The COMPASS-like complex modulates fungal development and pathogenesis by regulating H3K4me3-mediated targeted gene expression in *Magnaporthe oryzae*

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ABSTRACT

Histone H3K4 methylation is catalysed by the multi-protein complex known as the Set1/COMPASS or MLL/COMPASS-like complex, an element that is highly evolutionarily conserved from yeast to humans. However, the components and mechanisms by which the COMPASS-like complex targets the H3K4 methylation of plant pathogenic genes in fungi remain elusive. Here we present a comprehensive analysis combining biochemical, molecular, and genome-wide approaches to characterize the roles of the COMPASS-like family in *Magnaporthe oryzae*, a model plant fungal pathogen. We purified and identified six conserved subunits of COMPASS from the rice blast fungus *M. oryzae*, i.e., MoBre2 (Cps60/ASH2L), MoSpp1 (Cps40/Cfp1), MoSwd2 (Cps35), MoSdc1 (Cps25/DPY30), MoSet1 (MLL/ALL) and MoRbBP5 (Cps50), using an affinity tag on MoBre2. We determined the SPRY domain of MoBre2 can recognize directly with DPY30 domain of MoSdc1 *in vitro*. Furthermore, we found that deletion of the genes encoding COMPASS subunits of MoBre2, MoSpp1 and MoSwd2 caused similar defects regarding invasive hyphal development and pathogenicity. Genome-wide profiling of H3K4me3 revealed that the it has remarkable co-occupancy at the TSS regions of target genes.

Significantly, these target genes are often involved in spore germination and pathogenesis. Decreased gene expression caused by the deletion of *MoBre2*, *MoSwd2* or *MoSpp1* gene was highly correlated with decrease in H3K4me3. Taken together, these results suggest that *MoBre2*, *MoSpp1*, and *MoSwd2* function as a whole COMPASS complex, contributing to fungal development and pathogenesis by regulating H3K4me3-targeted genes in *M. oryzae*.

KEY WORDS: *Magnaporthe oryzae*, COMPASS complex, H3K4me3, Fungal, TSS, Pathogenesis, Target genes

INTRODUCTION

The eukaryotic genome is found in complexes with an equal mass of histone proteins to form nucleosomes, the basic units of chromatin (Kornberg, 1974; Smith, 2010). Chromatin assembly and folding into higher structures is a dynamic process that ultimately affects the activation or repression of gene expression. Chromatin remodelling and covalent modifications of histones are two means by which variation is introduced into the chromatin polymer, thereby regulating the structure and function of chromatin and ultimately gene expression (Luger et al., 1997; Strath, 2000; Lachner, 2002; Bhaumik et al., 2007; Shilatifard, 2008). Chromatin-modifying activities are often recruited to specific gene regulatory sequences, whereupon they cause localized changes in the chromatin structure and specific transcriptional effects (Ng et al., 2003; Allis, 2007). Several post-translational modifications are known to be present on the terminal domains of histones, such as acetylation, phosphorylation, ubiquitination, ADP ribosylation, and methylation (Workman et al., 2003; Berger et al., 2007; Kouzarides, 2007). Lysine methylation has been well documented on histones H3 and H4 *in vivo*, and residues 4, 9, 27, and 36 are typical methylation sites (Wang et al., 2010; Yang et al., 2012).

Histone H3K4 methylation is associated with active chromatin in a wide range of eukaryotic organisms. Many of the genes involved in H3K4 methylation encode proteins bearing a 130- to 140-amino-acid motif called the SET domain. This domain takes its name from the *Drosophila* proteins *Su(var)3-9*, *Enhancer of zeste (E(z))* and

63 *Trithorax*. Many SET domain-containing proteins have been shown to possess histone
64 or lysine methyltransferase activity (Tschiersch et al., 1994; Stassen et al., 1995;
65 Shilatifard, 2012). In *Saccharomyces cerevisiae*, the Set1/COMPASS (Complex
66 Proteins Associated with Set1), which consists of the seven polypeptides Set1,
67 Cps60/Bre2, Cps50/Swd1, Cps40/Spp1, Cps35/Swd2, Cps30/Swd3, and Cps25/Sdc1,
68 was the first H3K4 methylase to be identified (Miller et al., 2001; Krogan et al., 2003).
69 Cps60 is the product of the *BRE2/YLR015w* gene, which encodes a protein similar to
70 both *Drosophila* and human ASH2L proteins. ASH2L is a member of the Trx family
71 of homeodomain DNA-binding proteins that is thought to regulate gene expression,
72 morphogenesis, and differentiation in humans. Cps25 is encoded by the *YDR469* gene,
73 which has weak similarity to a DPY30 protein involved in dosage compensation of
74 expression of genes on the X chromosome. Some studies have revealed that Cps60
75 (Bre2) and Cps25 (Sdc1) form a globular structure sitting at the base of the Y-shaped
76 COMPASS structure sandwich (Chen et al., 2012; Li et al., 2016). Cps35 is the only
77 essential subunit of the COMPASS. In human embryonic kidney 293 cells, the
78 depletion of WDR5, (a Cps35 homologue) can result in a global loss of histone H3K4
79 methylation and concomitant loss of Hox gene expression (Li et al., 2016). The
80 mammalian Wdr82 (a Cps35 homologue) is required for the targeting of
81 Setd1A-mediated histone H3-Lys4 trimethylation near transcription start sites via
82 tethering to RNA polymerase II, an event that is a consequence of transcription
83 initiation (Lee et al., 2008; Palmer et al., 2013). Null mutants missing any one of the
84 six COMPASS subunits grow more slowly and display greater hydroxyurea
85 sensitivity than wild-type cells under normal conditions and they also exhibit a defect
86 in silencing gene expression near chromosomal telomeres (Roguev et al., 2001; Dover
87 et al., 2002).

88 Previous studies have shown that the COMPASS family of H3K4
89 methyltransferases is highly conserved from yeast to plants and humans (Jiang et al.,
90 2011; Shilatifard, 2012). In *Drosophila melanogaster*, there are three subclasses
91 within COMPASS-like complexes, namely SetD1A, Trx and trithorax-related (Trr),

all of which are homologues to yeast Set1 (Ardehali et al., 2011; Mohan et al., 2011; Shilatifard, 2012). While Set1D1A is homologous to yeast Set1, Trx and Trr are more distantly related (Czermin et al., 2002). dSet1 (*Drosophila Set1*), Trx and Trr each interact with a set of core complex subunits, with additional subunits unique to each (Mohan et al., 2011). In human cells, Set1A, Set1B, MLL1, MLL2, MLL3 and MLL4 are homologous to yeast Set1 (Cho et al, 2007). A biochemical study showed that human cells bear at least six COMPASS family members, each of which is capable of methylating H3K4 with non-redundant functions (Roguev et al., 2001; Dover 2002). These enzymes participate in diverse gene regulatory networks and are fully active in the context of an MLL/COMPASS-like complex that includes the proteins: WDR5, RbBP5, ASH2L and DPY-30 (WRAD). WRAD components function by engaging in physical interactions that recruit the SET1 family of proteins to target chromatin sites (Ernst et al., 2012; Ali et al., 2017; Hsu et al., 2018).

Recently, in filamentous fungi, such as *Fusarium spp.* and *Aspergillus spp.*, H3K4 methylation is also associated with active transcription of the majority of genes. Deletion of the COMPASS components CCLA and CCL1 in *Aspergillus spp.* and *Fusarium spp.*, respectively, results in increased expression of several, secondary metabolites (SMs) in these fungi (Palmer et al., 2013; Shinohara et al., 2016; Studt et al., 2016). Some studies have shown that H3K4 methylation is also associated with mainly secondary metabolism cluster genes, in the filamentous fungi, such as *Asperigillus spp.* and *Fusarium spp.* (Connolly et al., 2013; Palmer et al., 2013; Matthews et al., 2016). In *F. graminearum*, *FgSet1* is a Set1 orthologue and is one subunit of the COMPASS-like complex, which also plays an important role in regulating fungal growth and secondary metabolism (Connolly et al., 2013; Liu et al., 2015; Bachleitner et al., 2019).

M. oryzae functions as a model plant pathogenic fungus as the causal agent of rice blast disease, which is the most devastating and persistent disease of cultivated rice (Cao et al., 2016; Yan, 2016; Liu et al., 2018; Zhang et al., 2018). MoSet1 is the homologue of *S. cerevisiae* Set1. When the *MoSet1* is depleted ($\Delta moSet1$), H3K4

121 methylation becomes impaired, resulting in severe defects in infection-related
122 morphogenesis, including in conidiation and appressorium formation (Pham et al.,
123 2015). The homologues of COMPASS complex exist in multiple fungi, but other than
124 the Set1 subunit, no clear systemic functions of COMPASS have been reported in *M.*
125 *oryzae*.

