

Supplementary Table S2. Primers used in this study.

Primer	Sequence (5'-3')	Application
<i>MoSSBI</i> -Flank-F1	TAACTCGAGCAAGCCTTCCATGAACTG A	Amplifying <i>MoSSBI</i> 5' flank sequence for gene knock out
<i>MoSSBI</i> -Flank-R1	TAAATCGATCTTTGCGATGGTTTTGGGC	
<i>MoSSBI</i> -Flank-F2	TAAACTAGTGGTCGTGGCGTTTTGCATC CTAT	Amplifying <i>MoSSBI</i> 3' flank sequence for gene knock out
<i>MoSSBI</i> -Flank-R2	TAACCGCGGTCTTGTTCCAGGCACGCT	
<i>MoSSBI</i> -probe-F	TAACCTACGAGGGCAGCAATGTC	Amplifying probe <i>GENE</i> for southern blot and transformants screen
<i>MoSSBI</i> -probe-R	TAAAACCATGGCCGAGATCTCCT	
FL1111	GGAGGTCAACACATCAATG	Amplifying probe <i>HPH</i> for southern blot
FL1112	CTCTATTCCTTTGCCCTCG	
<i>MoSSBI</i> -OF	TAAATCGGGACCGGGCGGGTTGT	Transformants screen
<i>HPH</i> comR	GCTGATCTGACCAGTTGCCTA	
<i>MoSSBI</i> -comF	ACTCACTATAGGGCGAATTGGGTACTCA AATTGGTTATCGTTTCAGTAAAGCCGAC CT	<i>MoSSBI</i> complementation
<i>MoSSBI</i> -comR	CACCACCCCGGTGAACAGCTCCTCGCCC TTGCTCACGCGGGAAGACATGGCCTTGG T	
BD- <i>MoSSBI</i> -F	TAACATATGATGTCGACAGAAGTTTATGA C	Construction of pGBKT7- <i>MoSSBI</i>
BD- <i>MoSSBI</i> -R	TAAGAATTCTTAGCGGGAAGACATGGCC TT	
<i>MoSSBI</i> -CYFP-F	CGACTCACTATAGGGCGAATTGGGTACT CAAATTGATCGTTTCAGTAAAGCCGACC T	Construction of pHz65- <i>MoSSBI</i>
<i>MoSSBI</i> -CYFP-R	GCTCACCATCGTGGCGATGGAGCGGCGG GAAGACATGGCCTTGGT	
<i>MoSSBI</i> -V443F-F	GCCCTGGTAGACGGGGAAGTCTGTTCTGTT TGCTGCTGGTCAGCAAC	<i>MoSSBI</i> point mutation
<i>MoSSBI</i> -V443F-R	GTTGCTGACCAGCAGCAAACAGAACAG TTCCCCGTCTACCAGGGC	
<i>MoSSZI</i> -Flank-F1	CCCAAGCTTATTCAGCGGCCCAAGGCCG T	Amplifying <i>MoSSZI</i> 5' flank sequence for gene knock out
<i>MoSSZI</i> -Flank-R1	GATATCTTGAAAGCTCTGATTGGAG	
<i>MoSSZI</i> -Flank-F2	CCCAAGCTTTGATTTGAGTTATGACTATT	Amplifying <i>MoSSZI</i> 3' flank sequence for gene knock out
<i>MoSSZI</i> -Flank-R2	TCCCCGCGGAGGACATTGTGAGACAGA GC	
<i>MoSSZI</i> -probe-F	TAAGCTGTTGCGCCTGAGGACCG	Amplifying probe <i>GENE</i> for southern blot and transformants screen
<i>MoSSZI</i> -probe-R	TAACGGCCACATCAGACTTGGTG	
<i>MoSSZI</i> -OF	TAATGTGGCATGAGTAACCTCCTA	Transformants screen with <i>HPH</i> comR
<i>MoSSZI</i> -comF	ACTCACTATAGGGCGAATTGGGTACTCA AATTGGTTTGTGGCATGAGTAACCTCCTA	<i>MoSSZI</i> complementation

