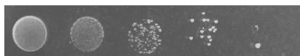
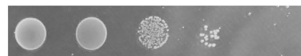
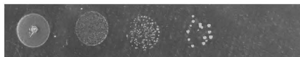
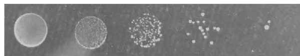
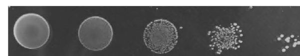
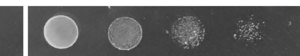
30℃ 37℃

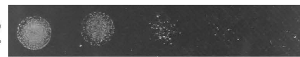
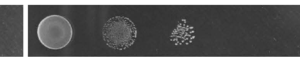
BY4741/pYES2  

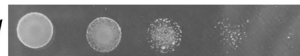
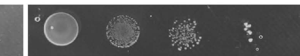
Δ*Scsit4*/pYES2 

Δ*Scsit4*/*MoPPE1* 

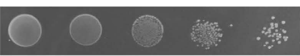
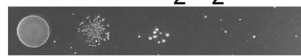
30℃ 37℃

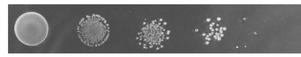
BY4741/pYES2  

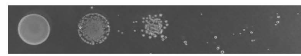
Δ*Scgln3*/pYES2  

Δ*Scgln3*/*MoNUT1*  

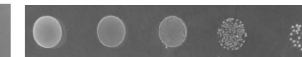
SD SD+H_2O_2

BY4741/pYES2  

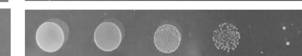
Δ*Scsap4*/pYES2 

Δ*Scsap4/MoSAP1* 

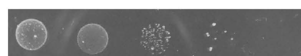
SD SD+ Hygromycin B

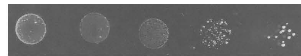
BY4741/pYES2 

Δ*Scsap155*/pYES2

Δ*Scsap155/MoSAP1* 

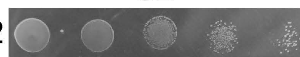
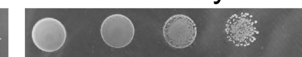
SD SD+ Hygromycin B

BY4741/pYES2 

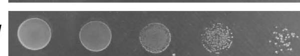
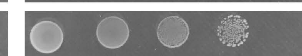
Δ*Scsap185*/pYES2 