126 Here, we report the identification of a conserved COMPASS-like complex that
127 contains six subunits: MoSet1, MoBre2, MoSwd1, MoSpp1, MoSwd2 and
128 MoSdc1 (Table 1). When the component genes, *MoBre2*, *MoSpp1* and *MoSwd2* were
129 deleted, H3K4me3 signals were dramatically decreased. Our results show that
130 MoBre2, MoSpp1 and MoSwd2 not only have unique regulation roles in fungal
131 development, but also function as a whole complex to methylate H3K4 in the TSS
132 region of its target genes. Moreover, most of these key target genes are involved in
133 conidia, mycelial growth and infectious hyphal formation in *M. oryzae*.

134

135 **RESULTS**

136 **Phylogenetic analysis and affinity purification of five subunits, MoBre2,** 137 **MoSwd1, MoSdc1, MoSet1 and MoJmjd2, from the COMPASS-Like complex of** 138 ***M. oryzae***

139 To explore whether homologues of the core COMPASS-like complex components are
140 present in *M. oryzae*, we performed a phylogenetic analysis of COMPASS-like
141 homologues from several animals and plants; the results suggested that the subunits of
142 the COMPASS-like complex are highly conserved in *M. oryzae*. We blasted yeast
143 homologues in the *M. oryzae* protein database with the amino acid sequences of
144 proteins such as Cps60/Bre2, Cps50/Swd1, Cps40/Spp1, Cps35/Swd2, Cps25/Sdc,
145 Cps30/WDR5 and Cps30/Swd3. We identified six homologues for these proteins,
146 namely MoSet1 (MGG_15053), MoBre2 (MGG_01427), MoSwd1 (MGG_04651),
147 MoSpp1 (MGG_03005), MoSwd2 (MGG_06406) and MoSdc1 (MGG_00350) (Fig.

1; Table 1). Orthologues of all the components in the COMPASS-like complex are single copies in *M. oryzae*, and their orthologues from representative species, including *S. cerevisiae*, *F. graminearum*, *Arabidopsis*, *Drosophila* and mammalian species (Fig.1; Supporting Information Fig. S1; Table 1; Supporting Information Table S1).

To purify the Set1/COMPASS-like complex in *M. oryzae*, we first generated strains able to stably express Flag-MoBre2 and performed an affinity purification using the total nuclear extracts from the Flag-MoBre2 strains (Fig. 1B; Supporting Information Fig. S2). Immunoprecipitated complexes were subjected to silver staining and protein identification using MudPIT (Multidimensional Protein Identification Technology). Tandem mass spectrometry analysis was used to identify the five proteins, MoBre2, MoSwd1, MoSdc1, MoSet1, and a novel JMJC domain-containing protein (MGG_09186) (Supporting Information Table S2). A sequence alignment of MGG_09186 revealed that it contains Jumonji (JmjC) catalytic domains, which can demethylate trimethylated lysine on histones, suggesting that this protein may act as a histone demethylase (Supporting Information Fig. S3A-C). We named MGG_09186 as MoJmjd2 (Smith et al., 2008; Huh et al., 2017). In addition, although we did not detect an obvious MoSet1 band in the SDS-PAGE gels, we did find that our MoBre2-Flag preparations contained significant amounts of MoSet1 (Supporting Information Table S2). However, the MudPIT did not result in peptides unique to other homologous subunits, such as MoSpp1, MoSwd2 and MoSdc1. The results clearly indicate that MoBre2, MoSwd1, MoSdc1, MoSet1 and MoJmjd2 have stable associations with COMPASS-like complexes of *M. oryzae* *in vivo*.

171

MoBre2 physically interacts with MoSdc1 via its SPRY domain 401-613 *in vitro*

173 **and the two subcellularly colocalize *in vivo***

174 In *M. oryzae*, MoBre2 shares 17.36.% and 12.70% of its identity with yeast Bre2 and
175 human ASH2L, respectively (Supporting Information Fig. S1C-E). In addition,
176 MoSdc1 and yeast Cps25 have a conserved DPY30 domain of approximately 37
177 amino acids with 68.57% shared identity (Supporting Information Fig. S1D). The
178 MoBre2 protein contains two SPRY domains (Fig. 2A). Recently, the ASH2L-DPY30
179 interaction was reported to be important for the regulation of H3K4 methylation
180 (Chen et al., 2012; Tremblay et al., 2014; Li et al., 2016).

181 To investigate whether MoBre2 interacts with MoSdc1 or MoSwd1, we expressed
182 and purified recombinant proteins corresponding to the full length, N-terminal and
183 C-terminal regions of MoBre2, MoSdc1 and MoSwd1. Our immunoprecipitation
184 experiments showed that MoBre2 can directly interact with MoSdc1 (Fig. 2A-G;
185 Supporting Information Table S3), but not with MoSwd1 (Supporting Information Fig.
186 S4A, B). To understand which residues are required for interactions between MoBre2
187 and MoSdc1, we constructed three deletion mutants of MoBre2: (amino acids 1-200,
188 termed MoBre2₁₋₂₀₀; amino acids 201-613, termed MoBre2₂₀₁₋₆₁₃; and amino acids
189 401-613, termed MoBre2₄₀₁₋₆₁₃), two deletion mutants of MoSdc1 (amino acids 1-100,
190 termed MoSdc1₁₋₁₀₀; amino acids 101-200, termed MoSdc1₁₀₁₋₂₀₀) (Fig. 2C-E). As
191 shown in Fig. 2, a pull-down assay showed that MoBre2 retained the binding activity
192 with MoSdc1₁₀₁₋₂₀₀ (Fig. 2C), but not MoSdc1₁₋₁₀₀ (Supporting Information Fig.
193 S4A).

194 In addition, MoSdc1₁₀₁₋₂₀₀ physically interacts with MoBre2₂₀₁₋₆₁₃ and MoBre2₄₀₁₋₆₁₃
195 (Fig. 2D, E), whereas this construct failed to bind to MoBre2₁₋₂₀₀ (Supporting
196 Information Fig. S4B), which suggests that the SPRY domain located between 401
197 and 613 of MoBre2 is required for recognition with MoSdc1. Furthermore, a western
198 blot analysis showed that MoSdc1 can interact with MoBre2₄₀₁₋₆₁₃ (Fig. 2F, G). Taken
199 together, these results clearly indicate that a 212-residue SPRY-C-terminal domain of
200 MoBre2 (residues 401-613) is required for interactions with MoSdc1 and that the
201 conserved DPY30-C-terminal regions of MoSdc1 are required for the binding activity

202 with MoBre2 (Fig. 2D, E). However, direct interactions between MoSwd1₁₋₃₇₅,
203 MoSwd1₁₅₀₋₃₇₅, MoSwd1₃₀₀₋₄₆₈ and MoBre2 was not detected in pulldowns of these
204 protein segments (Supporting Information Fig. S4D-F), suggesting that MoBre2
205 cannot interact with MoSwd1 directly *in vitro*.

206 To further explore whether MoBre2 can interact with MoSdc1 *in vivo*, the entire
207 coding regions of MoBre2 and MoSdc1 were fused with green fluorescent protein and
208 red fluorescent protein, respectively. These two constructs were co-transformed into
209 the wild-type strain P131. As shown in Fig. 3, the GFP-MoBre2 was co-localized with
210 the RFP-MoSdc1, clearly showing that MoBre2 can interact with MoSdc1 *in vivo* (Fig.
211 3). Moreover, these two proteins were localized to the nucleus in the mycelium (Fig.
212 3A-D), conidia (Fig. 3E-H), appressorium (Fig. 3I-L) and infectious hyphae (Fig.
213 3M-P). Taken together, these results indicate that MoBre2 can directly interact with
214 MoSdc1 *in vitro* and *in vivo*.

215

216 **MoBre2, MoSwd2 or MoSpp1 are critical for fungal development and invasive** 217 **hyphae formation during pathogenesis**

218 Next, we used a gene replacement approach to generate null mutants of *M. oryzae*
219 COMPASS-like complex genes. We successfully obtained three null mutant strains
220 for the *MoBre2*, *MoSpp1* and *MoSwd2* genes (Supporting Information Fig. S5). These
221 deletion mutants were confirmed by Southern blots (Supporting Information Fig. S5B,
222 E, I) and PCR analysis (Supporting Information Fig. S5C, F, J). We found that these
223 three gene deletion mutants had defects in colony growth on OTA plates (Fig. 4A).
224 Although the $\Delta mobre2$ deletion mutant displayed a reduction in colony growth, severe
225 defects were apparent during conidium formation (Fig. 4A, C, D). The $\Delta mobre2$
226 mutant produced approximately 30% of the conidia and $\Delta moswd2$ produced
227 approximately 10% of the conidia compared with the wild-type strain (Fig. 4C). By
228 contrast, the $\Delta mospp1$ null mutants showed a small reduction in colony growth
229 without conidium formation defects (Fig. 4C).