<i>MoSSZ1-comR</i>	CACCACCCCGGTGAACAGCTCCTCGCCC TTGCTCACCAATGTACCCCTGACACCAC C	
<i>MoSSZ1-S-F</i>	TTTCGTAGGAACCCAATCTTCAAATGA GCGAAAACGGCACAAAG	Construction of <i>MoSSZ1-S</i>
<i>MoSSZ1-S-R</i>	TTCGAATTTAGCAGCAGCGGTTTCTTTC AATGTACCCCTGACACCACC	
AD/BD- <i>MoSSZ1-F</i>	TAACATATGATGAGCGAAAACGGCACAA AG	Construction of pGADT7- <i>MoSSZ1</i> and pGBKT7- <i>MoSSZ1</i>
AD/BD- <i>MoSSZ1-R</i>	TAAGAATTCTCACAATGTACCCCTGACA CC	
<i>MoSSZ1-NYFP/ CYFP-F</i>	CGACTCACTATAGGGCGAATTGGGTACT CAAATTGTGTGGCATGAGTAACCTCCTA	Construction of pHz65- <i>MoSSZ1</i> and pHz68- <i>MoSSZ1</i>
<i>MoSSZ1-NYFP-R</i>	GCTCACCATCGTGGCGATGGAGCGCAAT GTACCCCTGACACCACC	
<i>MoSSZ1-CYFP-R</i>	GTTCGGGATCTTGCAGGCCGGGCGCAAT GTACCCCTGACACCACC	
<i>MoZUO1-Flank-F1</i>	CCGCTCGAGCTCGGCATGGCATCTGGCG T	Amplifying <i>MoZUO1</i> 5' flank sequence for gene knock out
<i>MoZUO1-Flank-R1</i>	CCAAGCTTTTTGCCTTCACTAACAAGA T	
<i>MoZUO1-Flank-F2</i>	GGACTAGTATATTTAGTTATGTGTTGAT	Amplifying <i>MoZUO1</i> 3' flank sequence for gene knock out
<i>MoZUO1-Flank-R2</i>	TCCCCGCGGCTCCGGGAACCATCACCAG C	
<i>MoZUO1-probe-F</i>	TAATACCGCTACAATAGTCAAGA	Amplifying probe <i>GENE</i> for southern blot and transformants screen
<i>MoZUO1-probe-R</i>	TAAATCCTCGTCCAGGTACTCGA	
<i>MoZUO1-OF</i>	TAAATGATGATTATGCTGGTGTTG	Transformants screen with <i>HPHcomR</i>
<i>MoZUO1-comF</i>	ACTCACTATAGGGCGAATTGGGTACTCA AATTGGTTATGATGATTATGCTGGTGTTG	<i>MoZUO1</i> complementation
<i>MoZUO1-comR</i>	CACCACCCCGGTGAACAGCTCCTCGCCC TTGCTCACAGCAAAGACTTGGCCTCGC	
<i>MoZUO1-S-F</i>	TTTCGTAGGAACCCAATCTTCAAATGG CTACCGCTACAATAGT	Construction of <i>MoZUO1-S</i>
<i>MoZUO1-S-R</i>	TTCGAATTTAGCAGCAGCGGTTTCTTTAG CAAAGACTTGGCCTCGC	
AD- <i>MoZUO1-F</i>	TAACATATGATGGCTACCGCTACAATAGT C	Construction of pGADT7- <i>MoZUO1</i>
AD- <i>MoZUO1-R</i>	TAAGAATTCCTAAGCAAAGACTTGGCC TC	
<i>MoZUO1-NYFP-F</i>	CGACTCACTATAGGGCGAATTGGGTACT CAAATTGCTCGGCATGGCATCTGGCGT	Construction of pHz65- <i>MoZUO1</i>
<i>MoZUO1-NYFP-R</i>	GCTCACCATCGTGGCGATGGAGCGAGCA AAAGACTTGGCCTCGC	
<i>MoMKK1-S-F</i>	TTTCGTAGGAACCCAATCTTCAAATGA AGAATCCCATATCTAGT	Construction of <i>MoMKK1-S</i>
<i>MoMKK1-S-R</i>	TTCGAATTTAGCAGCAGCGGTTTCTTCT	

	CTGCAGGCTTGGCTTCACCC	
<i>AD-MoMKK1-F</i>	TAACATATGATGCACGATCAAGAAGCTGCC	Construction of <i>pGADT7-MoMKK1</i>
<i>AD-MoMKK1-R</i>	TAAGAATTCTCACTCTGCAGGCTTGGCTT	
<i>MoMKK1-NYFP-F</i>	CGACTCACTATAGGGCGAATTGGGTACTCAAATTGGGTATGTTTCGCCGAAAATG	Construction of <i>pHz65-MKK1</i>
<i>MoMKK1-NYFP-R</i>	GCTCACCATCGTGGCGATGGAGCGCTCTGCAGGCTTGGCTTCA	
<i>MoMKK1-GFP-F</i>	ACTCACTATAGGGCGAATTGGGTACTCAAATTGGTTGGTATGTTTCGCCGAAAATG	Construction of <i>MoMKK1-GFP</i>
<i>MoMKK1-GFP-R</i>	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCTCTGCAGGCTTGGCTTCA	
<i>MoMKK1-GFP-qRT-F</i>	AGCTACTACATGGCGCCCGAG	qRT-PCR Primer of <i>MoMKK1-GFP</i>
<i>MoMKK1-GFP-qRT-R</i>	CTCACTATAGGGCGAATTGGG	

Supplementary Table S1. The putative MoMkk1-interacting proteins identified by co-IP

