Electronic supplementary materials

Table S1. Primers used in this study.

Primer	Sequence (5'- 3')	Notes
5929DF	GCTCTAGACAAACATCAGCGGTTAGCAA	For construction of gene disruption vector pBarMrst12
5929DR	GCTCTAGATTCCTCCTCTTCGTCGTTTC	
5929UF	CCGCTCGAGGGCGAGTGTAAATCGAGAGC	
5929UR	GGAATTCGCAACAGCACAAATTTTGAAAC	
5929SF	GCTCTAGAGCTGAGCTAAAAACCCCAGA	For construction of gene complementation vector pBen Mrst12
5929SR	GCTCTAGAAACGTCAGCCTGTCCAAGTT	
Mras1UF	GGACTAGTACCACCCAAGTTCATCTCAC	For construction of gene disruption vector pBarMras1
Mras1UR	GGACTAGTCGAGGACTCCTGTTTCTCAA	
Mras1DF	GCTCTAGA TTCAAGACCGCCAGAGTC	
Mras1DR	GCTCTAGA TTCAAGACCGCCAGAGTC	
Mras2UF	CCGCTCGAG CCCCCATAACTGAGAACGAA	For construction of gene disruption vector pBarMras2
Mras2UR	GGAATTC GAGCCACAGGGATTGTTTGT	
Mras2DF	CGAGCTCCATCCGTCATTCGTGACATC	
Mras2DR	CGAGCTCTGGTGGGCATTGTAACTTCA	
Mras3UF	CCGCTCGAGCTTGGTCTGGAGTTTCATGG	For construction of gene disruption vector pBarMras3
Mras3UR	CCGGAATTCGATGTCACGCTGTCTGATGA	
Mras3DF	GCTCTAGAGGTCTCCCTACTGCTGATGA	
Mras3DR	GCTCTAGAGGGAGTAGCAGTCCCAAGTT	
Mras4UF	GCTCTAGA GGCACGAGCTCTCATCCTAC	For construction of gene disruption vector pBarMras4
Mras4UR	GCTCTAGA ACTGCCCATTCCAATACTCG	
Mras4DF	CCGCTCGAGAGGTGGAAACCCACAGAATG	
Mras4DR	CCGCTCGAGGCCATTTATACGCTCGGCTA	
Mras5UF	CGAGCTCGCAACAACCAGCCAATAGTC	For construction of gene disruption vector pBarMras5
Mras5UR	CGAGCTCGAGTTGCTTGTTTGCAGGAT	
Mras5DF	GGAATTC CCTGGTCCAAAGAGCAATAA	
Mras5DR	CCGCTCGAG ATAAAACTCATCCGGGAAGC	
Mras6UF	CCGCTCGAG ATTTAGGGGCAGTTGAATGG	For construction of gene disruption vector pBarMras6
Mras6UR	GGAATTC CAAGGCATGGCTTTTGTTTT	
Mras6DF	CGAGCTCTGATTTGTTTGGTCGCTTCA	
Mras6DR	CGAGCTCATGAGCCAGAAATTGGCACT	
Mras7UF	CGAGCTC CTCTTGATCTCCGTGATTGC	For construction of gene disruption vector pBarMras7
Mras7UR	CGAGCTC TGATCATGCAGGTGTAGGG	
Mras7DF	CGGGATCC CAATGCTGCTGCTCCTAAC	
Mras7DR	CGGGATCC GATGAAATCCCTCTGTTCCA	
TF5929F	GCGTTGCTGATGCTATTTGA	For RT-PCR verifying
TF5929R	GATGCTCAGGTTGCACTTCA	
Mras1F	CCACGGTATTATCCGATCC	
Mras1R	CTGGACCTGCATAGCGTAGT	