230 To evaluate the virulence of $\Delta mobre2$, $\Delta moswd2$ and $\Delta mospp1$ null mutants,

conidia suspensions of these three null mutants and from wild-type P131 were inoculated onto the surface of barley. These three mutants showed severe defects in their invasive hyphae formation at 36 h after inoculation (Fig. 4B). At 36 h after inoculation, wild-type P131 formed bulbous infection hyphae and could extend to neighbouring host cells on the barley epidermis according to our microscopic observation (Fig. 4B, E). The infection ratio is 68%, 59% or 58.1% in the $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ deletion mutants, respectively, compared with wild-type strains (Fig. 4E). However, at 5 d after conidial suspension inoculation onto the seedlings of rice cultivar LTH, the typical robust lesions of rice blast were observed on all three null mutants and wild-type P131 (Supporting Information Fig. S6A, B), which suggests that MoBre2, MoSwd2 and MoSpp1 are required for fungal-host interaction in the early stages of pathogenesis. Taken together, these results indicate that MoBre2, MoSwd2 and MoSpp1 are required for in infection during invasive hyphae formation of the pathogen during fungal development in the early stages of pathogenesis .

MoBre2, MoSwd2 and MoSpp1 fine-regulate H3K4me3 distribution near TSS target regions

Considering that the COMPASS complex is highly conserved in its H3K4me3 methylation function and activates its target genes expression, we firstly examined the global levels of H3K4me3 in $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ gene deletion mutants. A western blot analysis showed that these three null mutants exhibited a robust H3K4me3 decrease throughout the entire genome (Fig. 5, panel A). To determine H3K4me3's chromatin distribution pattern at the genome-wide level, we performed ChIP-seq (chromatin IP-sequencing) experiments using antibodies against against H3K4me3 in the strains that were devoid of *MoBre2*, *MoSpp1* and *MoSwd2* genes.

In total, we identified 519, 1205, and 727 H3K4me3-decreased peaks in the $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ deletion mutants, respectively, compared with wild-type strains (Fig. 5B; Supporting Information Table S4). Remarkably, close to

60% (316) of the H3K4me3 decreased peaks in the *Δmobre2* deletion mutant overlapped with the *Δmospp1* deletion mutant and close to 59% (306) of the H3K4me3 decreased peaks in the *Δmobre2* deletion mutant overlapped with the *Δmoswd2* deletion mutant (Fig. 5B). Such striking similarity at the genome-wide level strongly suggests a functional connection among the MoBre2, MoSpp1 and MoSwd2.

Significantly, when we sorted all the genes according to their H3K4me3-ChIP-seq reads, we found that the peak distribution was in the TSS regions (-3000 bp to +3000 bp) (Fig. 5C-D). Furthermore, the *Δmobre2*, *Δmospp1* and *Δmoswd2* deletion mutants displayed a highly similar profile in the TSS regions (Fig. 5C-D). The signals of enriched H3K4me3-ChIP-seq reads in the TSS region were largely decreased in deletion mutants compared to the wild-type strain (Fig. 5C). In the *Δmobre2* mutant, the H3K4me3 signal was reduced 3.5 compared to the wild-type strain (Fig. 5C, left panel), while in the *Δmospp1* and *Δmoswd2* mutants, the signal displayed 2.6- and 2.2-fold changes respectively (Fig. 5C). These results are further corroborated with decreased intensity of peak signals in heatmaps comparing the knockout mutants with wild-type (Fig. 5D).

We observed that MoBre2, MoSwd2 and MoSpp1 are required for conidiation or infection defects during pathogenesis stage (Fig. 4), and thus we reasoned that MoBre2, MoSwd2 and MoSpp1 might specifically target to fungal development genes. We then performed the comparisons for enriched biological processes in the deletion mutants of *Δmobre2*, *Δmospp1* and *Δmoswd2* versus the wild-type strain which showed that many among their target genes are especially involved in mycelium development ((Fig. 5B ; Table 2 ; Supporting Information Fig. S7). In these hypothetical genes, we identified 69/519, 128/1205, 103/727 genes involved in mycelium development and pathogenesis in the *MoBre2*, *MoSpp1* and *MoSwd2* gene deletion mutants, respectively (Supporting Information Table. S5). Among them, there are 64 candidate genes most likely involved in mycelium development were shared by these three deletion mutants (Supporting Information Table S6). Moreover, we selected seven candidate genes from the 181 overlapping genes: *MoGln1*

(MGG_06888), *MoAbc7* (MGG_04855), *MoAgs1*(MGG_09639),
XDHI(MGG_01176), *MoPex1*(MGG_09299), *FZC55*(MGG_11764) and
CONx7(MGG_01127), all seven are involved in spore germination, fungal growth, or
pathogenesis (Fig. 5B ; Supporting Information Table S6). *MoABC7* has been
previously revealed to be involved in virulence, conidiation and abiotic stress
tolerance, and *MoPEX1* has been shown to be involved in infection-related
morphogenesis and pathogenicity. And were previously demonstrated to be involved
in mycelium development (Kim *et al.*, 2013a; 2013b; Deng et al., 2016; Kou et al.,
2016). We also selected three overlapped target genes, MGG_04682, MGG_14719
and MGG_02775, and confirmed that the H3K4me3 coverages are significantly
decreased in $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ deletion mutants (Fig. 5, panel D).
Such a striking similarity of phenotypes among the $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$
knockouts at the genome level strongly suggests a functional connection among
MoBre2, *MoSpp1* and *MoSwd2*.

Decreased target gene expression caused by deletion of *MoBre2*, *MoSwd2* or *MoSpp1* highly correlates with decreased H3K4me3

We further investigated whether the deletion of *MoBre2*, *MoSwd2* or *MoSpp1* would
changes in gene expression. We then performed RNA-seq in both wild-type strains
and each deletion mutant. Notably, the deletion of *MoBre2* showed relatively
moderate transcriptional changes, while the deletion of *MoSpp1* or *MoSwd2* caused
more obvious and consistent alterations with the thresholds above 2-fold changes
(deletion mutants vs. wild-types) and an False Discovery Ratio (FDR) less than 0.1,
including about 42.47% and 55.83% consistently up- and down-regulated genes in
 $\Delta mospp1$ or $\Delta moswd2$ versus wild-type (Fig. 6A; Supporting Information Table S7).

To investigate which biological processes are affected by the *MoBre2*, *MoSpp1* and
MoSwd2 genes, we further performed a gene ontology analysis for the downregulated
genes in each knockout strain (Fig. 6B-D; Supporting Information Table S7). Some

selected candidate genes such as *PTH11* (MGG_05871), *MoVelC* (MGG_14719) and MGG_03593 are all involved in spore germination and pathogenesis (Supporting Information Table S11; Kim *et al.*, 2013a; 2013b; Deng *et al.*, 2016; Kou *et al.*, 2016). These genes affected in $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ were more likely to be involved in developmental processes such as spore germination, transcriptional regulation (transcription factor activities) and pathogenesis (Fig. 6B; Supporting Information Table S8). More importantly, the genes showing decreased expression in the *MoSpp1* or *MoSwd2* deletion mutants are also involved in growth or development of symbionts on or near host, multi-organism processes or other aspects of pathogenesis (Fig. 6B-D). The functions of these selected genes are clearly consistent with the phenotypes we have observed in $\Delta mobre2$, $\Delta mospp1$ or $\Delta moswd2$ deletion mutants, that have defects in colony growth, infection, and other related aspects of early pathogenesis (Fig. 4B).

We detailed gene expression patterns of three genes, MGG_03593, *MoVelC* (MGG_14719) and MGG_09875. The read coverage of these genes indicated a significant drop in expression compared with the wild-type strain (Fig. 6E-G). Furthermore, qRT-PCR experiments for these genes selected indicate that expression in the wild-type was higher than in the knockouts (Fig. 6H-J).

To further observe the relationship between gene expression changes and H3K4me3 changes, we overlapped the downregulated genes in $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ deletion mutants. For each comparison between the deletion mutant and the wild-type (Fig. 7A), we observed that the downregulated genes were likely to have decreased the H3K4me3 peaks, which suggests that the downregulated genes that are overlapped targets of the MoBre2, MoSpp1 and MoSwd2. In $\Delta mobre2$ deletion mutants, there are 8 (3.5%) and 12 (5.3%) genes are overlapped with the $\Delta mospp1$ and $\Delta moswd2$ deletion mutants, respectively (Fig. 7, panel A). Notably, five overlapped genes that had decreased expression, MGG_02961, MGG_02775, MGG_00184, *MoVelC* (MGG_14719) and MGG_06148, followed the same trend in each of the $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ knockout lines (Fig. 7A, B; Supporting

Information Table S9). We identified expression levels through RNA-seq for those genes with decreased H3K4me3 peaks and found that these genes are also consistently down-regulated in the knockout strains (Fig. 7B). We further confirmed that a gene, we obtained (Fig. 7A), with reduced gene expression in RNA-seq had a similar H3K4me3 reduction pattern in ChIP-seq (Fig. 7A, C). For MGG_02775, *MoVelC* (MGG_14719) and MGG_04682, the read coverage of these genes indicated a significant drop both in gene expression and H3K4me3 occupancy (Fig. 7D, E). Significantly, the majority of H3K4me3-decreased peak target genes had significant expression changes, which correlates with the well-known effects of histone modification on gene expression.