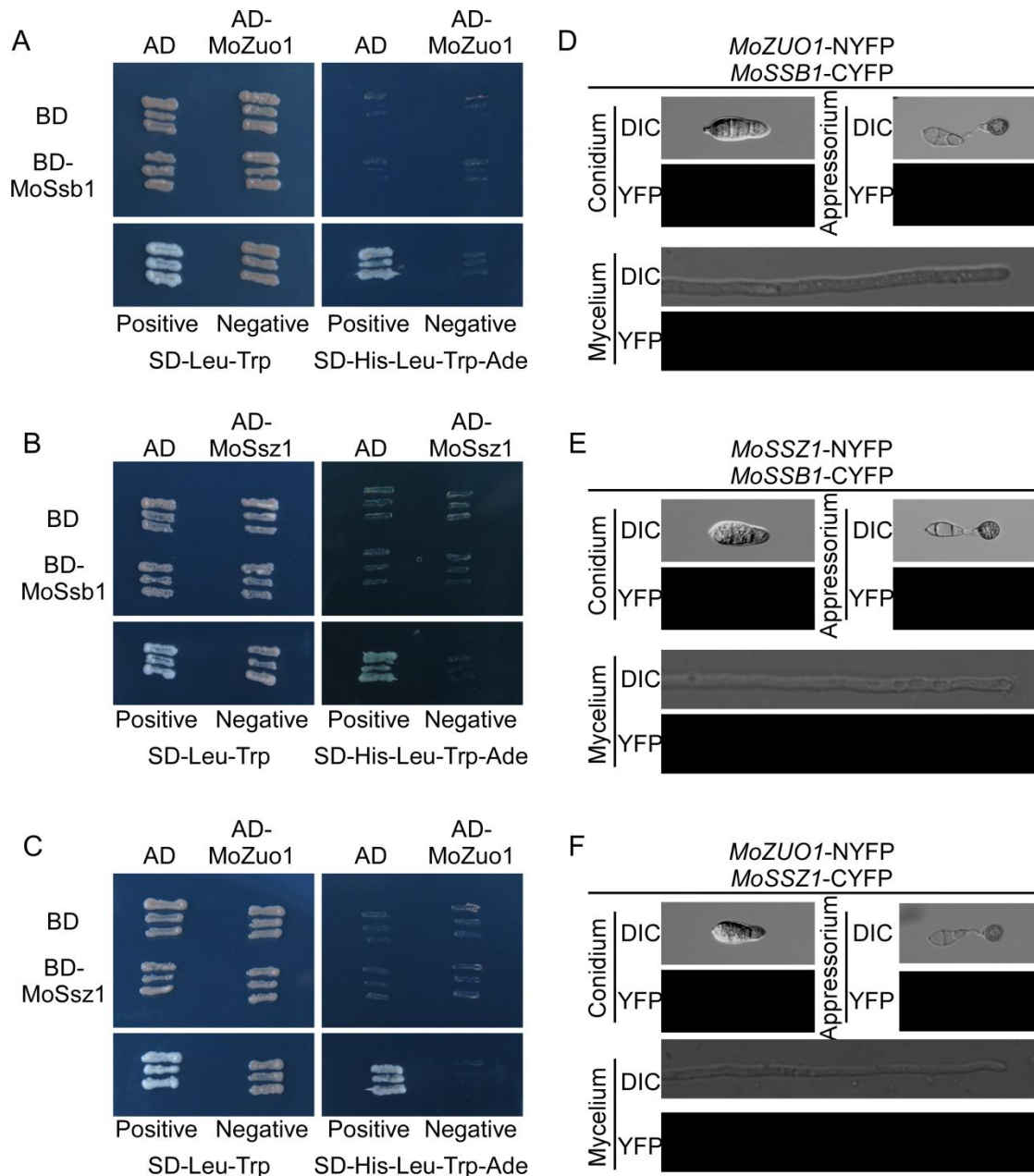
Gene ID	Putative protein name	
MGG_01084	glyceraldehyde-3-phosphate dehydrogenase	
MGG_11259	hypothetical protein	
MGG_02624	transaldolase	
MGG_04400	ATP-dependent RNA helicase eIF4A	
MGG_05673	40S ribosomal protein S3	
MGG_04467	60S acidic ribosomal protein P0	
MGG_00175	6-phosphogluconate dehydrogenase	
MGG_16149	ADP ATP carrier protein	
MGG_06530	aspartate aminotransferase	
MGG_08905	triosephosphate isomerase	
MGG_06712	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	S-
MGG_10265	hypothetical protein	
MGG_09952	GTP-binding nuclear protein GSP1/Ran	
MGG_08622	nucleoside diphosphate kinase	
MGG_10447	peptidyl-prolyl cis-trans isomerase	
MGG_02659	60S ribosomal protein L14	
MGG_04719	guanine nucleotide-binding protein subunit beta-like protein	
MGG_03641	elongation factor 1-alpha	
MGG_06269	60S ribosomal protein L2	
MGG_05155	adenosylhomocysteinase	
MGG_05063	phosphoglycerate kinase	
MGG_10607	enolase	
MGG_00349	40S ribosomal protein S13	
MGG_06919	40S ribosomal protein S3aE	
MGG_06720	ATP-citrate synthase subunit 2	
MGG_03653	60S ribosomal protein L20	
MGG_13806	14-3-3 family protein	
MGG_16204	60S ribosomal protein L12	
MGG_06958	hsp70-like protein	
MGG_03982	actin	