Mras2F	GCCAGGTTTGACTGGTGAGT	
Mras2R	CGGCAACCTCTCCATTACAT	
Mras3F	GCTCTTGCAGCTACTGCTTC	
Mras3R	GGACGTTCTGGGTGACTTC	
Mras4F	AAAGGCGTCTTGTGATGGAG	
Mras4R	ATTTAATTGCCACCCTTTTGC	
Mras5F	CAGGATGCATTCTTCAACCA	
Mras5R	ACCACAGCCCTTGGTATCAT	
Mras6F	GGTGGCCTTTTCTTCCAGTC	
Mras6R	GGACCAGCCCCGTTATTATT	
Mras7F	CTCCAGATGACGCTGCAC	
Mras7R	GCCAGTGTTGCCAGTGTT	
Hsp70F	CGGGCCCCCCCTCGAGGCAGTAGAGGTGTAGTTG	For pBar-Hsp70p-GFP-linker-Mrst12
Hsp70R	TGTGGTGGTTGTGATGCG	
5929GF	ATCACAACCACCACAATGGTGAGCAAGGGCGAGGA	
5929GR	CTTGTACAGCTCGTCCATGC	
5929CfusionF	GACGAGCTGTACAAGGGCGGAGCTGGTGCGGGCGCAGGCATGTACTCACAACATGCCA	
5929CfusionR	CGGGCTGCAGGAATTCTTACATCATGCCACTGGCGT	
Mras1CF	ATCACAACCACCACAATGCGTTACTCAATTGTCTT	For pBar-Hsp70p-Mras- GFP
Mras1CR	GCCCTTGCTCACCATCTCGTCGCGCTTCTGGAGAC	
Mras1GF	ATGGTGAGCAAGGGCGAGGA	
Mras1GR	CGGGCTGCAGGAATTCTTACTTGTACAGCTCGTCCATGC	
Mras2CF	ATCACAACCACCACAATGCGCTACTCCATTGTCTT	
Mras2CR	GCCCTTGCTCACCATATTGCTCTTGCTGCTGCCAA	
Mras3CF	ATCACAACCACCACAATGCGTTACGCTCTTGTTTC	
Mras3CR	GCCCTTGCTCACCATATCACGGTTGATTTCCATGC	
Mras4CF	ATCACAACCACCACAATGGCCTATCATTTAAGTCT	
Mras4CR	GCCCTTGCTCACCATATTGCCACCCTTTTGCTTGC	
Mras5CF	ATCACAACCACCACAATGCATTCTTCAACCATTTAC	
Mras5CR	GCCCTTGCTCACCATAGACTGAAACATGACAGCAC	
Mras6CF	ATCACAACCACCACAATGTACATGGCCCTGGTGG	
Mras6CR	GCCCTTGCTCACCATGAACTTGTTGGCAGCACCG	
Mras7CF	ATCACAACCACCACAATGTCCTTCAAGGCCATCTT	
Mras7CR	GCCCTTGCTCACCATTGCGGTGAACTCGACGGC	
5929HF	ATTCAGATCTACTAGTTGTACTCACAACATGCCACC	For pPc86-Mrst12
5929HR	CCGTTACTTACTTAGAGCTCACGCCAGTGGCATGATGTAA	
Mras1YF	AGTTATTACCCTCGAGCCAGATTCGTGAAATACG	For p178-promoter
Mras1YR	GGCGGATCTGCTCGAGGTGCCAACACCGTCCGTT	
Mras2YF	AGTTATTACCCTCGAGCCAAGCTCTCCTAACCGAAA	
Mras2YR	GGCGGATCTGCTCGAGGCCACACCTGTACCGGAAGTT	
Mras3YF	AGTTATTACCCTCGAGTAATTTGTCCTGCCGAGGTC	
Mras3YR	GGCGGATCTGCTCGAGTAATATCAACGGGAGCAGTTAATATCAACGGGAGCAGTTAA	