Together, these results suggest that the MoBre2, MoSpp1 and MoSwd2 may assemble into a functional COMPASS complex and mediate H3K4me3-regulated gene expression.

DISCUSSION

Highly conserved roles of COMPASS-Like H3K4 methyltransferase complexes in *M. oryzae*

This study is the first to uncover the molecular mechanism of the COMPASS-like complex in a plant pathogenic fungus providing new insight into the epigenetic regulatory mechanism of target gene expression during fungal development and infection-related pathogenesis.

In fungi, *F. graminearum* has been found to contain four COMPASS-like genes (Jiang et al., 2011; Mohan et al., 2011; Kanda et al., 2013; Liu et al., 2015). Our biochemical studies have demonstrated that the *M. oryzae* complexes are very similar to their mammalian counterparts in terms of subunit composition. We found that all the COMPASS family members are also highly conserved in *M. oryzae*. There are several common subunits, namely MoBre2, MoSwd1, MoSdc1, MoSet1, MoSpp1 and MoSwd2, that are homologues of Cps60, Cps50, Cps25, ySet1, Cps40 and Cps35,

374 respectively (Fig. 1; Table 1). A study showed that MoSet1, a component of
375 COMPASS, is required for global gene expression during infection-related
376 morphogenesis, which is consistent with the findings presented here (Pham et al.,
377 2015). However, we were unable to identify the homologues of yeast Cps30/Wdr5 in
378 *M. oryzae*. Moreover, we did not purify any Cps30-like protein from the
379 immunoprecipitation assay. Further study is required to determine whether there are
380 some Cps30-like proteins in the *M. oryzae* genome.

381 Previously published crystal structures of COMPASS or COMPASS-like in yeast
382 and humans revealed that a Y-shaped architecture with Cps50 and Cps30 localizes on
383 the top of two adjacent lobes and Cps60-Cps25, forming the base at the bottom
384 (Takahashi et al., 2011; Zhang et al., 2014). In *M. oryzae*, our results also showed that
385 MoBre2/Cps60 can interact directly with MoSdc1 *in vitro*, but not with MoSwd2.
386 Although we did not find that MoSwd1 can interact with MoBre2 in a pull down
387 assay, we did find that Flag-MoBre2 can be co-purified with MoSwd1 in an
388 immunoprecipitation assay *in vivo* (Supporting Information Table S4), which suggests
389 that MoSwd1 does indeed function in the COMPASS complex of *M. oryzae*.

390 Interestingly, we also found that our Flag-MoBre2 preparations contained
391 significant MoJmjd2 enrichment (Fig. 1; Supporting Information Table S5). A BLAST
392 protein search revealed a significant similarity between MoJmjd2 and UTX/UTY.
393 Recently, some studies have showed that histone demethylases, such as KdmA, an
394 H3K9/36me3 demethylase, play a role in transcriptional regulation in *A. nidulans*
395 (Agger et al., 2007 & 2019; Matthews et al., 2015). This finding suggests that these
396 UTX-like containing COMPASS-like complexes and their associated bivalent
397 H3K9/27 demethylase and H3K4me3 methyltransferase activities may be involved in
398 dynamic target gene activation (Bernstein et al., 2006; Amar et al., 2015; Bachleitner
399 et al., 2019).

400

401 **Contribution by COMPASS-Like complex components provides novel insights**
402 **into the regulation of target genes in TSS regions**

403 In this study, we found that the MoBre2, MoSpp1, and MoSwd2 are essential for
404 colony growth, conidiation, and invasive hyphae formation in *M. oryzae* (Fig. 6A-E).
405 The three gene deletion mutants exhibited pleiotropic defects as described for the
406 $\Delta moset1$ mutants (Pham et al., 2015). According to the ChIP-seq and RNA-seq results,
407 several previously characterized target genes, such as *MoABC7*, *MoPEX1*, *FZC55*,
408 *MoAGS1*, and *GLN1*, were among the COMPASS-like complex-downregulated genes
409 with H3K4me3-decreased peaks. In $\Delta moswd2$ strains, the expression of some target
410 genes involved in conidiation and pathogenesis is decreased, and this finding is
411 consistent with the mutant defects in invasive hyphae formation (Fig. 6B, E;
412 Supporting Information Table S8).

413 More interestingly, we also found downregulated expression of *MgHTF1*
414 (MGG_00184), a gene known to regulate conidial production. The $\Delta MgHTF1$ mutant
415 failed to form conidia and lost pathogenicity (Liu et al., 2010). The expression of
416 *MgCONx2* (MGG_02775), a gene that encodes a zinc finger C2H2-like protein, also
417 decreased. The *MgCONx2* mutant was previously deemed essential for conidiogenesis
418 and pathogenesis in *M. oryzae* (Zhang et al., 2014) (Fig. 7; Supporting Information
419 Table S8). The ChIP-seq results show that *MgHTF1* and *MgCONx2* both have
420 H3K4me3-decreased peaks in the $\Delta moswd2$ strains, which suggests that MoSwd2
421 activates H3K4me3 in the TSS regions to regulate the transcription of target genes
422 (Figs. 6 and 7; Supporting Information Table S4). The expression levels of *MgAnd1*
423 (MGG_02961) and *MgFox2* (MGG_06148) were also significantly downregulated in
424 the $\Delta mospp1$ mutant. *MgAnd1* encodes a cell cortex protein involved in nuclear
425 migration and positioning. Deleting of *MgAnd1* from *M. oryzae* leads to a delay in the
426 penetration of the host plants (Rahman et al., 2014). *MgFox2* encodes a
427 multifunctional enzyme that catalyses the second and third enzymatic reactions of the
428 β -oxidation pathway, and its disruption also leads to a significant reduction in
429 virulence (Patkar et al., 2012).

430 Notably, among the genes affected by the COMPASS-like complex, the gene
431 *MgVelC* (MGG_14719) was reported to be a positive regulator of conidiation and

432 pathogenicity in *M. oryzae* (Kim et al., 2014). The expression of *MgVelC* was
 433 downregulated in both $\Delta mobre2$ and $\Delta moswd2$ gene deletion strains. Furthermore, the
 434 ChIP-seq results also show that *MgVelC* has H3K4me3-negative peaks in both gene
 435 deletion mutants. These results suggest that *MgVelC* is a potential target gene that is
 436 regulated by the COMPASS-like complex. The transcriptional deficiency of these
 437 genes may be the cause of severe defects in terms of colony growth, conidiation, and
 438 pathogenicity in different mutants (Dang et al., 2001; Wang et al., 2012; D'Urso et al.,
 439 2016). Collectively, these findings suggest that the COMPASS-like complex controls
 440 a good number of genes for fungal pathogenicity and development in *M. oryzae*.
 441 However, the details of how these target genes regulate pathogenesis remain
 442 unknown.

443 In conclusion, our biological, biochemical, and proteomic studies identified novel
 444 components of the COMPASS-like complex and elaborated on its contribution to
 445 H3K4me3-mediated target gene expression in *M. oryzae*. This work serves as a
 446 functional resource for studying infection processes in plant pathogenic fungi and
 447 provides a greater breadth of knowledge in epigenetics regarding gene regulatory
 448 mechanisms.

449

450 **EXPERIMENTAL PROCEDURES**

451 **Strains and culture conditions**

452 The *M. oryzae* wild-type strain P131 was used in this study. The wild-type strain and
 453 corresponding transformants were grown on oatmeal tomato agar (OTA) medium and
 454 cultured at 25°C under light conditions. For extraction of genomic DNA, RNA, and
 455 protein, as well as the isolation of protoplasts, fresh mycelia were broken and cultured
 456 in liquid CM media (0.6% yeast extract, 0.3% enzymatic casein hydrolysate, 0.3%
 457 acidic casein hydrolysate, and 1% glucose) and shaken at 150 rpm at 25°C for 36 h.
 458 For measuring colony sizes, mycelial plugs of 5 mm were inoculated into the center
 459 of OTA plates. Colony diameter on each plate was measured for three days. Each
 460 experiment was repeated three times.

461

462 **Conidiation experiments and virulence tests of *M. oryzae***

463 The wild-type, deletion mutant and complementation strains were cultured on OTA
464 plates at 25 °C for four days. Then, 2 mL ddH₂O was added to each plate and mycelia
465 were collected with an inoculation loop and transferred to a new OTA plate and dried.
466 The plates were covered with three layers of gauze and cultured at 25°C for 12–16 h.
467 Two mL of ddH₂O was added to each dish and conidia were scraped with sterile
468 cotton swabs. Conidia suspensions were transferred into a new 50 mL tube and
469 centrifuged for 5 min at a minimum of 5,000 g at 25°C. The supernatant was removed
470 and pellets were resuspended to obtain 2×10^4 conidia per mL in 0.025% (v/v)
471 Tween-20 solution. A blood cell count board was used to count conidia under the
472 optical microscope. For the infection assay, four-week-old seedlings of rice (*Oryza*
473 *sativa* cv LTH) and eight-day-old seedlings of barley (*Hordeum vulgare*) cv. E9 were
474 used for spray infection assays. Plant incubation was performed as described
475 previously (Cao et al., 2016). All inoculation and conidiation experiments were
476 repeated three times independently.