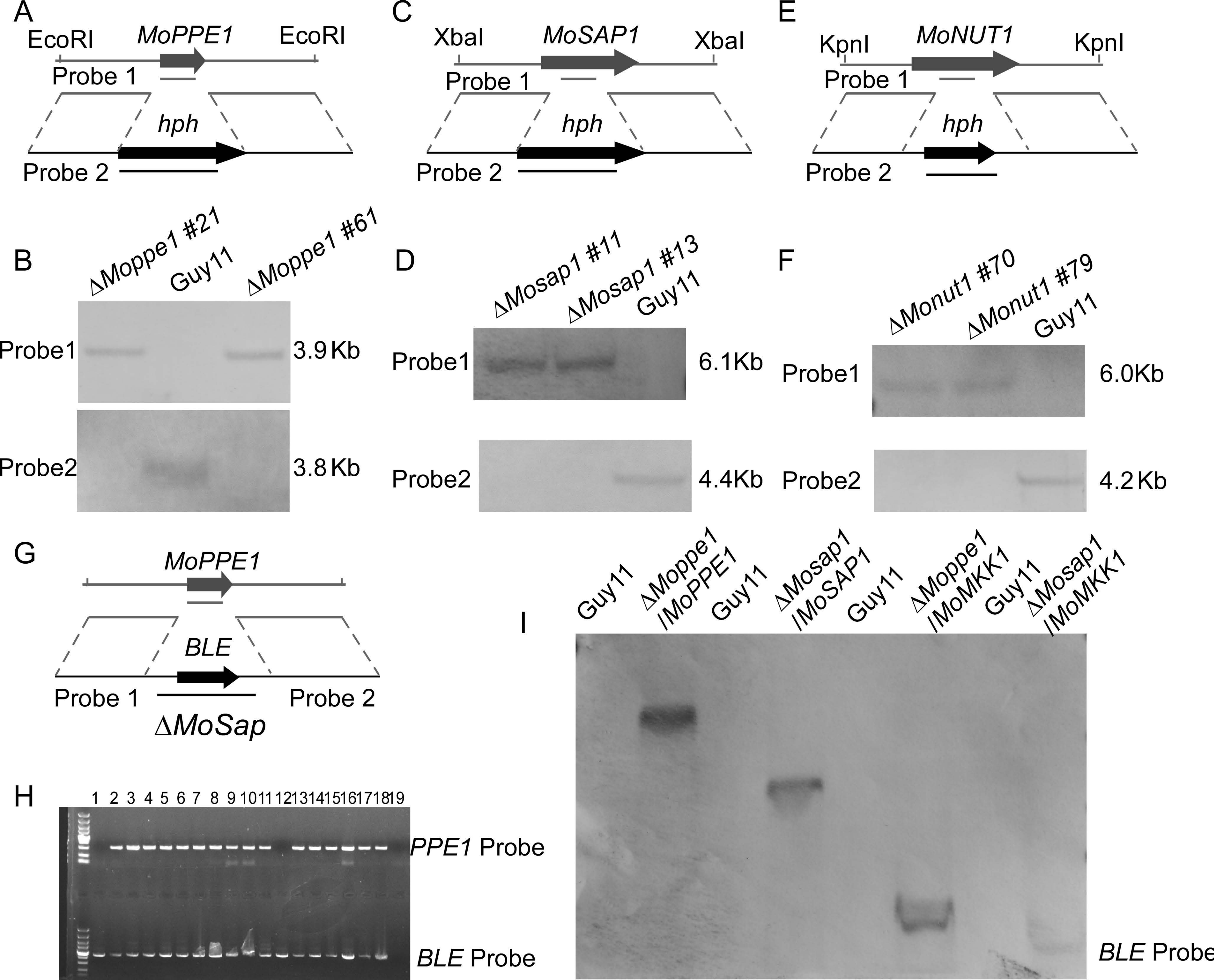
Δ*Scsap185/MoSAP1*

SD SD+Tunimycin

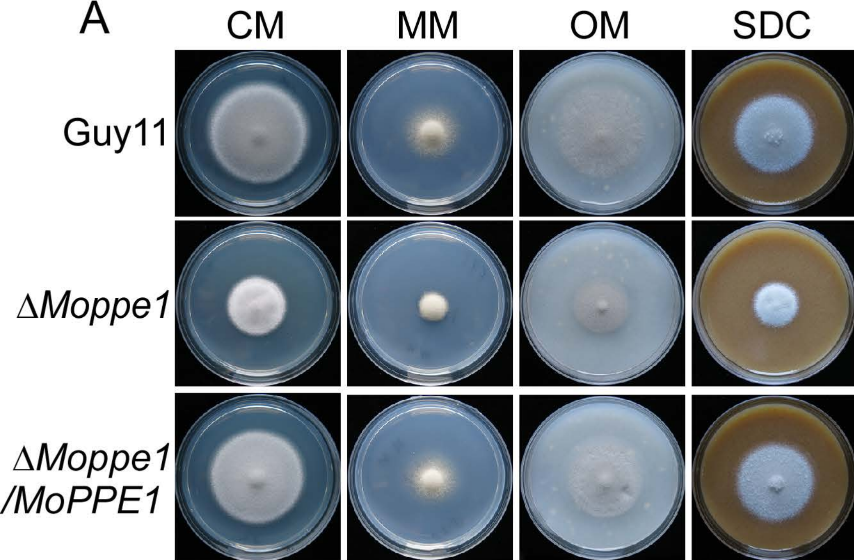
BY4741/pYES2  

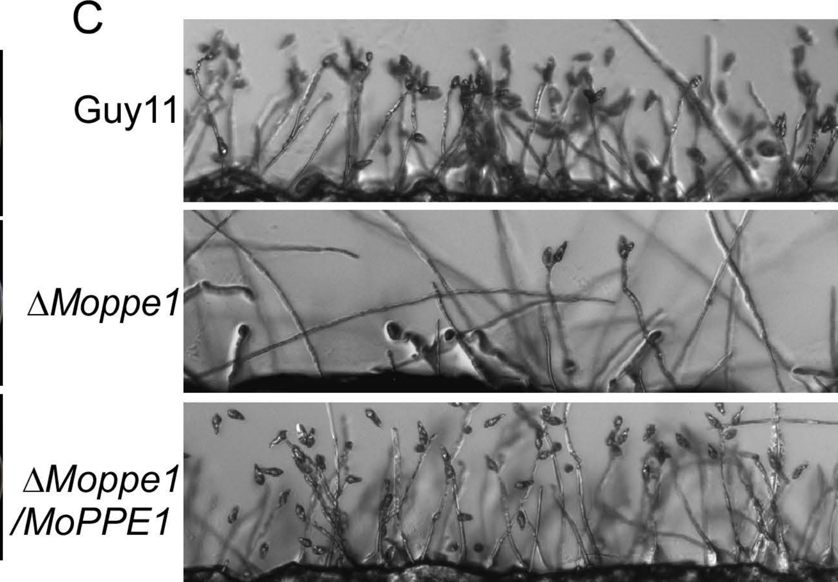
Δ*Scsap190*/pYES2

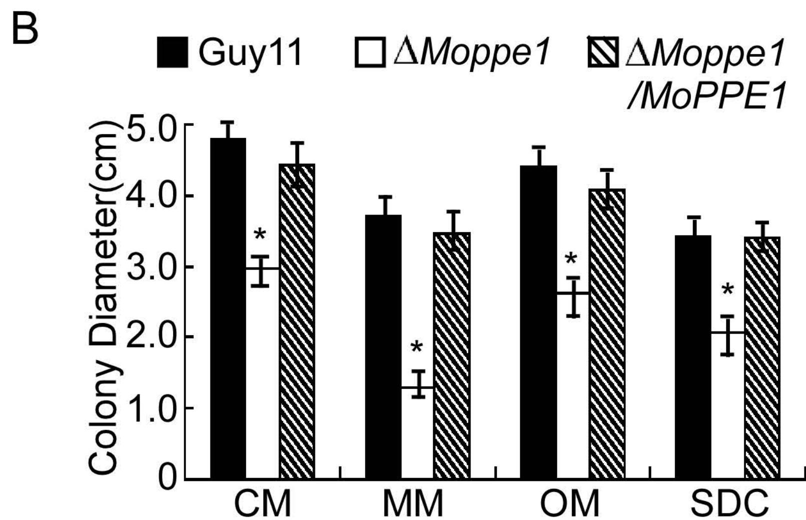
Δ*Scsap190/MoSAP1*  

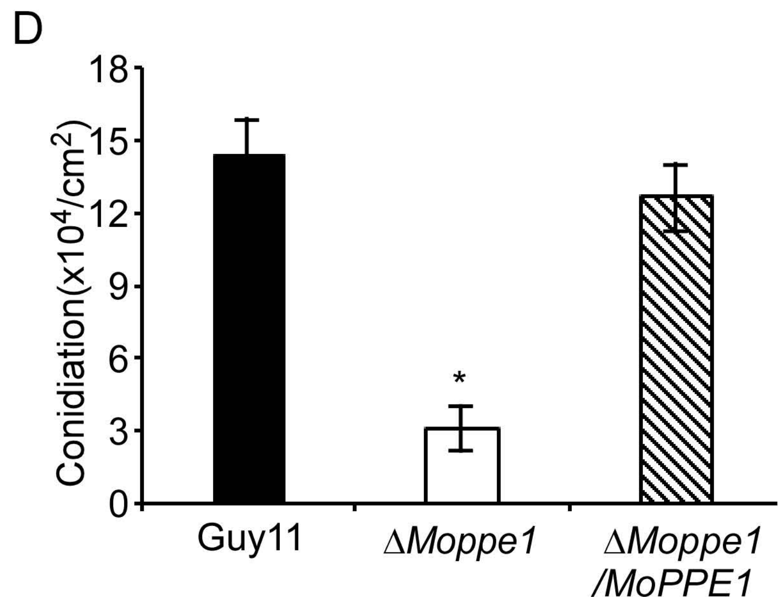


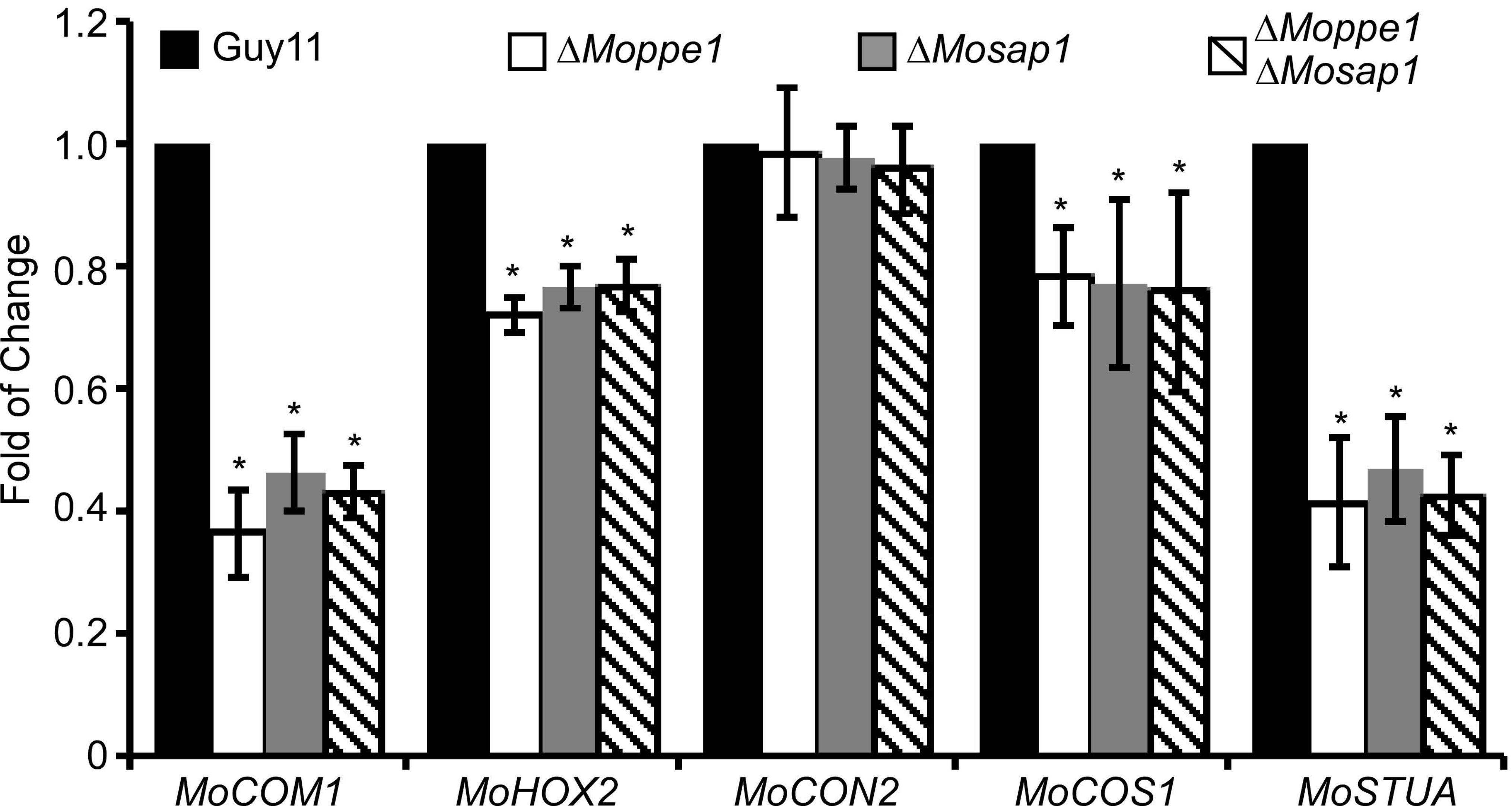
A
EcoRI
MoPPE1
EcoRI
Probe 1
hph
Probe 2
C
XbaI
MoSAP1
XbaI
Probe 1
hph
Probe 2
E
KpnI
MoNUT1
KpnI
Probe 1
hph
Probe 2
B
ΔMoppe1 #21
Guy11
ΔMoppe1 #61
Probe1
3.9 Kb
Probe2
3.8 Kb
D
ΔMosap1 #11
ΔMosap1 #13
Guy11
Probe1
6.1Kb
Probe2
4.4Kb
F
ΔMonut1 #70
ΔMonut1 #79
Guy11
Probe1
6.0Kb
Probe2
4.2 Kb
G
MoPPE1
BLE
Probe 1
Probe 2
ΔMoSap
H
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
PPE1 Probe
BLE Probe
I
Guy11
ΔMoppe1 /MoPPE1
Guy11
ΔMosap1 /MoSAP1
Guy11
ΔMoppe1 /MoMKK1
Guy11
ΔMosap1 /MoMKK1
BLE Probe


A
CM
MM
OM
SDC
Guy11
ΔMoppe1
ΔMoppe1
/MoPPE1

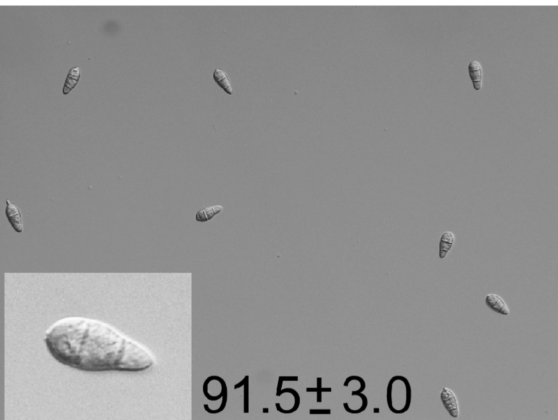

C
Guy11
ΔMoppe1
ΔMoppe1
/MoPPE1


B
Guy11
ΔMoppe1
ΔMoppe1
/MoPPE1
Colony Diameter(cm)
5.0
4.0
3.0
2.0
1.0
0
*
CM
MM
OM
SDC