MGG_04438	ADP-ribosylation factor
MGG_06936	elongation factor 1-gamma
MGG_02370	mitochondrial phosphate carrier protein
MGG_06685	cytochrome c
MGG_05661	40S ribosomal protein S18
MGG_00942	branched-chain-amino-acid aminotransferase
MGG_04104	60S ribosomal protein L22
MGG_07752	ATP synthase subunit alpha
MGG_04829	60S ribosomal protein L9-A
MGG_07753	60S ribosomal protein L11
MGG_03372	40S ribosomal protein S20
MGG_03226	cytochrome b-c1 complex subunit 2
MGG_17802	hypothetical protein
MGG_16486	hypothetical protein
MGG_06658	40S ribosomal protein S5
MGG_04484	60S ribosomal protein L18
MGG_05238	40S ribosomal protein S14
MGG_11513	heat shock protein SSB1
MGG_01592	eukaryotic translation initiation factor 2 subunit gamma
MGG_04489	non-histone chromosomal protein 6
MGG_04612	60S ribosomal protein L8-B
MGG_03185	ATP synthase subunit beta
MGG_08741	tRNA selenocysteine-associated protein 1
MGG_02660	nascent polypeptide-associated complex subunit alpha
MGG_05237	60S ribosomal protein L7
MGG_00968	mitochondrial outer membrane protein porin
MGG_10185	60S ribosomal protein L23a
MGG_07202	citrate synthase
MGG_11776	40S ribosomal protein S0
MGG_03670	subtilisin-like proteinase Spm1
MGG_03152	ATP synthase subunit 5
MGG_02872	40S ribosomal protein S27
MGG_16866	cytochrome b-c1 complex subunit Rieske

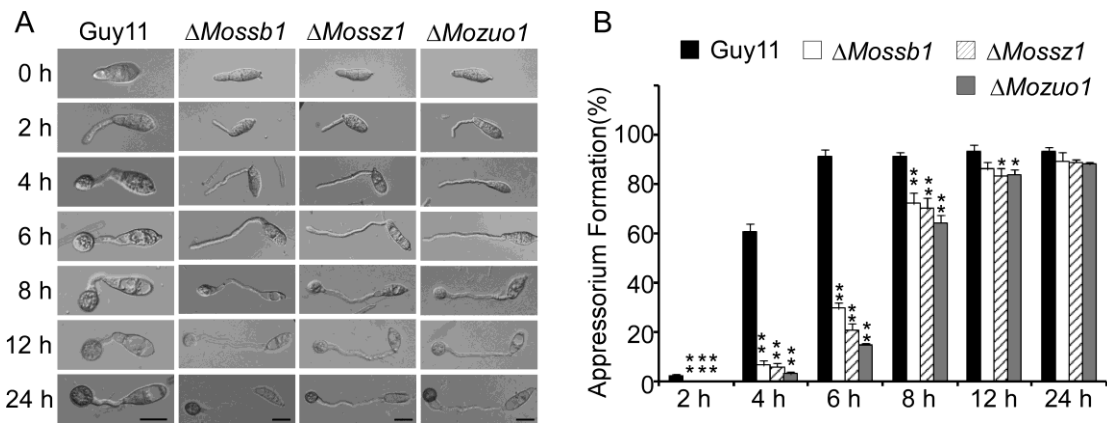
MGG_01113	40S ribosomal protein S10-A
MGG_06759	heat shock protein 90
MGG_06249	translationally controlled tumor protein
MGG_06693	60S ribosomal protein L27-A
MGG_09367	malate dehydrogenase
MGG_06668	hypothetical protein
MGG_03600	mitochondrial-processing peptidase subunit beta
MGG_04114	40S ribosomal protein S17
MGG_01588	14-3-3 family protein
MGG_03165	heat shock protein 60
MGG_08190	60S ribosomal protein L4-A
MGG_02747	40S ribosomal protein S4-A
MGG_03727	50S ribosomal protein L31e
MGG_05283	uricase
MGG_02710	peroxiredoxin type-2
MGG_02921	hypothetical protein
MGG_01742	elongation factor 2
MGG_13645	50S ribosomal protein L13e
MGG_06691	4-hydroxyphenylpyruvate dioxygenase
MGG_01712	isocitrate dehydrogenase subunit 2
MGG_07130	proteasome component PUP2
MGG_08835	malate dehydrogenase
MGG_12569	hypothetical protein
MGG_01995	isocitrate dehydrogenase subunit 1
MGG_13200	eukaryotic translation initiation factor 2 subunit alpha
MGG_10292	hypothetical protein
MGG_12797	hypothetical protein
MGG_09396	HET-C2 protein
MGG_15774	ketol-acid reductoisomerase
MGG_04135	mitochondrial F1F0 ATP synthase subunit F
MGG_15739	inorganic pyrophosphatase
MGG_03051	aspartate-semialdehyde dehydrogenase
MGG_13563	hypothetical protein