477

478 **Western blot analysis**

479 For all tested strains, total proteins were extracted as described previously (Cao et al.,
480 2016), then separated by 13% denaturing polyacrylamide gel (SDS-PAGE),
481 transferred to an Immobilon-P transfer membrane (Millipore, Billerica, MA, USA)
482 with a Bio-Rad electroblotting apparatus, and evaluated using the antibodies
483 anti-Histone H3 (Abcam, ab1791) and anti-H3K4me3 (Abcam, ab8580), respectively.
484 Samples were incubated with a secondary antibody (Santa Cruz, sc-2313) for
485 chemiluminescent detection.

486

487 **Co-IP and silver staining**

488 Immunoprecipitation experiments were performed using a $3 \times$ *MoBre2-Flag*
489 transgenic strain. About 0.5 g seven-day-old mycelia from the FLAG-MoBre2-
490 expressing strain were harvested and ground in liquid nitrogen. Subsequently, total
491 proteins were extracted with protein extraction buffer (50 mM Tris-HCl [pH=7.5],
492 100 mM NaCl, 5 mM EDTA, 1% Triton-X100, 2 mM PMSF). Then, 15 mL protein
493 extracts were incubated in a 50 µL slurry of anti-FLAG M2 affinity gel (Sigma,

494 A2220) and rotated at 4°C overnight. The beads were washed by 150 mL BC100 (20
495 mM Tris-HCl [pH=8.0], 100 mM NaCl, 1 mM EDTA, 0.1% NP-40)(Wang et al.,
496 2011). Subsequently, target proteins were eluted with the FLAG peptide
497 (DYKDDDDK). The samples were then boiled in the SDS-PAGE loading buffer and
498 separated in an SDS-PAGE gel. Silver staining experiments were performed using the
499 ProteoSilver plus silver stain kit (Sigma, PROT-SIL2). The silver stained bands were
500 analyzed by mass spectrometry.

501

502 **Protein expression in *E. coli* and purification**

503 The recombinant deletion vectors of MoBre2, MoSdc1 and MoSwd2 were
504 transformed into Rosetta (DE3) *E. coli* bacteria (Biomed, BC204-02, China). After
505 sequencing to determine positive clones, the bacteria were incubated at 37°C at 220
506 rpm to OD₆₀₀ = 0.6 with Luria-Bertani (LB) medium, then induced by the addition of
507 1 mM β-D-1-thiogalactopyranoside (IPTG) and incubated at 16°C for 14 h to express
508 GST-tagged fusion proteins. The fusion proteins were purified using
509 Glutathione-Sepharose 4B (GE Healthcare, 17-0756-01) following the manufacturer's
510 instructions.

511

512 **In vitro pull-down assay**

513 The fusion proteins were purified as described previously (Mao et al., 2014). Then,
514 equivalent protein of His-MoBre2_{FL} and GST-MoSdc1_{FL} fusion proteins were added to
515 a 1.5 mL centrifuge tube and the solution was brought to 400 μL by BC100 (20 mM
516 Tris-HCl [pH=8.0], 100 mM NaCl, 1 mM EDTA, 0.1% NP-40). Next, 40 μL
517 Glutathione-Sepharose 4B (GE Healthcare, 17-0756-01) was added to the solution.
518 The tubes were then rotated and incubated for 4 h at 4 °C to pull down GST-tagged
519 fusion proteins. This was followed by four washes with BC500 (20 mM Tris-HCl
520 [pH=8.0], 500 mM NaCl, 1 mM EDTA, 0.1% NP-40). The beads were boiled in SDS
521 loading buffer for 10 min, centrifuged at 3500 rpm at 4 °C for 5 min, and then the
522 supernatant was separated by 13% denaturing polyacrylamide gel (SDS-PAGE).
523 Finally, the gel was stained with Coomassie Brilliant Blue R-250 (AMRESCO,
524 0472-10G) and Western blotting using anti-His (Transgen Biotech, HT501) and
525 anti-GST antibodies (Transgen Biotech, HT601) for observation.

526

527 Subcellular localization analysis

528 For colocalization analysis of MoBre2 and MoSdc1, the full-length of *MoBre2* was
 529 amplified by PCR from wild-type P131 cDNA with primer pairs *MoBre2*-GFP-F and
 530 *MoBre2*-GFP-R. The resulting fragment and pKNTGRP27 vector were digested with
 531 *EcoRI* and *BamHI* and *MoBre2* was cloned into pKNTGRP27, which was generated
 532 by cloning the strong, constitutive RP27 promoter into pKNTG. The full-length of
 533 *MoSdc1* was amplified by PCR from wild-type P131 cDNA with primer pairs
 534 *MoSdc1*-RFP-F and *MoSdc1*-RFP-R. The resulting fragment and pKS vector were
 535 digested with *EcoRI* and *BamHI*, *MoSdc1* was cloned into pKS and the RFP-MoSdc1
 536 fusion construct was generated. The GFP-MoBre2 and RFP-MoSdc1 fusion
 537 constructs were digested with *Not I* and co-transformed into wild-type P131.
 538 Colocalization of GFP-MoBre2 and RFP-MoSdc1 fusion proteins was performed with
 539 a Nikon A1 laser scanning confocal microscope.

540

541 ChIP-seq

542 Protoplasts for ChIP sequencing were prepared as described previously (Cao et al.,
 543 2016). Briefly, the protoplasts were crosslinked with 37% formaldehyde for 10 min,
 544 followed by termination of the crosslinking reaction with 10 × glycine for 5 min.
 545 Protoplasts were collected by centrifugation at 4000 rpm for 15 min, washed with 0.7
 546 M NaCl, and diluted to 1 × 10⁸ /mL. Centrifugation and suspension of protoplasts
 547 occurred with 750 μL RIPA buffer (50 mM Tris-HCl [pH=8.0], 150 mM NaCl, 2 mM
 548 EDTA, 1% NP-40, 0.5% sodium dextranolate, 0.1% SDS). The chromatin was sheared
 549 by sonication with JY 92-IIDN (SCIEN TZJY, China) for 8 min (25% W, output 3S,
 550 Stop 5S). Chromatin immunopurification with anti-H3K4me3 antibody (abcam
 551 ab8580) and Dynabeads Protein A (Invitrogen 10002D) were incubated overnight at
 552 4 °C as previously described (Mao et al., 2014). Subsequently, DNA fragments were
 553 extracted using the phenol-chloroform method for constructing an Illumina
 554 sequencing library and sequenced with single-ends on a Hiseq 2000.

555

556 Mass spectrometry (LC-MS/MS) and data analysis

557 Samples were destained in Proteo Silver Destainer A and Proteo Silver Destainer B,
 558 and then rinsed with pipetted water several times. Proteins were digested with 500 ng
 559 of sequencing-grade modified trypsin (Promega), and the resulting peptides were

resolved on a nano-capillary reverse phase column. Using a water: acetonitrile gradient system, at 300 nL/min, sample was directly introduced into an ion-trap mass spectrometer in the data-dependent mode to collect MS/MS spectra on the five most intense ions from each full MS scan. MS/MS spectra were matched to peptides in a database containing *M. oryzae* protein sequences. All proteins with a Protein Prophet probability score of 0.9 were considered positive identifications.

RNA-seq

Total RNA was isolated from the tissue of wild-type strain P131, Δ *Mobre2*, Δ *mospp1* and Δ *moswd2* deletion mutants, and mRNA was then isolated using a Poly (A) Purist MAG kit (Ambion). Each sample was analysed in technical triplicates, and three experiments were replicated. DNA was removed by RNase-free DNase (Qiagen) followed by column clean-up according to the manufacturer's instructions. Illumina TruSeq RNA Sample Preparation kits were used to construct RNA-seq libraries; cDNA was sequenced on an Illumina HiSeq 2000.

Bioinformatics

ChIP-seq read quality was assessed using FASTQC (0.11.7) and aligned to *M. oryzae* genome assembly (MG8) using Bowtie2 software (2.3.4.3). Only uniquely mapped reads were kept for analysis. H3K4me3 peaks were called by MACS2 (2.1.1.20160309) with read extension to average fragment size and the threshold of $FDR \leq 1E-6$. Based on the MACS2-called peaks and aligned read files, DiffBind (v2.6.6) was used to identify differential binding peaks of H3K4me3 with $FDR < 0.01$ and binding fold change ≥ 2 .

RNA-seq reads were aligned to *M. oryzae* transcriptome annotation (MG8) with the STAR aligner (2.6.0c). Differentially expressed genes and quantification were done using the edgeR package (3.20.9). Plots and charts were drawn with R software and MS Excel.