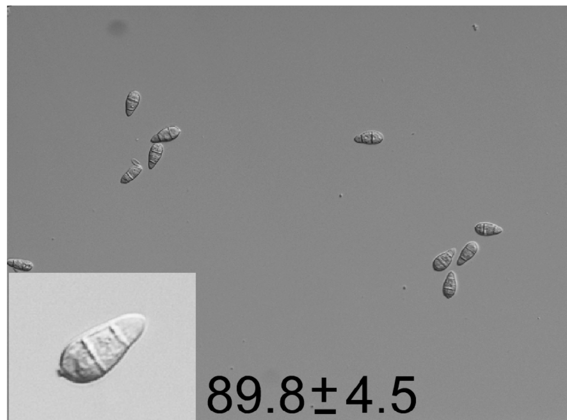

D
Conidiation(x10⁴/cm²)
18
15
12
9
6
3
0
*
Guy11
ΔMoppe1
ΔMoppe1
/MoPPE1


Guy11
ΔMoppe1
ΔMosap1
ΔMoppe1 ΔMosap1
Fold of Change
1.2
1.0
0.8
0.6
0.4
0.2
0
MoCOM1
MoHOX2
MoCON2
MoCOS1
MoSTUA

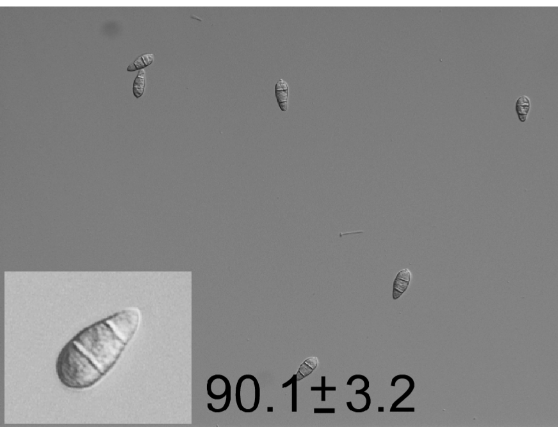
Guy11



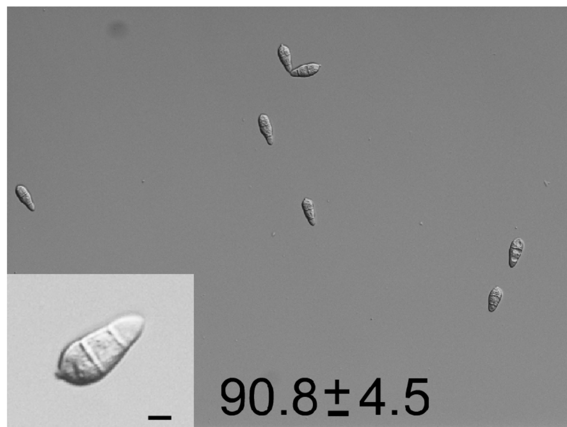
ΔMoppe1



ΔMosap1

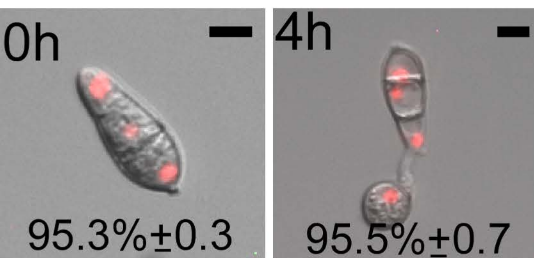


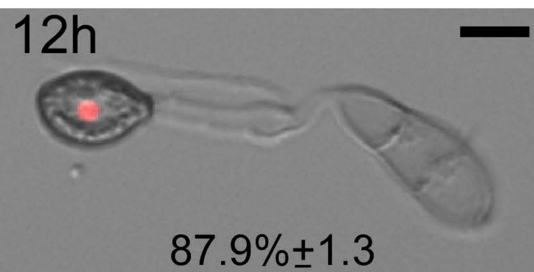
ΔMoppe1 ΔMosap1

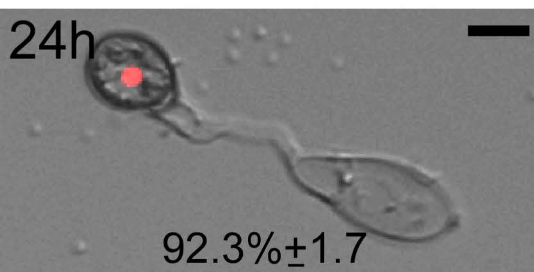


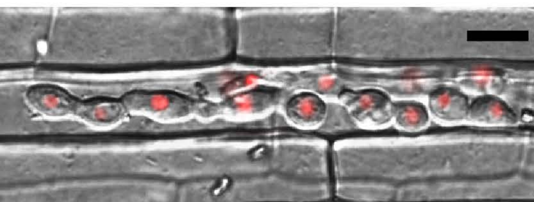
Guy11

ΔMoppe1

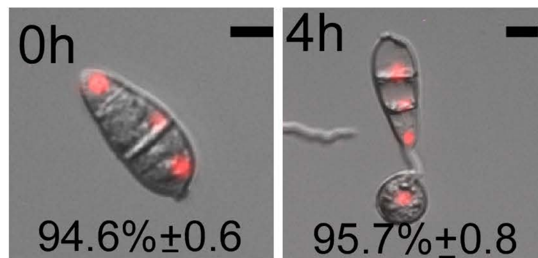


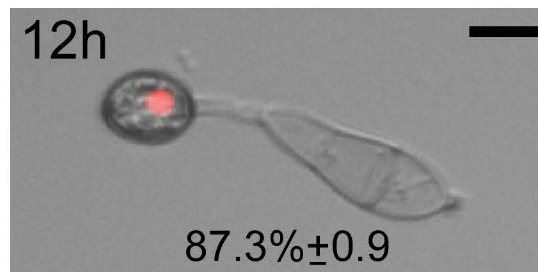




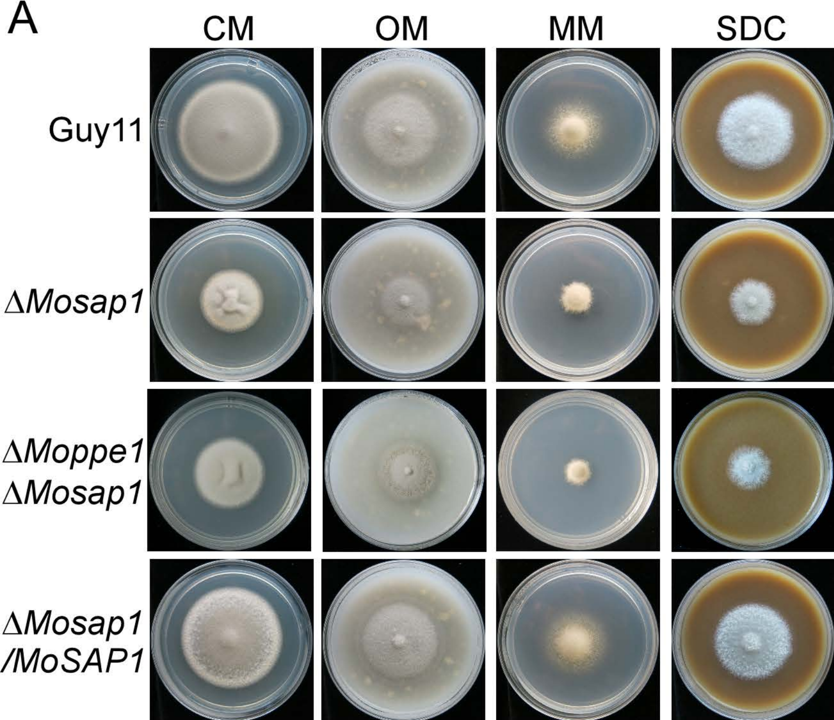


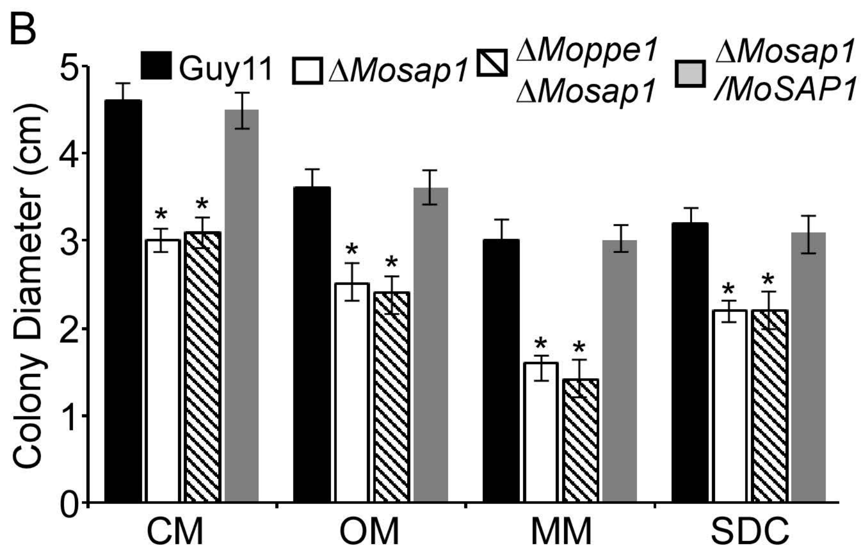
Nuclei number of per length infection hyphae 0.68±0.08