	TATCAACGGGAGCAGTAGATGCCATCCCAATAGTGC	
Mras4YF	AGTTATTACCCTCGAGATACAGGGCGCGAGTAAGAA	
Mras4YR	GGCGGATCTGCTCGAGTGAATGCCAGAGCTGATCG	
Mras5YF	AGTTATTACCCTCGAGTCGCTTCTGTTTTGTCTACCA	
Mras5YR	GGCGGATCTGCTCGAGTTAATTTGTCCTGCCGAGGTC	
Mras6YF	AGTTATTACCCTCGAGAAGAACCGCTACGTCGGAC	
Mras6YR	GGCGGATCTGCTCGAGACGTCTGTTCAATCCGCT	
Mras7YF	AGTTATTACCCTCGAGAGAGACGGGGCCGCCGCCT	
Mras7YR	GGCGGATCTGCTCGAGGAGGGTGGTCATCCTGCTT	
Mras1EMSAF	AGCCAGTGGCGATAAGCCAGATTCGTGAAATACG	
Mras1EMSAR	AGCCAGTGGCGATAAGTGCCAACACCGTCCGTT	
Mras2EMSAF	AGCCAGTGGCGATAACCAAGCTCTCCTAACCGA	
Mras2EMSAR	AGCCAGTGGCGATAAGCCACACCTGTACCGGAA	
Mras3EMSAF	AGCCAGTGGCGATAATAATTTGTCCTGCCGAGG	
Mras3EMSAR	AGCCAGTGGCGATAATAATATCAACGGGAGCA	
Mras4EMSAF	AGCCAGTGGCGATAAATACAGGGCGCGAGTAAG	
Mras4EMSAR	AGCCAGTGGCGATAATGAATGCCAGAGCTGATC	
Mras5EMSAF	AGCCAGTGGCGATAATCGCTTCTGTTTTGTCTAC	For EMSA
Mras5EMSAR	AGCCAGTGGCGATAATTAATTTGTCCTGCCGAG	analysis
Mras6EMSAF	AGCCAGTGGCGATAAAAGAACCGCTACGTCGGA	
Mras6EMSAR	AGCCAGTGGCGATAAACGTCTGTTCAATCCGCT	
Mras7EMSAF	AGCCAGTGGCGATAAAGAGACGGGGCCGCCGCC	
Mras7EMSAR	AGCCAGTGGCGATAAGAGGGTGGTCATCCTGCT	
Mras7EMSAFm1	CCACGAGCCTTAAAACGCAGCATG	
Mras7EMSARm1	CATGCTGCGTTTTAAGGCTCGTGG	
Mras7EMSAFm2	CCACGAGCCTAAAAAAACAGCATG	
Mras7EMSARm2	CCACGAGCCTTTTTTTTACAGCATG	

Table S2. Comparison of the LT_{50} values between the WT and *Mrst12* mutants against the last instar larvae of silkworm.

Strains	**Topic infection**		**Injection**	
	LT_{50} (days)	Statistic test*	LT_{50} (days)	Statistic test*
WT	3.02 ± 0.07	–	2.11 ± 0.09	–
Δ*Mrst12*	–	χ^2=81.38; *P*<0.0001	2.31 ± 0.08	χ^2=1.93; *P*=0.1653
Comp	2.99 ± 0.07	χ^2=0.05; *P*=0.8317	2.07 ± 0.07	χ^2=0.18; *P*=0.6677

*, Log-Rank statistic tests were performed between the WT and *Mrst12* mutants.

Supporting figures:

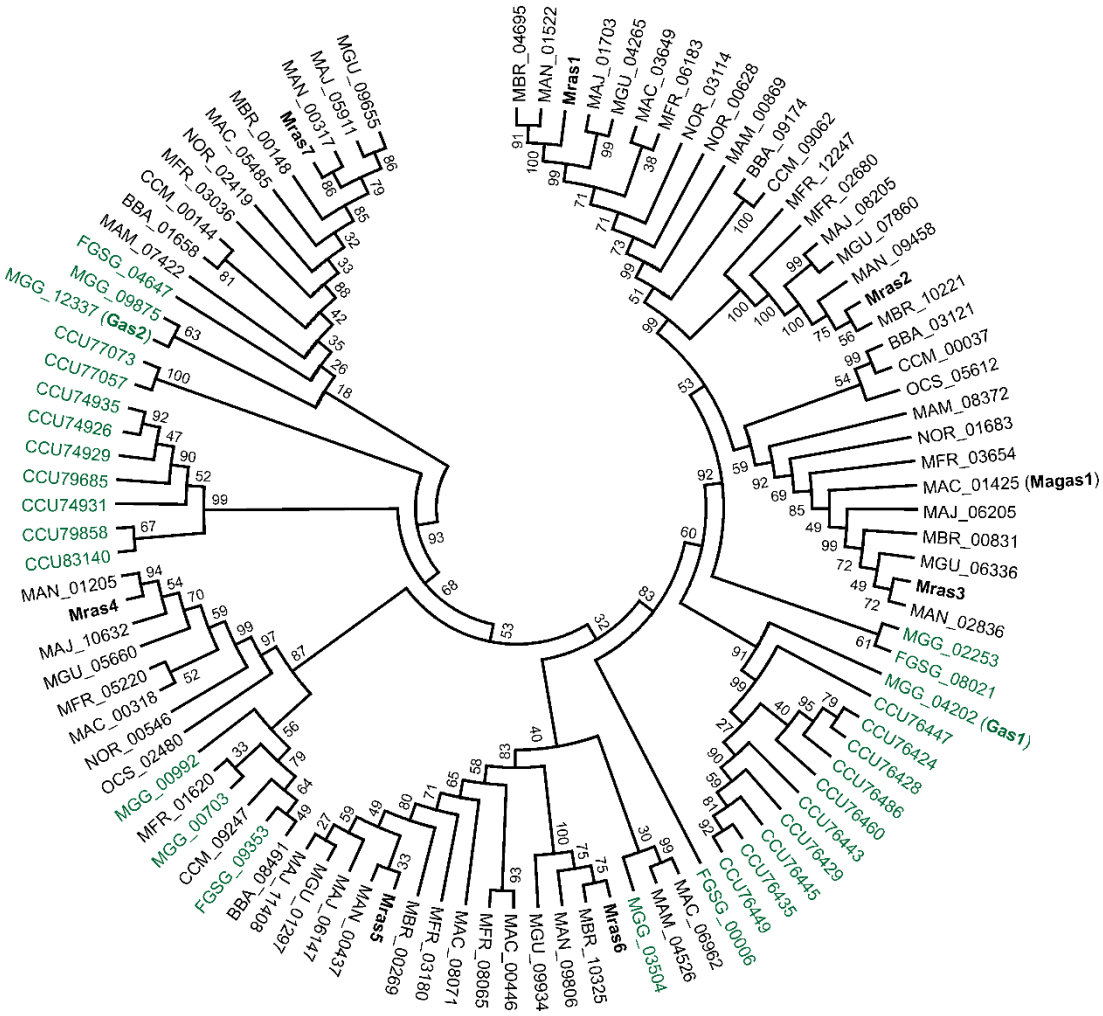

Figure S1. Phylogenetic analysis of the DUF3129 domains retrieved from the proteins from the selected insect and plant fungal pathogens. The selected insect pathogens include: MAC, *Metarhizium acridum*; MAN, *M. anisopliae*; MBR, *M. brunneum*; MGU, *M. guizhouense*; MAJ, *M. majus*; BBA, *Beauveria bassiana*; CCM, *Cordyceps militaris*; OCS, *Ophiocordyceps sinensis*. The selected plant pathogenic fungi (genes labelled in green) include: MGG, *Magnaporthe oryzae*; FGSG, *Fusarium graminearum*; CCU, *Blumeria graminis* f. sp. *hordei*.