Sequence alignment and structural modeling

The sequences of the Unknown and JMJD2A protein sequences were aligned in ClustalX2 and then coloured by ESPript 3.0 (<http://esprict.ibcp.fr/ESPript/ESPript/>;

Robert et al., 2014). Using the online phyre2 modelling machine (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index; Kelley et al., 2015), we constructed and selected the complex of the core Tudor domain of Unknown and H3K27me3 with the highest score, based on the template of the JMJD2A Tudor domain–H3K4me3 complex (PDB code: 2006; Ng et al., 2007).

597

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606

607 **Author contributions**

608 W.X.W designed the experiments. S.D.Z., W.Y.S., M.Y.Z., S.P., X.Y.Z., Y.Y., D.H., 609 M.S., J.Y., L.E.C., and Y.X. performed the biological experiments. X.Y.L. and Q.Z. performed the bioinformatics analysis. W.X.W wrote the manuscript.

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Figure legends

Fig. 1. Neighbour-joining tree of candidate COMPASS or COMPASS-like orthologues from *M. oryzae* and other species. Panel A: Co-immunoprecipitation and mass spectrometry analysis of the COMPASS-like complex from *M. oryzae*. Panel B: The boxes contain the peptide sequences identified using tandem mass spectrometry.

Fig. 2. MoBre2 interacts directly with MoSdc1 *in vitro*. Panel A: Domain organization of MoBre2, MoSdc1 and MoSwd1. In MoSwd1, the WD40-repeat domains are shown in red; in MoBre2, the SPRY domains (SPLA and RY anodine receptor domains) are shown in yellow; in MoSdc1, the DPY30 domain is shown in green. Numbers indicate residue numbers at the boundaries of various subdivisions. Protein interactions are indicated with grey areas. Panel B: GST pull-down assay with MoBre2 and MoSdc1. GST-tagged MoSdc1 was incubated with His-tagged MoBre2. The eluates were subjected to SDS-PAGE. Panel C-D: Full-length MoBre2 interacts with the C-terminal region of MoSdc1 (residues 101–200) but not with the N-terminal region of MoSdc1 (residues 1–100). Panel E: The fragments of MoSdc1 (residue 101–200) interact with the two deletion mutants (MoBre2₂₀₁₋₆₁₃, MoBre2₄₀₁₋₆₁₃) Panel F-G: Western blot analysis showing that His-tagged MoBre2₂₀₁₋₆₁₃ interacts with GST-tagged full-length MoSdc1.

Fig. 3. Dual-colour imaging of GFP-MoBre2 and RFP-MoSdc1 in *M. oryzae* by confocal laser scanning microscopy. The GFP-MoBre2 and RFP-MoSdc1 fusion constructs were transformed into the protoplasts of wild-type P131. The GFP-MoBre2 and RFP-MoSdc1 signals were merged. HY, vegetative hyphae; CO, conidia; AP, appressoria; IH, infection hypha. DIC, differential interference contrast. Scale bars = 20 μ m.

Fig. 4. Deletion of *MoBre2*, *MoSpp1* and *MoSwd2* results in defects in hyphal growth,

887 and pathogenic infection. Panel A: Five-day-old cultures of the P131, $\Delta mobre2$,
888 $\Delta mospp1$ and $\Delta moswd2$ strains grown on oatmeal tomato agar (OTA) medium. Panel
889 B: Barley leaves were sprayed with conidial suspensions (1×10^5 spores/mL) of
890 wild-type P131, the $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ deletion mutants 36 h after
891 inoculation. Scale bars = 20 μ m. Panel C-E: Microscopic observation of conidium
892 formation in the wild-type strain P131 and the $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$
893 deletion mutants. Means and SDs were calculated from three independent
894 experiments. *, $P < 0.01$, $n > 100$.

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896 **Fig. 5.** The *MoBre2*, *MoSwd2* and *MoSpp1* deletion mutants display significantly
897 decreased H3K4me3 profiles in TSS target regions. Panel A: Western blot analysis of
898 histone H3K4me3 modifications in the wild-type P131 and $\Delta mobre2$, $\Delta mospp1$ and
899 $\Delta moswd2$ strains. Panel B: The number in the Venn diagram shows the H3K4me3
900 decreased peaks in three deletion mutants, compared with the wild-type. Panel C: The
901 H3K4me3-ChIP-seq distribution of enriched peaks around the TSS of the target genes
902 in *MoBre2*, *MoSwd2* and *MoSpp1* deletion mutants. Panel D: The heatmaps show the
903 distribution of H3K4me3-ChIP-seq peak signal density in different samples. Panel E:
904 The read coverage shows that the peaks in these three deletion mutants are significantly
905 decreased compared with the wild-type strain in selected sample genes.

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907 **Fig. 6.** The *MoBre2*, *MoSwd2* or *MoSpp1* gene deletion mutants exhibit gene
908 expression decrease. Panel A: The heat map shows all the differentially expressed
909 genes in the comparison between the wild-type and the three deletion mutants. Panels
910 B-D: The differentially expressed genes are shown in volcano plots, the x-axis shows
911 the fold-changes transferred by $\log_2$, and the y-axis shows the False Discovery Ratio
912 (FDR) transferred by minus $\log_{10}$. Panels E-G: The read coverage shows that the
913 expression signals of selected targets in these three deletion mutants are significantly
914 decreased compared with the wild-type strain in some selected genes involved in
915 spore germination. Panels E-G: qRT-PCR experiment for spore germination genes
916 show the expression in WT decreased compared with knockouts.

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918 **Fig. 7.** Decreased gene expression in the *MoBre2*, *MoSwd2* or *MoSpp1* gene deletion
919 mutants is highly correlated with decreased H3K4me3. Panel A: Venn diagrams show

changes in down-regulated gene expression in knockouts compared to the wild-type strains. The numbers of down regulated genes are indicated. Panel B: Heat map of differentially expressed genes between knockouts and the wild-type strains. Panels C-E: Examples of genomic tracks showing genes with decreased H3K4me3-ChIP-seq signal levels and downregulated RNA-seq reads. Panels F-H: qRT-PCR experiment for selected genes show the expression in WT decreased compared with deletion mutants. Identified genes show decreased signals and the expression level was reduced in deletion mutants.

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941 **Table 1. Subunit composition of COMPASS-like family from yeast to human.**

Species		Conserved subunits of COMPASS-like Complexes				
<i>Saccharomyces cerevisiae</i>	ySet1	Cps60 (Bre2)	cps40 (Spp1)	cps35 (Swd2)	cps25 (Sdc1)	cps50 (Swd1)
<i>Drosophila</i>	SetD1A/T rithorax(tr x)/Trithor ax-related (Trr)	Ash2	dCxxC1	dWdr82	dDpy30	dRbbp5
<i>Mammalian</i>	Set1A/Set 1B/MLL1 /MLL2/M LL3/MLL	ASH2	CxxC1	Wdr82	hDPY30	RbBP5

<i>Fusarium graminearum</i>	FgSet1 (FGSG_0 7445)	FgBre2 (FGSG_ 09564)	FgSpp1 (FGSG_ 09543)	FgSwd 2(FGSG_ 05413)	FgSdc1 (FGSG_0 7407)	——
<i>Magnaporthe oryzae</i>	MoSet1(MGG_01 5053)	MoBre2(MGG_01 427)	MoSpp1 (MGG_0 3005)	MoSwd2(MGG_06 406)	MoSdc1(MGG_00 350)	MoSwd1(MGG_046 51)
Gene locus	$\Delta mobre2$	$\Delta mospp1$	$\Delta moswd2$	Protein	Function	

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956 **Table 2. List of the some downstream target genes involved in conidiation,**
957 **mycelium development, or pathogenesis in fungal development and infection**
958 **in the $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ deletion mutants.**

MGG_06888 (<i>MoGln1</i>)	√	-	-	A glutamine synthetase-enc oding orthologue	Appressorium formation; Mycel ium development
MGG_04855 (<i>MoAbc7</i>)	√	√	-	ABC transporters	Conidiation
MGG_09639 (<i>MoAgs1</i>)	√	-	√	The a-1,3-glucan synthase gene	Infection
MGG_01176(<i>XDH</i> <i>I</i>)	√	√	√	Xylitol dehydrogenase	Mycelium growth
MGG_09299 (<i>MoPex1</i>)	√	√	-	Encodes a putative homologue to peroxin 1	Infection
MGG_11764 (<i>FZC55</i>)	√	-	-	Zn2Cys6 Transcription factors	Appressorium formation
MGG_01127(<i>CON</i> <i>x7</i>)	√	-	√	The Cys2-His2 (C2H2) zinc finger protein	Conidial differentiation
MGG_05871(<i>PTH1</i> <i>I</i>)	√	√	√	G-protein-coup led receptor	Appressoria-like structures; Infection
MGG_14719 (<i>MoVelC</i>)	√	√	√	The velvet gene	Pathogenesis; Spore germination
MGG_03593	√	√	√	Unknown	Hypothetical protein
MGG_09875	√	√	√	Unknown	Hypothetical protein
MGG_00450	√	-	√	Unknown	Pathogenesis
MGG_11261	√	√	-	Unknown	Pathogenesis