MGG_01689	rho GDP dissociation inhibitor
MGG_01092	homocitrate synthase
MGG_04444	60S ribosomal protein L6-B
MGG_07076	oxidoreductase
MGG_02953	60S ribosomal protein L21-B
MGG_08810	2 5-diketo-D-gluconic acid reductase A
MGG_17072	dihydrolipoyl dehydrogenase
MGG_01111	cytochrome c oxidase subunit 6B
MGG_04752	ATP synthase subunit 4
MGG_08637	mitochondrial import receptor subunit tom-40
MGG_10569	pyruvate dehydrogenase E1 component subunit beta
MGG_03065	V-type proton ATPase proteolipid subunit 2
MGG_05980	pyridoxine biosynthesis protein PDX1
MGG_15868	argininosuccinate synthase
MGG_06127	profilin
MGG_18003	ATP synthase subunit D
MGG_03149	dihydrolipoyllysine-residue succinyltransferase
MGG_13499	acetyl-CoA acetyltransferase
MGG_01807	NEDD8-conjugating enzyme UBC12
MGG_02252	tetrahydroxynaphthalene reductase
MGG_05892	hypothetical protein
MGG_02525	alanine-glyoxylate aminotransferase 1
MGG_13438	xaa-Pro dipeptidase
MGG_10583	12-oxophytodienoate reductase 1
MGG_06479	40S ribosomal protein S22
MGG_10568	sterol 24-C-methyltransferase
MGG_15540	hypothetical protein
MGG_07048	60S ribosomal protein L5
MGG_10252	hypothetical protein
MGG_15527	hypothetical protein
MGG_03521	aconitate hydratase
MGG_12574	aspartyl-tRNA synthetase
MGG_09194	60S ribosomal protein L17

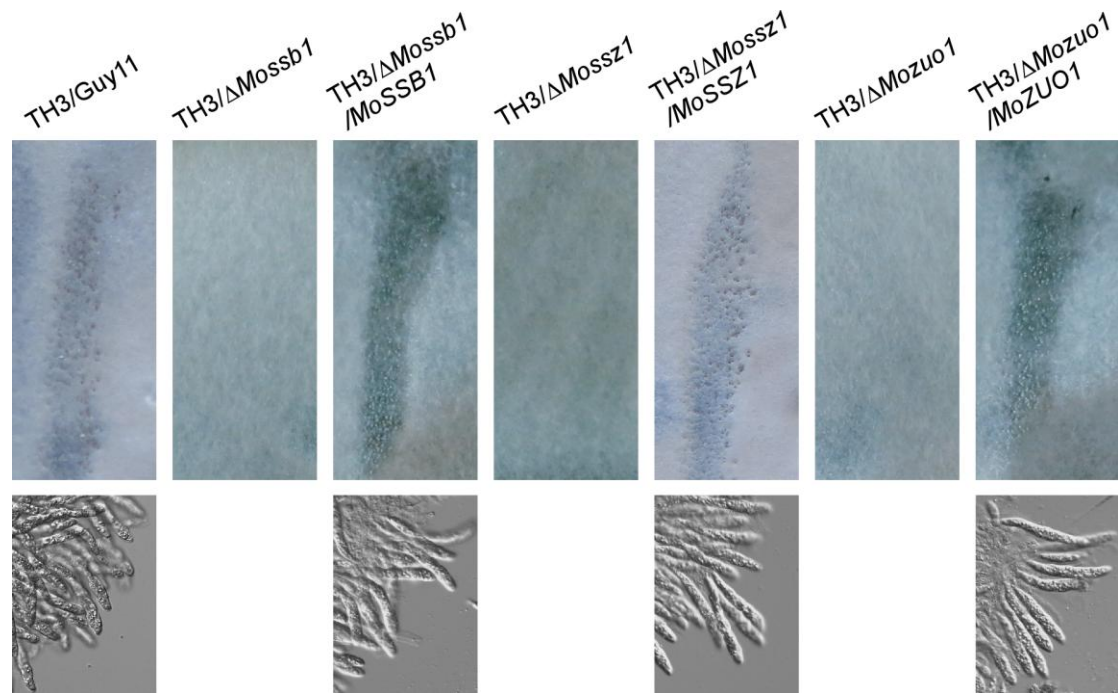
MGG_12868	enoyl-CoA hydratase
MGG_01521	cell division control protein 3
MGG_02697	serologically defined colon cancer antigen 1
MGG_00221	40S ribosomal protein S7
MGG_01631	UTP-glucose-1-phosphate uridylyltransferase
MGG_03578	histone H2B