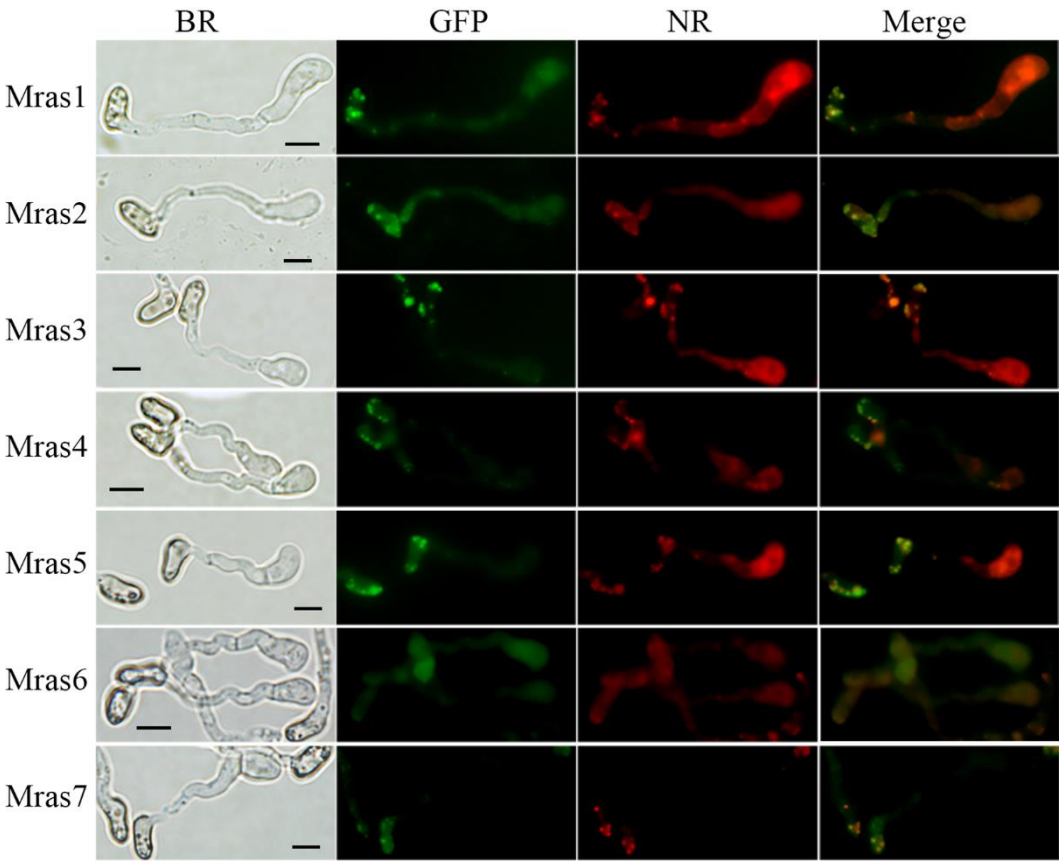

Figure S2. Localization of Mras proteins on LD surface during fungal formation of appressoria. The spores of the mutants expressing the Mras-GFP fusion protein were induced for 18 hrs on the hydrophobic plastic plate surface containing a minimum medium supplemented with 1% glycerol. Fungal cells were then stained with the dye Nile red (NR). BR, bright field image. Bar, 5μm.

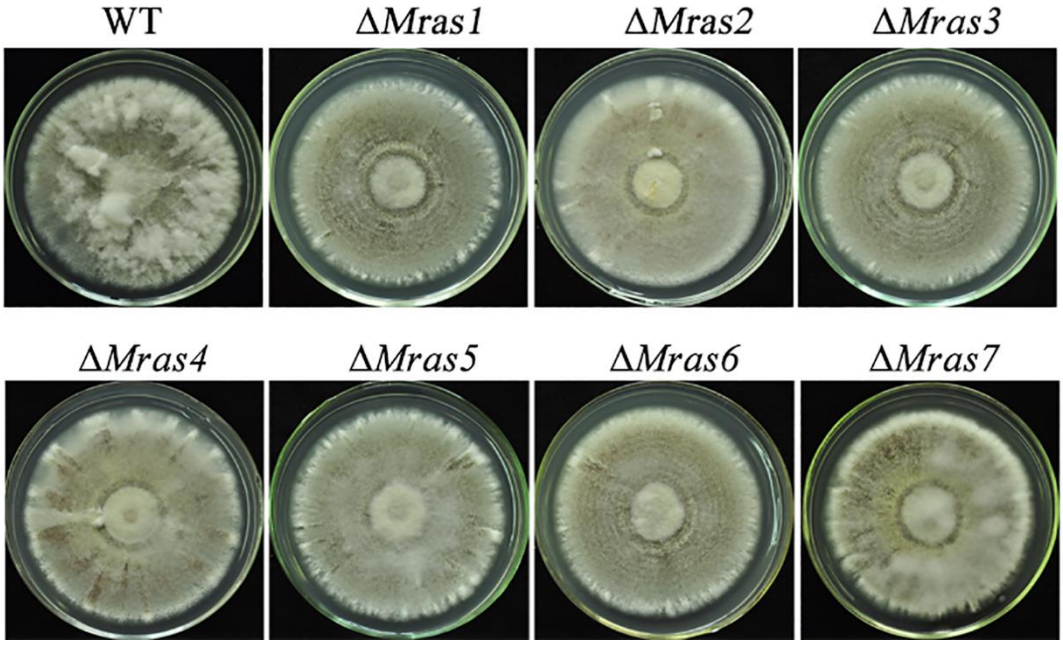

Figure S3. Phenotyping of gene deletion mutants. Both the WT and *Mras* null mutants were inoculated on PDA and incubated at 25 °C for two weeks.