MGG_00501(<i>TDG2</i>)	√	-	-	Unknown	Pathogenesis
MGG_03451(<i>GCF6</i>)	√	√	√	Unknown	Pathogenesis;Sp orulation
MGG_07833; MGG_10570; MGG_07975	√	-	√	Unknown	Mycelium development
MGG_09640; MGG_08710; MGG_04206	√	-	-	Unknown	Mycelium development
MGG_12574	√	√	-	Unknown	Mycelium development
MGG_04676	-	√	-	Unknown	Spore germination;Path ogenesis
MGG_04495; MGG_07259	-	√	-	Unknown	Spore germination
MGG_11236; MGG_04527	-	√	-	Unknown	Fungal-type vacuole
MGG_12462 (<i>MoLPP5</i>)	-	√	-	Unknown	Fungal-type vacuole
MGG_07478	√	√	√	Unknown	Fungal-type vacuole
MGG_00993	√	√	-	Unknown	Fungal-type vacuole
MGG_00450	√		√	Unknown	Sexual reproduction;Spo rulation
MGG_07535; MGG_01818(<i>magA</i>) ; MGG_02240	-	-	√	Unknown	Sexual reproduction
MGG_07535; MGG_09499	-	-	√	Unknown	Sporulation

MGG_00450	√	-	√	Unknown	Sporulation
MGG_07930	√	-	√	Unknown	Developmental process
MGG_02961	√	√	√	Unknown	hypothetical protein
MGG_02775	√	√	√	Unknown	hypothetical protein
MGG_00184	√	√	√	Unknown	Regulation of biosynthetic process
MGG_06148	√	√	√	Unknown	hypothetical protein
MGG_12574; MGG_04855; MGG_13691	√	√	-	Unknown	Mycelium development
MGG_09548; MGG_04689; MGG_00990; MGG_05631; MGG_03039	-	√	√	Unknown	Mycelium development
MGG_02835; MGG_03929; MGG_04966; MGG_01782; MGG_10681; MGG_00341; MGG_12944	-	√	-	Unknown	Mycelium development

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961 Supporting Information

962 **Fig. S1.** Identification of the H3K4 methyltransferase MoSet1, MoBre2, MoSdc1 and
963 MoSpp1 in *M. oryzae*. Panel A. Schematic representation of MoSet1. Each functional
964 domain is highlighted differently. RRM, RNA recognition motif, N-SET, N-Set

965 domain, SET, SET domain. Panel B. The MoSet1 SET domain is evolutionarily
 966 conserved. Sequence alignment of the SET domains from mammals (Set1A),
 967 *Saccharomyces cerevisiae* (ySet1) and *M. oryzae* (MoSet1). Positions with 100% and
 968 99-75% amino acid conservation are represented in black and blue, respectively.
 969 Panel C. Alignment of MoBre2 from *M. grisea*, yBre2 from *S. cerevisiae* and ASH2L
 970 from mammals. Numbers refer to amino acid residues. Identical residues among these
 971 proteins are shaded in black, whereas similar residues are shaded in grey. Panel D.
 972 Alignment of the conserved domains of MoSdc1 from *M. grisea*, ySdc1 from *S.*
 973 *cerevisiae* and DPY30 from mammals. Panel E. Alignment of the conserved domains
 974 of MoSpp1 from *M. oryzae*, Cps40 from *S. cerevisiae* and DPY30 from mammals.

975 **Fig. S2.** Identification of 3 × MoBre2-Flag mutant strains by western blotting.
 976 Immunoblotting with anti-FLAG antibody. Lanes 1 to 5 are five transformants, and
 977 lane 6 is a positive control.

978 **Fig. S3.** Modelled structure of the core tudor domain of MoJmjd2 in complex with
 979 histone H3K9me3. Shown are side view (A), the key residues caging the
 980 trimethylated H3K27 (C); and top view (B) a modelled MoJmjd2 (cyan)- H3K27me3
 981 (gold) complex.

982 **Fig. S4.** GST-tag-based pull-down assay of MoBre2 and MoSwd1. Panel A.
 983 Full-length MoBre2 cannot interact with the N-terminal region of MoSdc1 (residues
 984 1-100). Panel B. The fragments of MoSdc1 (residues 101-200) cannot interact with
 985 MoBre2₁₋₂₀₀. Panel C. GST-based pull-down assay with MoBre2 and MoSwd1.
 986 GST-tagged MoSwd1 was incubated with His-tagged MoBre2. The eluate was
 987 subjected to SDS-PAGE. Panels D-F. Full-length MoBre2 interacts with the three
 988 deletion mutants of MoSwd1₁₋₃₇₅ (residues 1-375), MoSwd1₁₅₀₋₃₇₅ (residues 150-375)
 989 and MoSwd1₃₇₅₋₄₆₈ (residues 375-468).

990 **Fig. S5.** Schematic diagram of the deletion strategies for *MoBre2*, *MoSpp1* and
 991 *MoSwd2*. Panel A. Schematic diagram of the *MoBre2* deletion strategy. P, Sp, E, Sa,
 992 and K indicate the recognition sites of *Pst* I, *Spe* I, *Eco* RI, *Sal* I, and *Kpn* I,

993 respectively. HYG, hygromycin phosphotransferase gene. P1 to P4 are the PCR
994 primers *MoBre2LF*, *MoBre2LR*, *MoBre2RF*, and *MoBre2RR*. Panel B. Southern blot
995 analysis of the *MoBre2* deletion mutants. *Pst* I-digested genomic DNA was hybridized
996 with the probe (marked in A). Lane M, *HindIII*-digested fragments of λ DNA as
997 markers; lane 1, wild-type strain P131; lanes 2 to 4, *MoBre2* deletion mutants
998 Δ *mobre2KO1*, Δ *mobre2KO2*, and Δ *mobre2KO3*. C. The Δ *mobre2KO2* deletion
999 mutant was verified with the primer pairs *MoBre2F*/*HYBLBCK* and
1000 *MoBre2R*/*HYBRBCK*. Lane M, marker; lane 1, Δ *mobre2KO2* amplification product
1001 with *MoBre2F*/*HYBLBCK*; lane 2, Δ *mobre2KO2* amplification product with
1002 *MoBre2R*/*HYBRBCK*; lanes 3 and 4 are repeats of lanes 1 and 2. Panel D. Schematic
1003 diagram of the *MoSpp1* deletion strategy. EV, S, P, C, and K indicate the recognition
1004 sites of *EcoRV*, *SpeI*, *PstI*, *Cla* I, and *Kpn* I, respectively. HYG, hygromycin
1005 phosphotransferase gene. P1 to P4 are the PCR primers *MoSpp1LF*, *MoSpp1LR*,
1006 *MoSpp1RF*, and *MoSpp1RR*. Panel E. Southern blot analysis of the Δ *mospp1* deletion
1007 mutants. *EcoRV*-digested genomic DNA was hybridized with the probe (marked in D).
1008 Lane M, *HindIII*-digested fragments of λ DNA as markers; lanes 1 to 3, *MoSpp1*
1009 deletion mutants Δ *mospp1KO3*, Δ *mospp1KO2*, and Δ *mospp1KO1*; lane 4, wild-type
1010 strain P131. Panel F. The Δ *mospp1KO1* and Δ *mospp1KO3* deletion mutants were
1011 verified by PCR with the primer pairs *MoSpp1F*/*HYBLBCK* and
1012 *MoSpp1R*/*HYBRBCK*. Lane M, marker; lane 1, Δ *mospp1KO1* amplification product
1013 with *MoSpp1F*/*HYBLBCK*; lane 2, Δ *mospp1KO1* amplification product with
1014 *MoSpp1R*/*HYBRBCK*; lane 3, Δ *mospp1KO3* amplification product with
1015 *MoSpp1F*/*HYBLBCK*; lane 4, Δ *mospp1KO3* amplification product with
1016 *MoSpp1R*/*HYBRBCK*. Panel G. Schematic diagram of the Δ *moswd2* deletion strategy.
1017 A, B, P, C, and K indicate the recognition sites of *Apa* I, *Bam* HI, *Pst* I, *Cla* I, and
1018 *Kpn* I, respectively. HYG, hygromycin phosphotransferase gene. P1 to P4 are the PCR
1019 primers *MoSwd2LF*, *MoSwd2LR*, *MoSwd2RF*, and *MoSwd2RR*. Panel H. Southern
1020 blot analysis of the *MoSwd2* deletion mutants. *Apa* I-digested genomic DNA was
1021 hybridized with the probe (marked in G). Lane M, *Hind* III-digested fragments of

1022 λ DNA as markers; lane 1, wild-type strain P131; lanes 2 and 3, $\Delta moswd2$ deletion
1023 mutants $\Delta moswd2KO1$ and $\Delta moswd2KO2$. Panel I. The $\Delta moswd2KO2$ deletion
1024 mutant was verified by PCR with the primer pairs *MoSwd2F*/*HYBLBCK* and
1025 *MoSwd2R*/*HYBRBCK*. Lane M, marker; lane 1, $\Delta moswd2KO2$ amplification product
1026 with *MoSwd2F*/*HYBLBCK*; lane 2, $\Delta moswd2KO2$ amplification product with
1027 *MoSwd2R*/*HYBRBCK*.