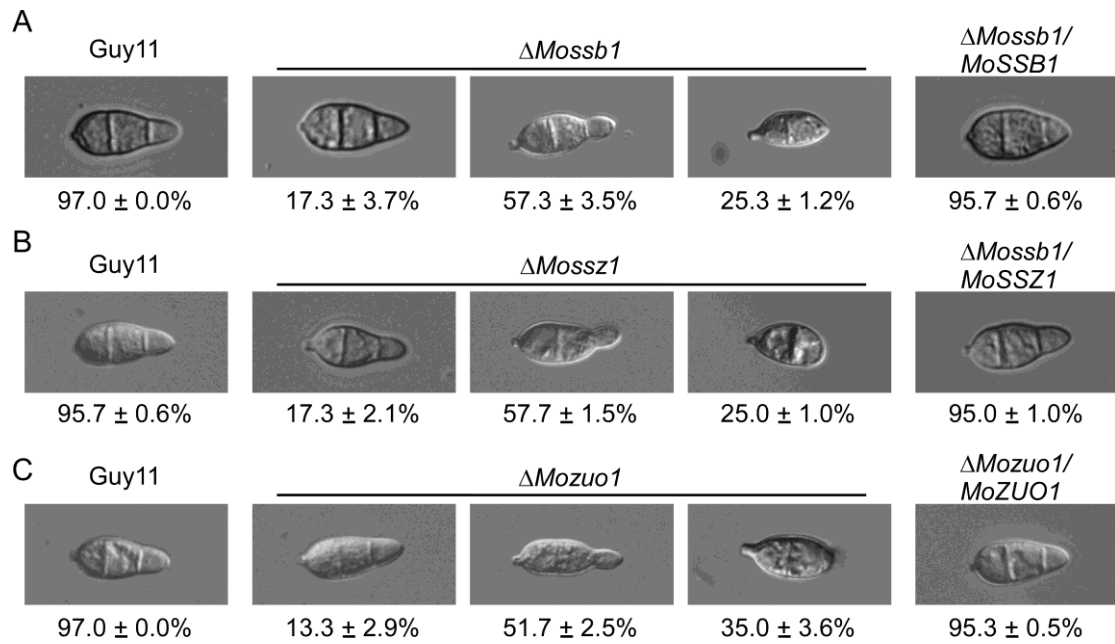
Supplementary Figure S8. The interactions among MoSsb1, MoSsz1 and MoZuo1 could not be established by yeast two-hybrid and BiFC. A-C, Yeast two hybrid assays for the interaction among MoSsb1, MoSsz1 and MoZuo1. The transformants were grown on SD-Leu-Trp and on selective SD-Leu-Trp-His-Ade for 3 d. No yeast cells expressing the pGADT7-*MoZUO1*/pGBKT7-*MoSSB1*, pGADT7-*MoSSZ1*/pGBKT7-*MoSSB1* and pGADT7-*MoZUO1*/pGBKT7-*MoSSZ1* were grown on selective SD-Leu-Trp-His-Ade. D-F, BiFC assays for the interaction among MoSsb1, MoSsz1 and MoZuo1. Mycelium, conidium and appressorium stages of transformants expressing the *MoZUO1*-NYFP/*MoSSB1*-CYFP, *MoSSZ1*-NYFP/*MoSSB1*-CYFP and *MoZUO1*-NYFP/*MoSSZ1*-CYFP constructs were tested by both DIC and epifluorescence microscopy. More than 100 samples of every stage were observed and no yellow fluorescent was detected.