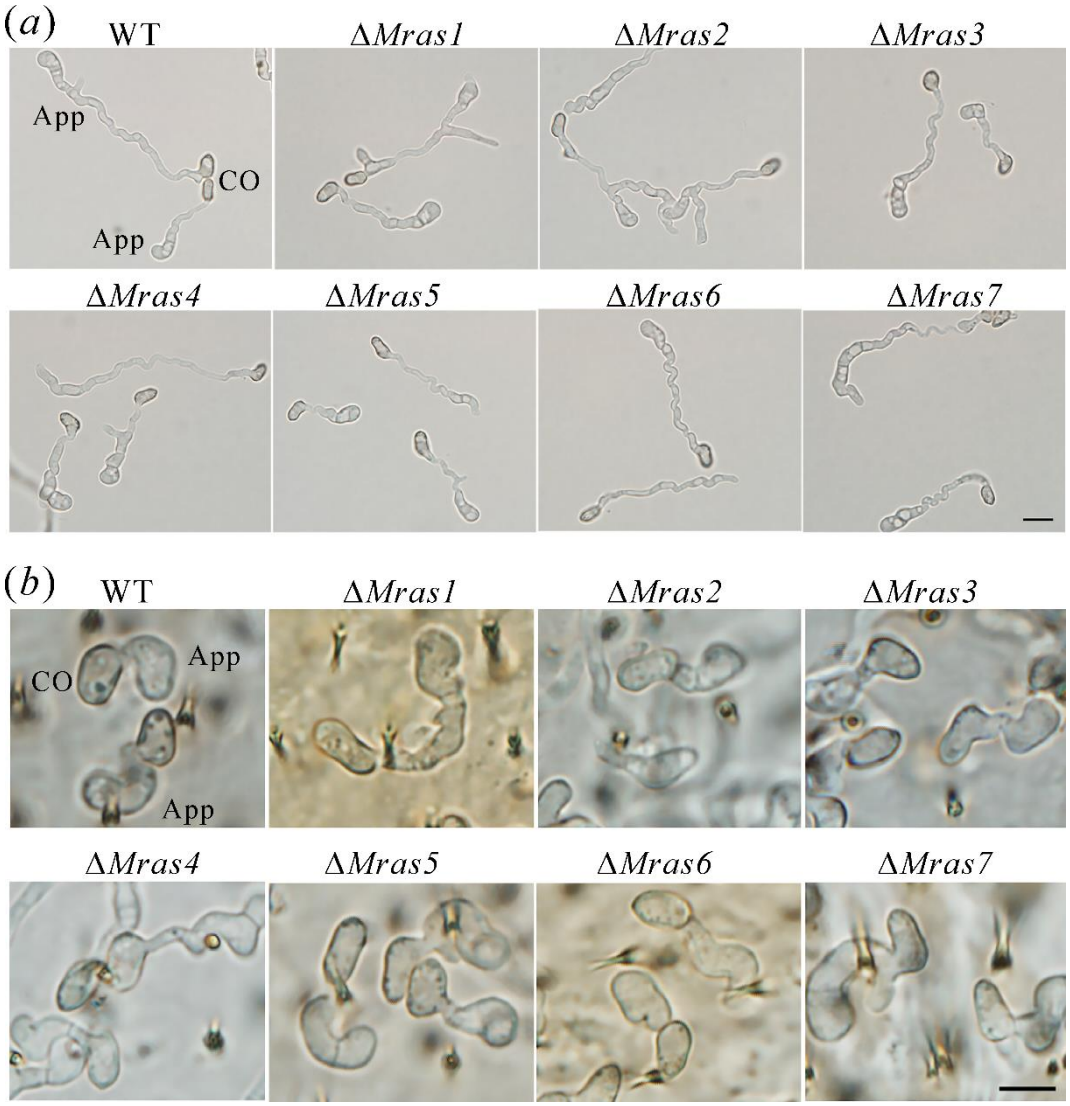

Figure S4. Appressorium induction. (*a*) Conidia of the WT and *Mras* null mutants were induced for 18 hrs on the hydrophobic plate surface containing a minimum medium supplemented with 1% glycerol. Bar, 10 μm. (*b*) Fungal spores were induced for 18 hrs on the hind wings of mealworm. App, appressorium; CO, conidia. Bar, 5 μm.