1028 **Fig. S6.** Inoculation test of the $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$ deletion mutants.
1029 Rice leaves were sprayed with conidial suspensions (1×10^5 spores/mL) of the
1030 wild-type P131, or of the deletion mutants $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$. The
1031 inoculated leaves were photographed 5 d post inoculation.

1032 **Fig. S7.** GO term analysis of the candidate genes involved in mycelium development
1033 with H3K4me3-ChIP-seq peaks decreased in $\Delta mobre2$, $\Delta mospp1$ and $\Delta moswd2$
1034 mutants. The value shown on the table represents the frequency of the GO term in the
1035 Gene Ontology annotation database. The colour represents the enrichment p-value
1036 (Fisher's exact test) of each GO term. The scaled relative color are shown in the
1037 heatmap.

1038 **Table S1.** Names and sequences of the 29 COMPASS-like proteins from
1039 *Magnaporthe oryzae*, *Saccharomyces cerevisiae*, *Fusarium graminearum*,
1040 *Arabidopsis*, *Drosophila*, and mammals shown in Figure 1.

1041 **Table S2.** Tandem mass spectrometry analysis to identify five subunits of
1042 COMPASS-like proteins.

1043 **Table S3.** Primers used in this study.

1044 **Table S4.** List of the hypothetical genes with decreased H3K4me3-ChIP-seq peaks
1045 compared with the wild type strain in the $\Delta mobre2$, $\Delta mospp1$ or $\Delta moswd2$ mutants.

1046 **Table S5.** Gene Ontology analysis of the hypothetical genes with decreased
1047 H3K4me3-ChIP-seq peaks compared with the wild type strain in the $\Delta mobre2$,
1048 $\Delta mospp1$ or $\Delta moswd2$ mutants.

1049 **Table S6.** Gene Ontology analysis of the pathogenesis-related genes with decreased
1050 H3K4me3-ChIP-seq peaks in the $\Delta mobre2$, $\Delta mospp1$ or $\Delta moswd2$ mutants.

1051 **Table S7.** List of the hypothetical genes decreased in H3K4me3-ChIP-seq levels
1052 overlapped with downregulated expression genes in RNA-seq in the $\Delta mobre2$,
1053 $\Delta mospp1$ or $\Delta moswd2$ mutants.

1054 **Table S8.** GO term analysis of candidate genes involved in spore germination with
1055 decreased in both H3K4me3-ChIP-seq signals and RNA-seq signals in the $\Delta mobre2$,
1056 $\Delta mospp1$ or $\Delta moswd2$ mutants.

1057 **Table S9.** List of genes decreased in H3K4me3-ChIP-seq levels and downregulated
1058 expression in RNA-seq in the $\Delta mobre2$, $\Delta mospp1$ or $\Delta moswd2$ mutants.

1059

[illegible]

Control

A

B

C

D

E

F

G

A

B

WT P131

 $\Delta mobre2$ $\Delta mospp1$ $\Delta moswd2$

C

D

E

A

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B

C

F

D

G

E

H

A

B

MoSET1_(M.grisea).seq	SDALRENLKRRKKPVKERSIHNWGLYAMEHFPKDDMI	1127-1167
ySet1_(S.cerevisiae).seq	SELIISLNQINRRKKPVKERSIHNWGLYALDSIAAKEMI	924-964
Set1A(Mammalian).seq	SDLLKLNQLKRRKKLRFGRSRIHEWGLFAMEFIADDMV	1558-1598
Consensus	s nql rkk f rs ih wgl a i m	
MoSET1_(M.grisea).seq	IEYVGEEVRFPSVAQVRERARYDRSGIGSSYLFRVDEDVID	1168-1208
ySet1_(S.cerevisiae).seq	IEYVGERIRQEVAEERKRYLKNIGIGSSYLFRVIDENTVID	965-1005
Set1A(Mammalian).seq	IEYVGQNIQQLVADMRERRYVQEGIGSSYLFRVLDHTIID	1599-1639
Consensus	ieyvg r va re ry gigssylfr d id	
MoSET1_(M.grisea).seq	ATKKGGLARFINHSCMPNCTAKIIRVEGTRKRVIIYALRDI	1209-1249
ySet1_(S.cerevisiae).seq	ATKKGGLARFINHCCDPNCTAKLIKVGRRRRVVIYALRDI	1006-1246
Set1A(Mammalian).seq	ATKCGNLARFINHCCPNQYARVITIESQKKRVIIYSKQFI	1640-1680
Consensus	atk g arfinh c pnc ak i iviy i	
MoSET1_(M.grisea).seq	ARNEETIDYDYKEELPEKEEDRVPCLCGTTNCRGFL	1250-1282
ySet1_(S.cerevisiae).seq	AASEETIDYDYKEERPKDDEERIPCLCGAPNCRGFL	1246-1079
Set1A(Mammalian).seq	GVDEETIDYDYKEFLP...DNKIPCLCGTESCRGSL	1681-1710
Consensus	ee tydykf e pclcg c g l	

C

MoBRE2(M.oryzae).seq	AVPNTISTGP...SAPPTVKPMFERYNDQIAEDIVYDIIDE	136
yBRE2_(S.cerevisiae).seq	EYLDQIRSAVCVDGNSKVNTLDTLYKQIAEDIVWDIIDE	160
ASH2L(Mammalian).seq	KYPKDLTY.....RPMSDMGWGAHVHTLAVLYHVETE	127
Consensus	e d e	

D

MoSDC1_(M.grisea).seq	RYVYINIKVITGVLLLEGMKIIAKDCPHDELRLVIGEF	
ySDC1_(S.cerevisiae).seq	RYVYININVTPELLAGMRLIAVQQPEDELRLVIGEF	
DPY30(Mammalian).seq	RAYLDQTVVPEILLQGLAVLAKERFPNIEFLASY	
Consensus	r yl v ll g a p p l	

E

MoSPP1_(M.oryzae).seq	YCLCRGPDHRRMISCEACEDWFHGEQVDVSKDVGETLIQ	30-70
Cps40(S.cerevisiae).seq	YCLCKRPDYGEIMVGDCGDDWFHFTCLHIPEQFKDLVFS	26-66
CFP1(Mammalian).seq	YCLCRKPLINCFMIGDCNCEWFHGDICIRITEKMAKAIRE	197-237
Consensus	yc c pd m c c wfh c	

1**2****3****4****5****6****67 kDa —**

A

B

C

A

P131 $\Delta mobre2$ $\Delta mospp1$ $\Delta moswd2$ CK

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B

P131 $\Delta mobre2$ $\Delta mospp1$ $\Delta moswd2$ CK

GO_Term

Anatomical structure development

Mycelium development

Multicellular organismal development

Regulation of transcription

Regulation of gene expression

Regulation of RNA metabolic process

Regulation of transcription, DNA-dependent

Regulation of macromolecule biosynthetic process

Regulation of biosynthetic process

Regulation of cellular biosynthetic process

MoBre2KO**MoSpp1KO****MoSwd2KO**

2.40E-04

1.30E-01

2.30E-04

4.90E-04

1.70E-01

1.00E-03

6.20E-04

2.00E-01

8.90E-04

2.30E-02

1.10E-03

3.70E-01

2.50E-02

7.80E-04

3.90E-01

2.80E-02

3.30E-02

6.20E-01

2.80E-02

3.30E-02

6.20E-01

2.90E-02

1.10E-03

4.20E-01

3.00E-02

1.10E-03

4.20E-01

3.00E-02

1.10E-03

4.20E-01

GO_Term	<i>P-value</i>		
	MoBre2KO	MoSpp1KO	MoSwd2KO
Spore germination	2.70E-06	4.60E-02	1.80E-01
Developmental process	2.00E-02	8.40E-01	6.70E-01
Growth or development of symbiont on or near host	4.20E-01	9.90E-06	1.70E-05
Multi-organism process	5.70E-01	1.00E-02	5.20E-03
Biological regulation	7.40E-01	1.70E-06	4.00E-06
Regulation of nitrogen compound metabolic process	7.60E-01	2.30E-06	3.70E-07
Regulation of macromolecule biosynthetic process	7.60E-01	2.60E-06	8.80E-07
Regulation of biosynthetic process	7.70E-01	2.90E-06	9.90E-07
Regulation of cellular biosynthetic process	7.70E-01	2.90E-06	9.90E-07
Regulation of macromolecule metabolic process	7.80E-01	4.90E-06	1.70E-06
Regulation of primary metabolic process	7.80E-01	5.50E-06	1.90E-06
Regulation of cellular process	8.00E-01	6.90E-07	3.20E-06
Regulation of cellular metabolic process	8.00E-01	2.50E-06	8.50E-07
Regulation of metabolic process	8.10E-01	3.80E-06	6.30E-07
Regulation of biological process	8.20E-01	1.70E-06	4.00E-06