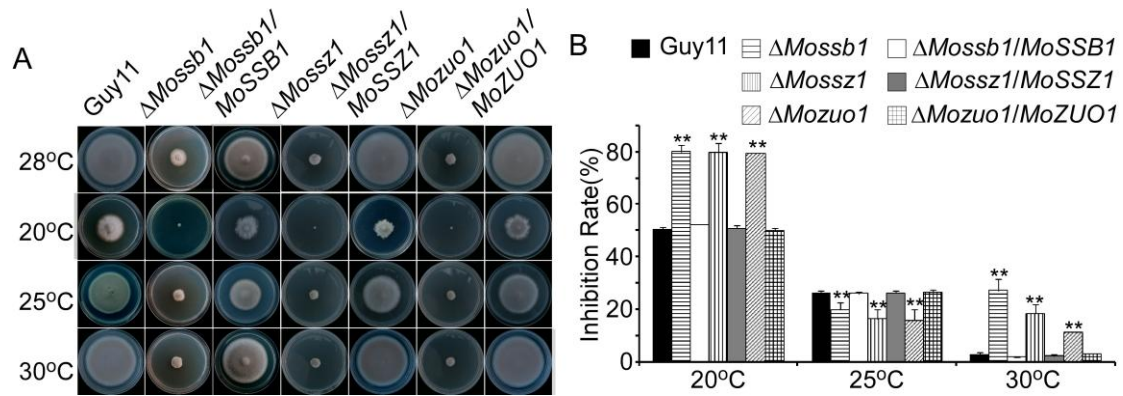
Supplementary Fig. S7. Appressorium formation assay. Conidia of the indicated strains were harvested from 10-day-old SDC agar cultures, filtered through one-layer Miracloth. Appressorial formation were observed and counted on hydrophobic slides at 0, 2, 4, 6, 8, 12 and 24 h and then photographed. At least 100 conidia per replicate were examined. The experiment was performed three times with similar results. Asterisks indicate significant differences at $P < 0.01$, according to Duncan's range test.



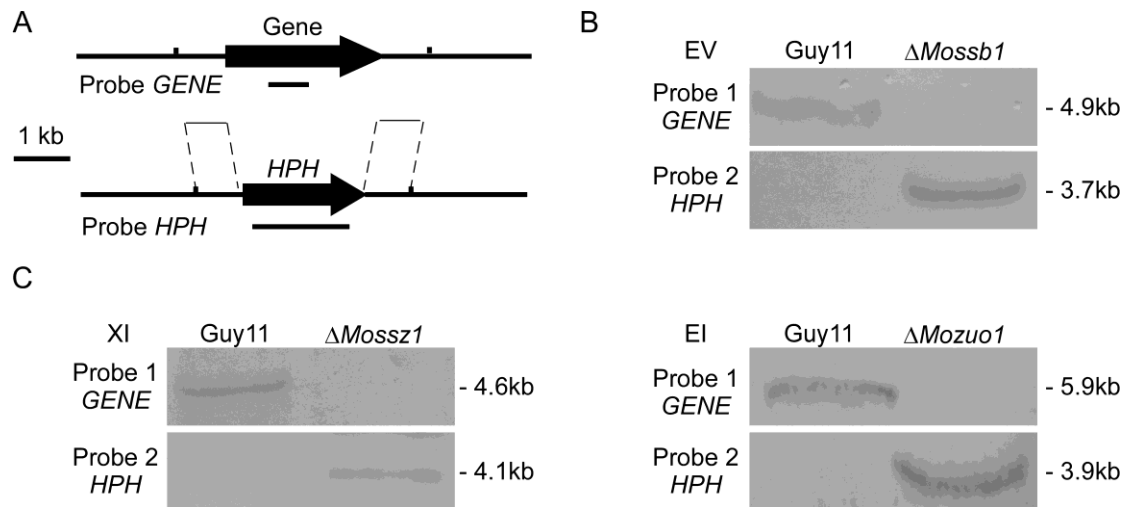
Supplementary Fig. S6. MoSsb1, MoSsz1 and MoZuo1 are involved in mating. Perithecia production was photographed following three weeks of incubation. Cross between TH3 (*MATI-1*) and Guy11 (*MATI-2*) represents the positive control. Cross between TH3 and each mutant failed to produce perithecia or ascospores. Cross between the complemented strain and TH3 produce normal perithecia and ascospores. The experiment was performed three times with similar results.



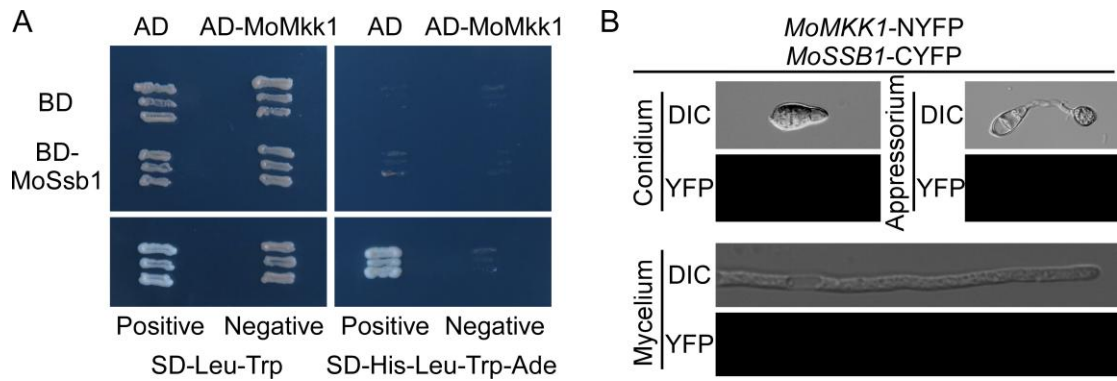
Supplementary Fig. S5. Conidia shape comparison. A-C, Conidia of the indicated strains were harvested from SDC medium and observed under a microscope.



