGG_13797 Predicted protein
MGG_13799 Hypothetical protein
MGG_13806 DNA damage checkpoint protein Rad24
MGG_13917 Sorbitol dehydrogenase
MGG_14097 Predicted protein
MGG_14472 Methylthioribose-1-phosphate isomerase
MGG_14547 Hypothetical protein
MGG_14858 Ribosomal protein L24
MGG_14886 Cyclopropane-fatty-acyl-phospholipid synthase
MGG_14947 Arsenical pump-driving ATPase
MGG_14971 Elongation factor 3
MGG_14972 Predicted protein
MGG_15088 Cytochrome c oxidase polypeptide VI, variant 2
MGG_15202 3-hydroxybutyryl-CoA dehydrogenase