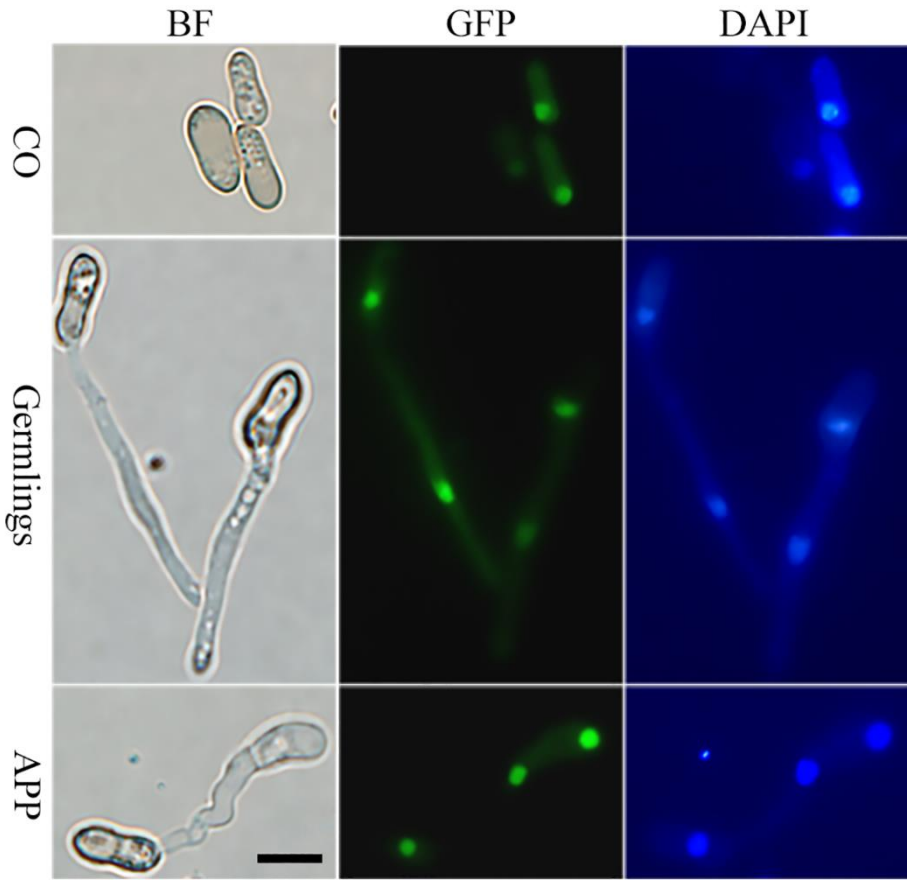

Figure S5. Nuclear localization of Mrst12 in different type of cells. The GFP-fused Mrst12 signal are co-localized with the nuclenus-staining dye DAPI in the conidia (CO), germlings (spores germinated in SDB for 18 hrs) and appressorium (App). Bar, 5 μm.