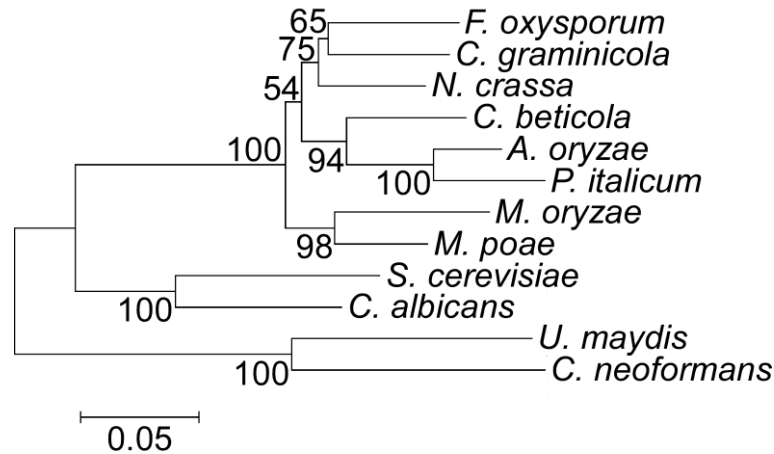
Supplementary Fig. S4. Resistance of $\Delta Mossb1$, $\Delta Mossz1$ and $\Delta Mozuo1$ mutants to temperature stress inducer. A, Growth of Guy11, $\Delta Mossb1$, $\Delta Mossz1$, $\Delta Mozuo1$ mutants and their complemented strains in media cultured in 20, 25, 28 and 30°C after 10 days before being photographed. B, Colony diameters were measured in each independent biological experiment after 10 days. The growth inhibition in 20, 25 and 30°C is relative to the growth rate in 28°C. Three independent biological experiments were performed with three replicates each time. Error bars represent standard deviation and asterisks represent significant differences ($P < 0.01$)



Supplementary Fig. S3. Southern blot analysis of the *MoSSBI*, *MoSSZI* and *MoZUOI* gene deletion mutants. A, Strategy of knockout in the *M. oryzae* genome. Thick arrows indicate the orientations of the genes and *HPH* genes. Thin lines below the arrows indicate the probe sequence of each gene. B, Southern blot analysis of *MoSSBI* gene knockout mutant with the gene-specific probe and hygromycin phosphotransferase (*HPH*) probe. The restriction enzyme *EcoRV* was used for Southern blot. C, Southern blot analysis of *MoSSZI* and *MoZUOI* gene knockout mutants with the gene-specific probe and *HPH* probe. The restriction enzyme *XhoI* and *EcoRI* were used for Southern blot, respectively.



Supplementary Fig. S2. The interactions between MoSsb1 and MoMkk1 could not be established by yeast two-hybrid and BiFC. A, Yeast two hybrid assays for the interaction between MoSsb1 and MoMkk1. MoSsb1 was inserted into vector pGBKT7 and MoMkk1 was inserted into pGADT7. The transformants were plated on SD-Leu-Trp and on selective SD-Leu-Trp-His-Ade for 3 d. Yeast cells expressing the pGADT7-*MoMkk1* and pGBKT7-*MoSsb1* failed to grow on selective SD-Leu-Trp-His-Ade. B, BiFC assays for the interaction between MoSsb1 and MoMkk1. Mycelium, conidium and appressorium stages of transformants expressing the *MoMkk1*-NYFP/*MoSsb1*-CYFP constructs were tested by both DIC and epifluorescence microscopy. More than 100 samples of every stage were observed and no yellow fluorescent was detected.



Supplementary Fig. S1. Phylogenetic analysis of MoSsb1. Neighbour-joining tree with 1000 bootstrap replicates of phylogenetic relationships between MoSsb1 homologues in fungi. The sequences were collected from the National Center for Biotechnology Information (NCBI) database. Abbreviations numbers correspond to species names and GenBank accession numbers are as follows: *F. oxysporum* (*Fusarium oxysporum* XP_018233273.1), *C. graminicola* (*Colletotrichum graminicola* XP_008089456.1), *N. crassa* (*Neurospora crassa* XP_964602.3), *A. oryzae* (*Aspergillus oryzae* OOO07910.1), *S. cerevisiae* (*Saccharomyces cerevisiae* NP_014190.1), *C. albicans* (*Candida albicans* XP_716208.1), *U. maydis* (*Ustilago maydis* XP_011391166.1), *C. neoformans* (*Cryptococcus neoformans* XP_012046659.1), *P. italicum* (*Penicillium italicum* KGO65718.1), *C. beticola* (*Cercospora beticola* XP_023452832.1) and *M. poae* (*Magnaportheopsis poae* KLU88584.1).