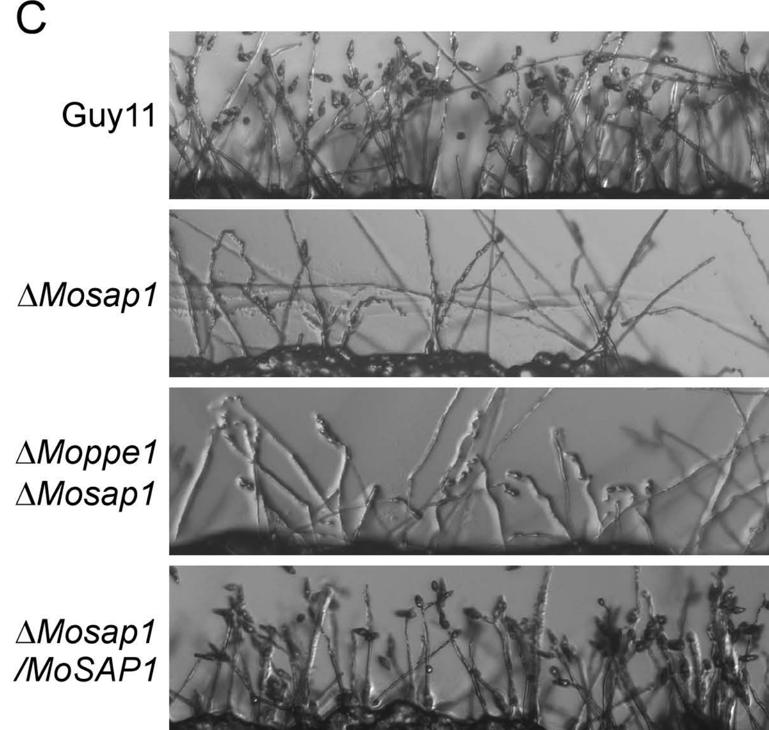


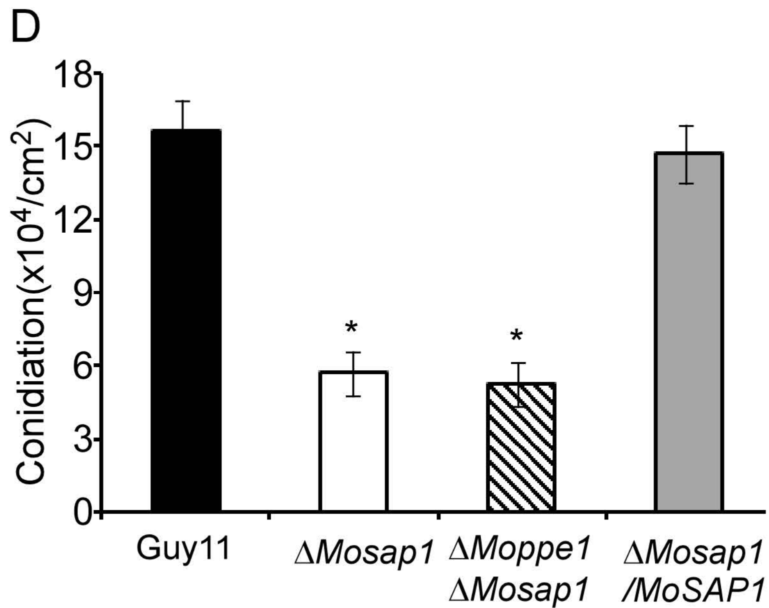


Nuclei number of per length infection hyphae 0.66±0.07


A
CM
OM
MM
SDC
Guy11
ΔMosap1
ΔMoppe1
ΔMosap1
ΔMosap1
/MoSAP1


B
Guy11
ΔMosap1
ΔMoppe1
ΔMosap1
ΔMosap1
/MoSAP1
Colony Diameter (cm)
0
1
2
3
4
5
CM
OM
MM
SDC


C
Guy11
ΔMosap1
ΔMoppe1
ΔMosap1
ΔMosap1
/MoSAP1


D
Conidiation(×10⁴/cm²)
0
3
6
9
12
15
18
Guy11
ΔMosap1
ΔMoppe1
ΔMosap1
ΔMosap1
/MoSAP1

A

CK

Congo Red (μg/ml)

200 400 600

CFW (μg/ml)

200 400 600

Guy11

ΔMoppe1

ΔMoppe1/MoPPE1

B

Guy11 ΔMoppe1 ΔMoppe1/MoPPE1

Inhibition rate (%)

0 20 40 60 80 100

200 400 600

Congo Red(μg/ml)

200 400 600

CFW(μg/ml)

A

Congo Red(μg/ml)

CFW(μg/ml)

CK 200 400 600 200 400 600

Guy11

Δ*Mosap1*

Δ*Mosap1*/*MoSAP1*

B

Guy11 Δ*Mosap1*

Δ*Mosap1*/*MoSAP1*

Inhibition rate (%)

0 20 40 60 80 100

*

200 400 600 200 400 600

Congo Red(μg/ml)

CFW(μg/ml)