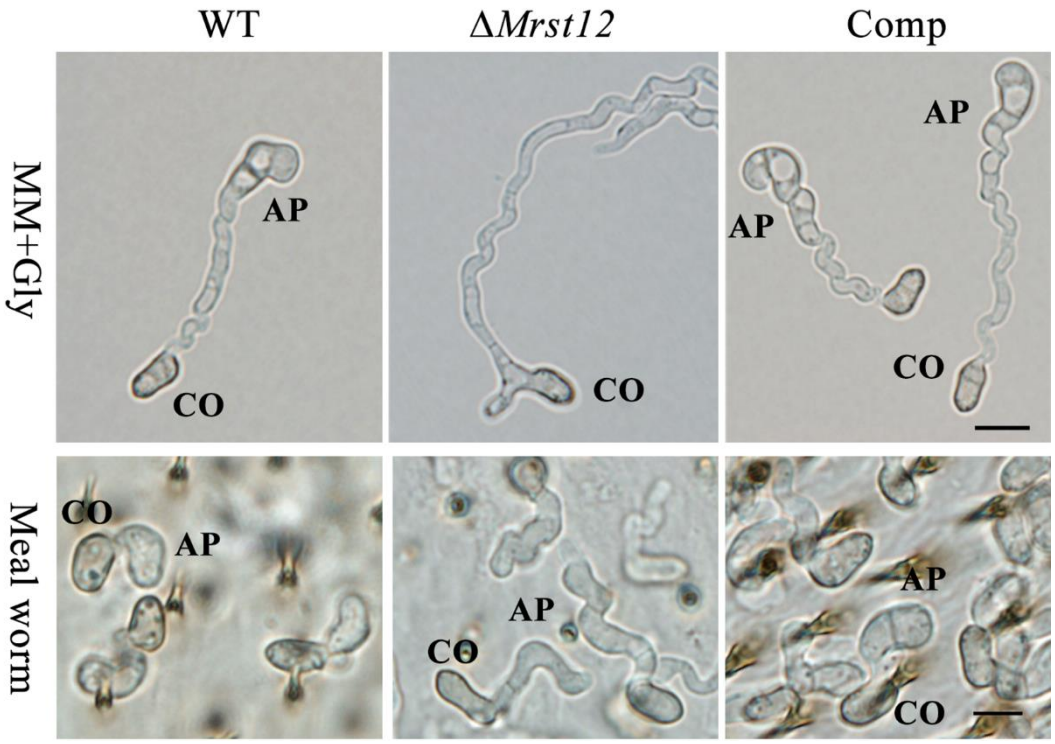

Figure S6. Appressorium induction. Conidia of the WT, Δ*Mrst12* and the complemented mutant (Comp) of Δ*Mrst12* were induced for 18 hrs on the hydrophobic plate surface containing a minimum medium supplemented with 1% glycerol (MM+Gly; Bar, 10 μm) and the hind wings of mealworm, respectively. AP, appressorium; CO, conidia. Bar, 5 μm.

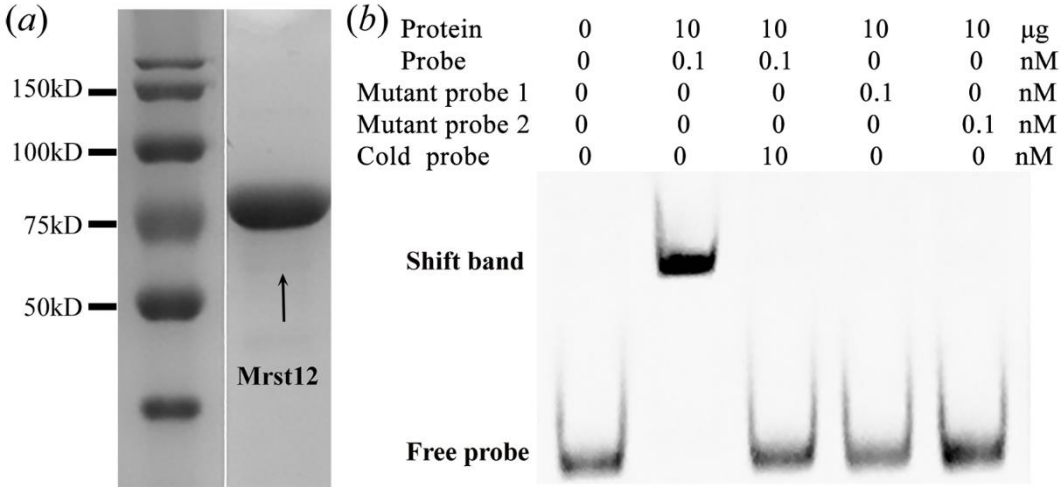

Figure S7. Mrst12 purification and negative treatment of EMSA analysis. (*a*) Protein expression and purification of Mrst12 (arrowed on gel). (*b*) A negative control assay of EMSA analysis. The consensus binding motif YGAMAMR of the *Mras7* (MAA_05223) gene promoter was mutated to CGACAAG (for mutant probe 1) and AAAAAA (for mutant probe 2) for EMSA analysis. Competition was achieved by adding 10 nM of unlabeled (cold) WT probe.