Relative Biomass

1.2
1.0
0.8
0.6
0.4
0.2
0

*

**

Guy11

Δ*Moppe1*

Δ*Moppe1*
/*MoMKK1*^{DD}

Relative Biomass

1.2
1.0
0.8
0.6
0.4
0.2
0

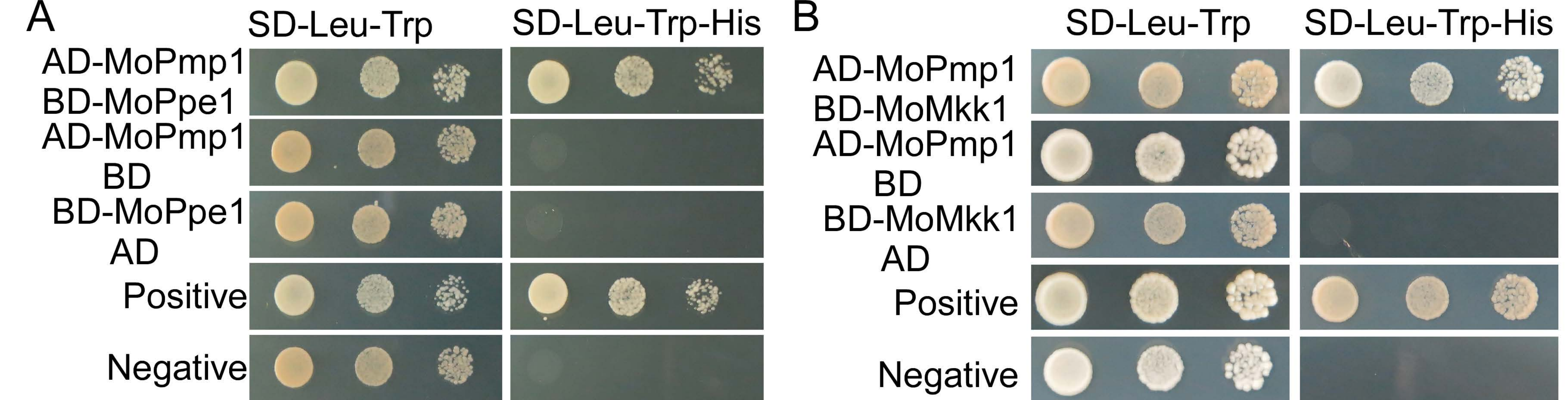
*

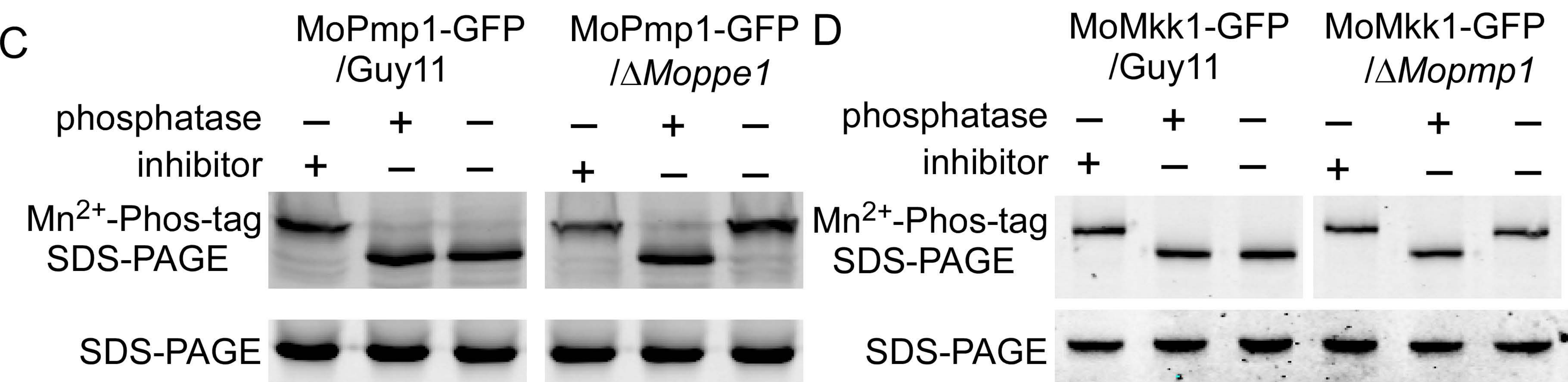
**

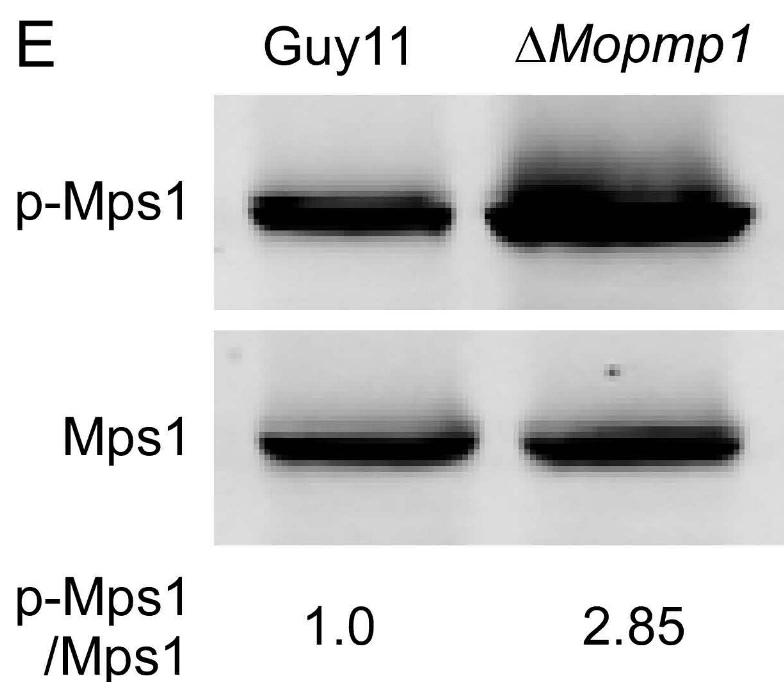
Guy11

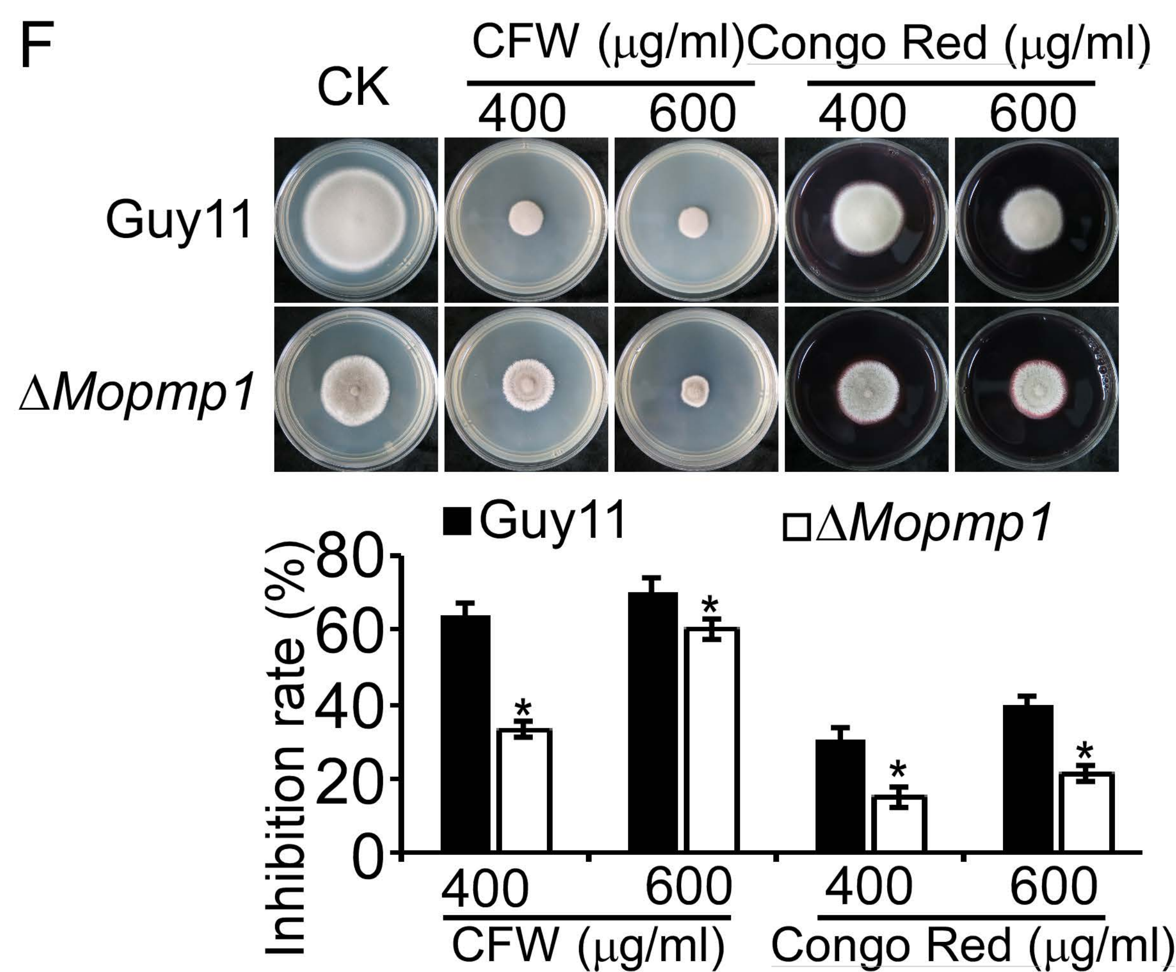
Δ*Mosap1*

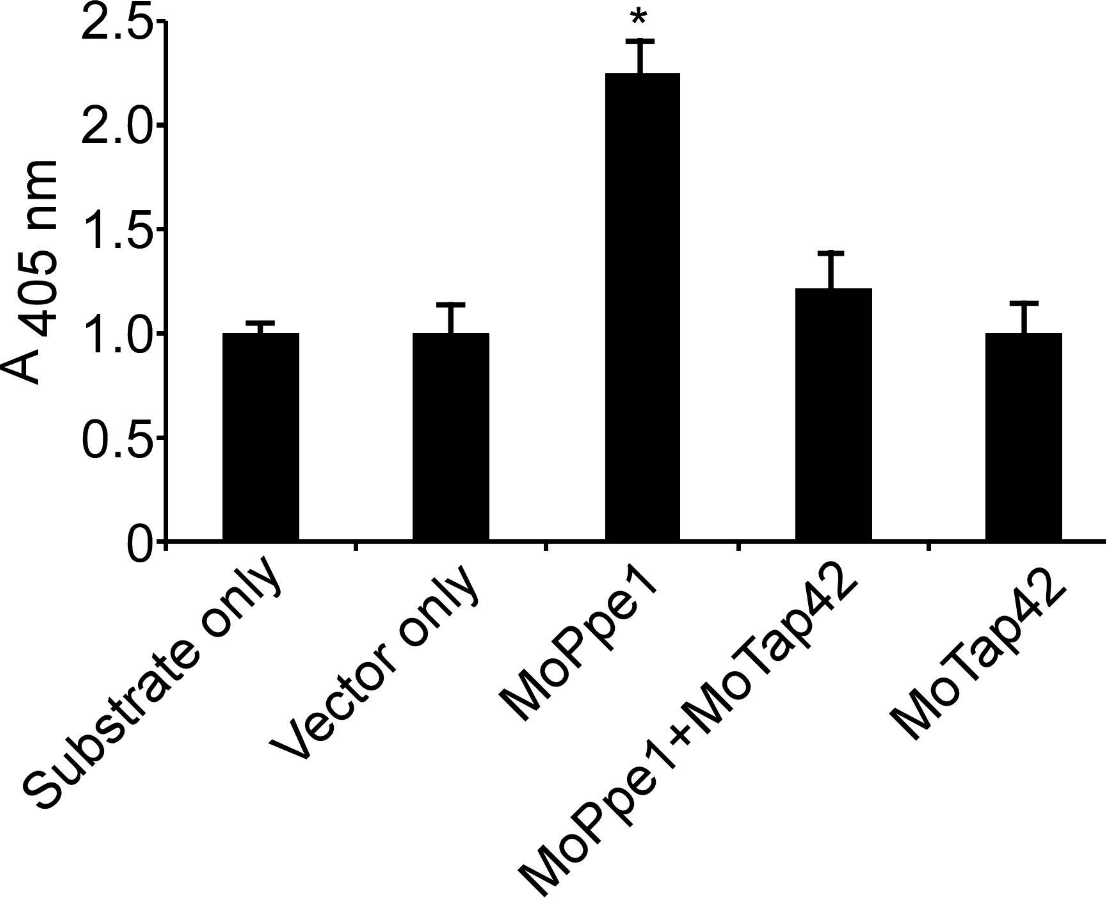
Δ*Mosap1*
/*MoMKK1*^{DD}


A
SD-Leu-Trp
SD-Leu-Trp-His
AD-MoPmp1
BD-MoPpe1
AD-MoPmp1
BD
BD-MoPpe1
AD
Positive
Negative
B
SD-Leu-Trp
SD-Leu-Trp-His
AD-MoPmp1
BD-MoMkk1
AD-MoPmp1
BD
BD-MoMkk1
AD
Positive
Negative


C
MoPmp1-GFP
/Guy11
MoPmp1-GFP
/ΔMoppe1
phosphatase
inhibitor
Mn2+-Phos-tag
SDS-PAGE
SDS-PAGE
D
MoMkk1-GFP
/Guy11
MoMkk1-GFP
/ΔMopmp1
phosphatase
inhibitor
Mn2+-Phos-tag
SDS-PAGE
SDS-PAGE


E
Guy11
ΔMopmp1
p-Mps1
Mps1
p-Mps1
/Mps1
1.0
2.85


F
CK
CFW (μg/ml)
Congo Red (μg/ml)
400
600
400
600
Guy11
ΔMopmp1
Guy11
ΔMopmp1
Inhibition rate (%)
80
60
40
20
0
400
600
400
600
CFW (μg/ml)
Congo Red (μg/ml)


2.5
2.0
1.5
1.0
0.5
0
A 405 nm
*
Substrate only
Vector only
MoPpe1
MoPpe1+MoTap42
MoTap42

A

Guy11 | ΔMoppe1 | ΔMosap1 | ΔMoppe1 ΔMosap1

CM

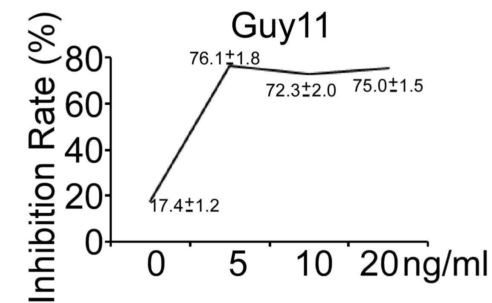
only CFW

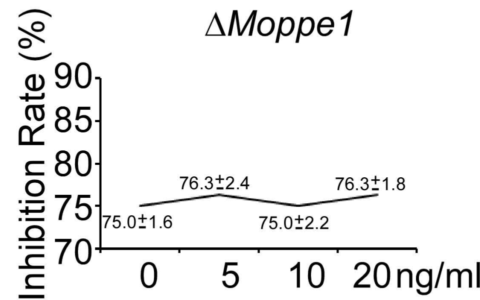
CFW 5 ng/ml Rap

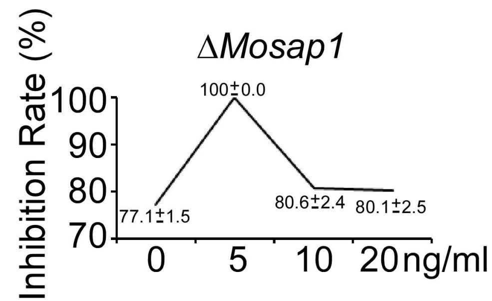
CFW 10 ng/ml Rap

CFW 20 ng/ml Rap

B







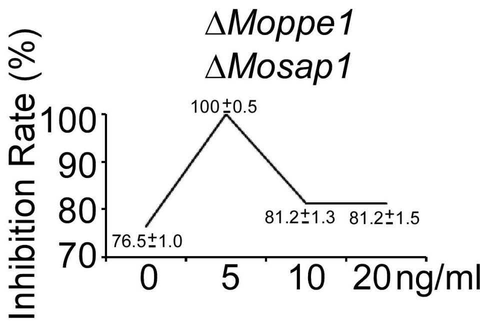


Fig. S1 . Complementation of the yeast mutants with *Magnaporthe oryzae* gene counterparts. (A) *MoPPE1* could completely suppress the growth defect of yeast SIT4 deletion mutant at 37 °C. The yeast *SIT4* mutant was complemented with *MoPPE1* cDNA to generate the strain BY4741Δ*sit4* + pYES2-*MoPPE1.* The WT strain BY4741 and SIT4 mutant transformed with empty pYES2 vector, respectively, were used as controls. Serial dilutions of cell suspension of each strain were spotted under different stresses as indicated in the Fig. (B) MoNut1 partially repressed the *S. cerevisiae GLN3* mutant. (C) *MoSAP1* could completely suppress the yeast mutant *SAP190* in 1 mM tunicamycin and could partially repress the yeast mutant *SAP185* which exhibit an increased resistance in 300 µg/ml hygromycin B, *SAP155* which was sensitive to 200 µg/ml hygromycin B and *SAP4* with the stress of 1 mM H_2O_2 that mutant is more resistant.

Fig. S2. Targeted genes knockout strategy and confirmation by Southern blot analysis. (A) Strategy of knocking out target genes in *M. oryzae* genome. Thin lines below the arrows indicate the probe sequence of each gene. (B) Southern blot analysis was used to confirm the *MoPPE1* deletion and the copy of the *HPH* gene. The genomic DNA of Guy11 and Δ*Moppe1* mutant was digested with *Eco*R I and hybridized with probes. (C-F) Strategies of knocking out *MoSAP1* and *MoNUT1* and verified by southern blot. The genomic DNA of Guy11 and Δ*Mosap1* mutant was digested with *Xba* I and Δ*Monut1* mutant was digested with *Kpn* I. (G) The Δ*Moppe1* Δ*Mosap1* double mutant strain was generated with a 3.4 kb fragment, which included the flanking sequences of *MoPPE1* and the bleomycin sequence transformed into Δ*Mosap1* mutant protoplasts then using the PCR to identify the putative double mutant. (H) The PCR results of verification the double mutant, number #1 and #12 were the double mutants. (I) The southern blot used to detect the copy of the bleomycin gene in different strains.

Fig. S3. MoPpe1 is involved in the vegetative growth and conidiation. (A) Guy11, Δ*Moppe1* mutant and complemented strain were inoculated on CM, MM, OM and SDC media cultured at 28 °C for 7 days, then photographed. (B) Statistical analyses of the colony diameter from wild-type Guy11, Δ*Moppe1* mutant and the complemented strain on different medium. Error bars represent the standard deviations; Asterisks denote statistical significances ($p < 0.01$). (C) Conidia were observed under a light microscope after illumination for 24 h then photographed. (D) The conidia were harvested from the Guy11, Δ*Moppe1* mutant and complemented strain incubated on SDC medium for 7 days. The number of conidia were calculated and analysed. Error bars represent the standard deviations. Asterisks represent significant difference ($p < 0.01$).

Fig. S4. Expression levels of conidiation-related genes. The expression results of *MoCOM1*, *MoHOX2*, *MoCON2*, *MoCOS1* and *MoSTUA* genes that were shown previously to be important in the process of conidial development. Histogram shown the results of three biology repeats, error bars denote standard errors of three biology experiments. Asterisk denote values that are not significantly different at ($p < 0.05$).

Fig. S5. There is no great difference in conidial morphology between wild-type and mutants. Conidia were harvested from different mutants the observed by light microscopy. Bars = 10 µm.

Fig. S6. MoPpe1 is dispensable function in nuclear division in *M. oryzae.* (A) Nucleus was viewed, photograph and calculated during appressorium formation at 0, 4, 12, 24 h time point and infection phase with the transformation of H1-RFP into Δ*Moppe1* mutant and Guy11 respectively. The merged image shows H1: RFP and DIC. Bars = 10 µm.

Fig. S7. MoPpe1 and MoSap1 are important for the vegetative growth and conidia formation of *M. oryzae*. (A) Guy11, Δ*Mosap1* single mutant, Δ*Moppe1* Δ*Mosap1* double mutant and Δ*Mosap1* complemented strain were inoculated on CM, MM, OM and SDC media cultured at 28 °C for 7 days, then photographed. (B) Statistical analyses of the colony diameter of four different strains on different medium. Error bars represent the standard deviations, asterisks denote statistical significances ($p < 0.01$). (C) The conidia were photographed under a light microscope after illumination for 24 h. (D) Conidia production of Guy11, Δ*Mosap1* single mutant, complemented strain and Δ*Moppe1* Δ*Mosap1* double mutant were collected after 7 days on SDC medium, then calculated and analysed. Error bars represent the standard deviations. Asterisks denote statistical significances ($p < 0.01$).

Fig. S8. MoPpe1 is involved in the cell wall stress response of *M. oryzae*. (A) Guy11, Δ*Moppe1* mutant and the complemented strain were incubated on complete medium (CM) plates containing different concentrations of Congo Red (CR), Calcofluor white (CFW) and sodium dodecyl sulfate (SDS) at 28 °C for 7 days. (B) The inhibition rate was determined by plotting the percentage of colonies in the presence of various concentrations of CR, CFW and SDS against regular CM. The asterisks denote statistical significances ($p < 0.01$)

Fig. S9. MoSap1 is important for cell wall stress responses of *M. oryzae*. (A) The wild-type strain, Δ*Mosap1* mutant and the complemented strain were incubated on complete medium (CM) plates with different concentrations of Congo

Red (CR), Calcofluor white (CFW) and sodium dodecyl sulfate (SDS) at 28 °C for 7 days. (B) The inhibition rate was determined by plotting the percentage of colonies in the presence of various concentrations of CR, CFW and SDS against regular CM, asterisks denote statistical significances ($p < 0.01$)

Fig. S10. The relative fungal growth assay. Diseased rice leaves were collected after 7 days inoculation. Total DNA was extracted from per 1.5 g disease leaves and test by qRT-PCR (HiScript II Reverse Transcriptase, Vazyme Biotech Co., Nanjing, China) with 28S/Rubq1 primers. The results were of three biology repeats. Single asterisks denote statistical significances ($p < 0.05$), double asterisks represent statistical significances ($p < 0.01$)

Fig. S11. MoPpe1 regulates the CWI pathway via MoPmp1. (A) Yeast two hybrid assay for the interaction between MoPpe1 and MoPmp1. The AD-MoPmp1 and BD-MoPpe1 vectors were co-introduced into yeast strain AH109, and the transformants were plated with serial dilutions of yeast cells on SD-Leu-Trp for 3 days and on selective SD-Leu-Trp-His added with 2 mM 3-AT (3-amino-1,2,4-triazole) for 10 days. (B) Interaction between MoMkk1 and MoPmp1. The AD-MoPmp1 and BD-MoMkk1 vectors were co-introduced into yeast strain AH109, and the transformants were plated with serial dilutions of yeast cells on SD-Leu-Trp for 3 days and on selective SD-Leu-Trp-His added with 2.5 mM 3-AT (3-amino-1,2,4-triazole) for 12 days. (C) MoPmp1 was hyperphosphorylated in Δ*Moppe1* mutant. (D) MoPmp1 dephosphorylate the MoMkk1 in *M. oryzae*. (E) The MoMps1 phosphorylayion increased in Δ*Mopmp1* mutant. (F) The Δ*Mopmp1* mutant exhibited increased resistance to cell wall stress.

Fig. S12. Protein phosphatase MoPpe1 possesses the phosphatase activity that is reduced upon MoTap42 addition. Recombinant His-tagged MoPpe1 and MoTap42 was expressed in bacteria and purified. Phosphatase activity was determined using the indicated proteins and p-nitrophenyl phosphate (pNPP) as a substrate.

Fig. S13. Rapamycin treatment affects the fungal susceptibility to calcofluor white. (A) The wild-type strain, Δ*Moppe1*, Δ*Mosap1* and Δ*Moppe1* Δ*Mosap1* mutant were incubated with 150 μg/ml Calcofluor white (CFW) stress then added with different concentrations of rapamycin 5, 10, 20 ng/ml, respectively, the strains on complete media (CM) as a control. (B) The broken line graph of each strain's inhibition rate with different treatment. Detailed inhibition rate along with positive and negative SD was shown in each graph.

Table S1. MoMkk1 and MoPpe1 putative interacting proteins identified by affinity capture assays

Table S2. Total of primers were used in this study

Table S3. Comparison of mycological characters

Table S4. Utilization rate of the strains with different nitrogen source

S1 Table. Partial of MoMkk1 and MoPpe1 interacting proteins identified by affinity capture assays in *Magnaporthe Oryzae*

Proteins	Putative functions	# of unique peptide
MoMkk1 interacting proteins		
MGG_03911	serine/threonine-protein phosphatase ppe1	7 / 5
MGG_09470	myosin regulatory light chain cdc4	5 / 6
MGG_04143	Ras-like protein Rab-6A	4 / 3
MGG_00450	phosphoenolpyruvate carboxykinase	5 / 5
MGG_06952	hypothetical protein	9 / 11
MGG_15140	tyrosine-protein phosphatase pmp1	3 / 2
MoPpe1 interacting proteins		
MGG_12709	MoPpe1 associated protein MoSap1	8 / 12
MGG_01540	MoTap42	11 / 13
MGG_02755	nitrogen regulatory protein NUT1	12 / 9
MGG_06362	small COPII coat GTPase	3 / 6
MGG_15140	tyrosine-protein phoaphatase pmp1	4 / 3
MGG_09480	conserved hypothetical protein	5 / 3
MGG_01742	elongation factor 2	8 / 13
MGG_01490	conserved hypothetical protein	11 / 6

S2 Table. Primers used in this study

Primer name	Sequence (5'-3')	Remark
PPE1 F1	TAACTCGAGTGCCACACCTCAAGCTGGTGTT	amplify *MoPPE1* 5' flank sequence
PPE1 F2	TAAGATATCTAGCTGGTGTCCCAGGTTGCTG	amplify *MoPPE1* 5' flank sequence
PPE1 F3	TAAACTAGTGATACCGATATGGCAAAGTGGC	amplify *MoPPE1* 3' flank sequence
PPE1 F4	TAAGAGCTCAGTGTTCCTTCAAGTCCGCAGT	amplify *MoPPE1* 3' flank sequence
PPE1 KO-L	CAGAAATCACGGATCCCAAGCTG	amplify *MoPPE1* probe sequence
PPE1KO-R	CATGAACACAGAGCACTGAACC	amplify *MoPPE1* probe sequence
PPE1 BY	GTCCATCAAAGGCATGACATAC	validation of *MoPPE1* deletion
HPH R	GCTGATCTGACCAGTTGCCTA	(HPH)
PPE1 HB-F1	ACTCACTATAGGGCGAATTGGGTACTCAAATTGGTTCATTTG CGTCTTCCCATTGAGC	*MoPPE1* complementation
PPE1 HB-F2	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCAAGAA ATAGTCCCCTGGGCCTC	*MoPPE1* complementation
PPE1 Flag F1:	CTATAGGGCGAATTGGGTACTCAAATTGGTTCATACTCCGTTC TGAGAAGATGC	Construction of *MoPPE1*-Flag
PPE1 Flag F2:	CTTTATAATCACCGTCATGGTCTTTGTAGTCCAAGAAATAGTC CCCTGGGCCTC	Construction of *MoPPE1*-Flag
PPE1 Stag F1:	TTTCGTAGGAACCCAATCTTCAAAATGGCTTCTACCGTGCCGA AG	Construction of *MoPPE1*-Stag
PPE1 Stag F2:	TTCGAATTTAGCAGCAGCGGTTTCTTTCAAGAAATAGTCCCCT	Construction of *MoPPE1*-Stag

	GGGCCTC	
SAP1 F1:	TAAGTCGACGAGTTAGTTCGCTGGTTGGC	amplify *MoSAP1* 5' flank sequence
SAP1 F2:	TAAGAATTCCTTGGCGCGCACTCAAGCAG	amplify *MoSAP1* 5' flank sequence
SAP1 F3:	TAAGGATCCGCAGGGAGAAACGATTGTCCC	amplify *MoSAP1* 3' flank sequence
SAP1 F4:	TAAACTAGTTCATCATAATCACATCGCGG	amplify *MoSAP1* 3' flank sequence
SAP1 KO-L	GCGAACTCATGGCCGAACTTCTTCACTG	amplify *MoSAP1* probe sequence
SAP1 KO-R	GAAGCATCGGTCATCATCACATCAGAACC	amplify *MoSAP1* probe sequence
SAP1 BY	CTATCTGGCCTTATCTACCTGG	validation of *MoSAP1* deletion
SAP1 HB-F1	ACTCACTATAGGGCGAATTGGGTACTCAAATTGGTTCTATCT GGCCTTATCTACCTGG	*MoSAP1* complementation
SAP1 HB-F2	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACAGCAAG CTCCCTTATGTTCTC	*MoPPE1* complementation
NUT1 F1:	TAACTCGAGCGGCTGCTCCTTGTAAAGCAAAG	amplify *MoNUT1* 5' flank sequence
NUT1 F2:	TAAGAATTCGTTGCGGCTGGATCCTTTATTC	amplify *MoNUT1* 5' flank sequence
NUT1 F3:	TAATCTAGAACTTCTCCCCCAAAACAACAGGG	amplify *MoNUT1* 3' flank sequence
NUT1 F4:	TAACCGCGGCCTAGGAAAGAAGTCCTTCACTG	amplify *MoNUT1* 3' flank sequence
NUT1 KO-L	GTACGAACAGCAAGGCGTGCAAG	amplify *MoNUT1* probe sequence
NUT1 KO-R	CGTTAGCGCTTCCTGCTCTGCTC	amplify *MoNUT1* probe sequence
NUT1 BY	CATCTTCGATGTGATTGCGGATCG	validation of *MoNUT1* deletion
NUT1 HB-F1	ACTCACTATAGGGCGAATTGGGTACTCAAATTGGTTCATCTTC GATGTGATTGCGGATCG	*MoNUT1* complementation
NUT1 HB-F2	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCAGACT CATGGTCAACCAATCCCAC	*MoNUT1* complementation
NUT1 NGFP ProF1	ACTCACTATAGGGCGAATTGGGTACTCAAATTGGTTCATCTTCGA TGTGATTGCGGATCG	Construction of GFP-NUT1
NUT1 NGFP ProR1	TGTTGCGGCTGGATCCTTTATTC	Construction of GFP-NUT1
Gln3GFPF	GAATAAAGGATCCAGCCGCAACAATGGTGAGCAAGGGCGAGG A	Construction of GFP-NUT1
Gln3GFPR	CTTGTACAGCTCGTCCATGC	Construction of GFP-NUT1
MoGln3GeneF1	GCATGGACGAGCTGTACAAGATGAATCCCACAATAACAGAGC	Construction of GFP-NUT1
MoGln3GeneR1	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACTTACAGA CTCATGGTCAACCAATCCCAC	Construction of GFP-NUT1
MoTap42 GFPF1:	ACTCACTATAGGGCGAATTGGGTACTCAAATTGGTTGTACATA CAACCACCTCCTGCTCCTG	Construction of *TAP42*-GFP
MoTap42 GFPR1:	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACACCCCTGT TCAGAGTGTTTCC	Construction of *TAP42*-GFP
AD/BD-PPE1 F1:	TAACATATGATGGCTTCTACCGTGCCGAAG	Construction of AD/BD-PPE1
AD/BD-PPE1 R1:	TAAGAATTCTCACAAGAAATAGTCCCCTGGG	Construction of AD/BD-PPE1
AD/BD-SAP1 F1:	TAACATATGATGTTCTGGCGGTTTGGCGGCTA	Construction of AD/BD-SAP1
AD/BD-SAP1 R1:	TAAGAATTCTCAAGCAAGCTCCCTTATGTTC	Construction of AD/BD-SAP1
AD/BD-TAP42F1	TAACATATGATGGAGCAAGATCAGACCCAGGA	Construction of AD/BD-TAP42
AD/BD-TAP42R1	TAAGAATTCTTAACCCCTGTTCAGAGTGTTTC	Construction of AD/BD-TAP42
PPE1 RFPF1	ACTCACTATAGGGCGAATTGGGTACTCAAATTGGTTCATTTGC GTCTTCCCATTGAGC	Construction of *PPE1*-RFP

PPE1 RFPR1	CAAGAAATAGTCCCCTGGGCCTC	Construction of *PPE1*-RFP
RFPF:	GAGGCCCAGGGGACTATTTCTTGATGGCCTCCTCCGAGGACGT	Construction of *PPE1*-RFP
RFPR:	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACTTAGGC GCCGGTGGAGTGGC	Construction of *PPE1*-RFP
PPE1 DBF1:	GTATCGATAAGCTTGATATC CTTGCCTGGCAACATTCGTAC	Construction of double mutant
PPE1 DBR1:	GGTATCGTTGCGGTCTTCG	Construction of double mutant
bleF	CGAAGACCGCAACGATACCCGAGGGTACCTGAAGGAGCAT	Construction of double mutant
bleR	AGATGAGCTGTATCTGGAAG	Construction of double mutant
PPE1 DBF2	CTTCCAGATACAGCTCATCT GATAAAGACAAAAGAATGTC	Construction of double mutant
PPE1 DBR2	GGTGGCGGCCGCTCTAGAACTAGTCAGTGTTCCTTCAAGTCCG CAG	Construction of double mutant
28S rDNA LL	TACGAGAGGAACCGCTCATTCAGATAATTA	qRT-PCR
28S rDNA RR	TCAGCAGATCGTAACGATAAAGCTACTC	qRT-PCR
Rubq1 LL	GTGGTGGCCAGTAAGTCCTC	qRT-PCR
Rubq1 RR	GGACACAATGATTAGGGATCA	qRT-PCR
Rice_ EF1α_QF	CTTCAACACCCCTGCTATG	qRT-PCR Primer of *EF1α*
Rice_EF1α_QR	CCGTTGTGGTGAATGAGTAA	qRT-PCR Primer of *EF1α*
Rice_Cht1-F	CGTGGTGACCAACATCATCA	qRT-PCR Primer of *Cht1*
Rice_Cht1-R	GAGTTGAAAGGCCTCTGGTTGT	qRT-PCR Primer of *Cht1*
Rice_PR1a_QF	TCTTCATCACCTGCAACTACTC	qRT-PCR Primer of *PR1α*
Rice_PR1a_QR	ATTCATCGGATTTATTCTCACC	qRT-PCR Primer of *PR1α*
Rice_PBZ1_QF	CTACTATGGCATGCTCAAGAT	qRT-PCR Primer of *PBZ1*
Rice_PBZ1_QR	ATAGAAAGGCACATAAACACAA	qRT-PCR Primer of *PBZ1*

S3 Table. Comparison of mycological characters

Strain	Germination rate(%)[a]	Appressrium formation (%)[b]	Appressrium formation (%)[c]
Guy11	95.2±1.1	94.7±1.4	93.8±1.5
Moppe1	95.0±1.2	95.1±1.8	94.5±1.2
Δ*Moppe1*/*MoPPE1*	95.2±1.3	95.0±1.1	94.4±1.2
Δ*Mosap1*	94.5±1.6	94.7±1.7	94.1±1.3
Δ*Mosap1*/ *MoSAP1*	95.1±0.8	95.4±1.3	93.8±1.5
ΔΔ*Moppe1Mosap1*	93.4±1.8	94.5±1.6	94.7±1.2

[a] Percentage of conidial germination on artificial surface at 4 hpi.

[b] Percentage of appressorium formation on artificial surface at 24 hpi.±SD was calculated from three repeated experiments.

[c] Percentage of appressorium formation on artificial surface at 24 hpi.±SD, treated with 10 ng/ml

rapamycin, calculated from three repeated experiments .

S4 Table. **The relative growth rate of the tested strains with different nitrogenous source compared to CM media respectively, following seven days growth**

Growth media	Utilization rate %				
	Guy11	Δ*Moppe1*	Δ*Mosap1*	Δ*Moppe1* Δ*Mosap1*	Δ*Monut1*
GMM+YE	90±0.8	91.3±1.2	90.6±1.5	92±1.3	91.3±1.0
GMM+PE	66.7±1.5	70.1±1.3	71.2±1.1	68.1±0.9	75±2.0*
GMM+CA	60±1.3	62±2.0	60±0.0	63.3±1.2	69.3±0.8*
GMM+YNB	70±1.2	65.5±0.7*	66.6±1.0*	66.7±1.0*	0±0*
GMM+Va	75±1.0	63.7±0.2*	63.5±2.0*	60%±1.5*	0±0*
GMM+$(NH_4)_2SO_4$	30±1.0	33.3±2.0*	33.3±1.2*	33.3±1.2*	50±2.0*
GMM+$(NH_4)_2C4H4O_6$	48.2±1.5	47±0.5	48.4±1.6	46±1.0	48±1.2
GMM+Gln	37.5±0.8	54.5±1.2*	54.8±1.0*	55±2.0*	48.8±2.0*
GMM+NH_4NO_3	28.8±1.5	27.3±1.3	25±2.0	26±2.0	24.6±2.0
GMM+$NaNO_3$	67.3±2.0	33.3±0.0*	35.5±0.8*	36±1.0*	0±0*
GMM+$NaNO_2$ 1mM	61.5±0.0	46.8±1.0*	48±2.0*	48.8±0.8*	0±0*
GMM+$NaNO_2$ 5mM	38.5±1.5	18.8±0.0*	16.6±1.2*	0±0*	0±0*

Supplements, such as yeast extract (YE), peptone (PE), vitamins (VA), casamino acids (CA), yeast nitrogen base without amino acids (YN-AA), L-Glutamine (Gln) and other nitrogen sources, NH_4^+ or NO_3^- were added into GMM with a same concentration in complete media (CM). The relative growth rate was [Utilization rate = (the diameter of treated strain) / (the diameter of strain in CM) x 100%]. $NaNO_2$ (1 mM and 5 mM, the wild type could not grow on 25 mM). The experiments were repeated three times. GMM [1% glucose minimal medium: 0.52 g/L KCl, 0.52 g/L $MgSO_4·7H_2O$, 1.52 g/L KH_2PO_4, 10 g/L glucose, 0.001% (W/V) thiamine and 0.1% (W/V) trace elements; containing 10 mM NH_4^+]. Asterisks indicate a significant difference ($p < 0.05$).