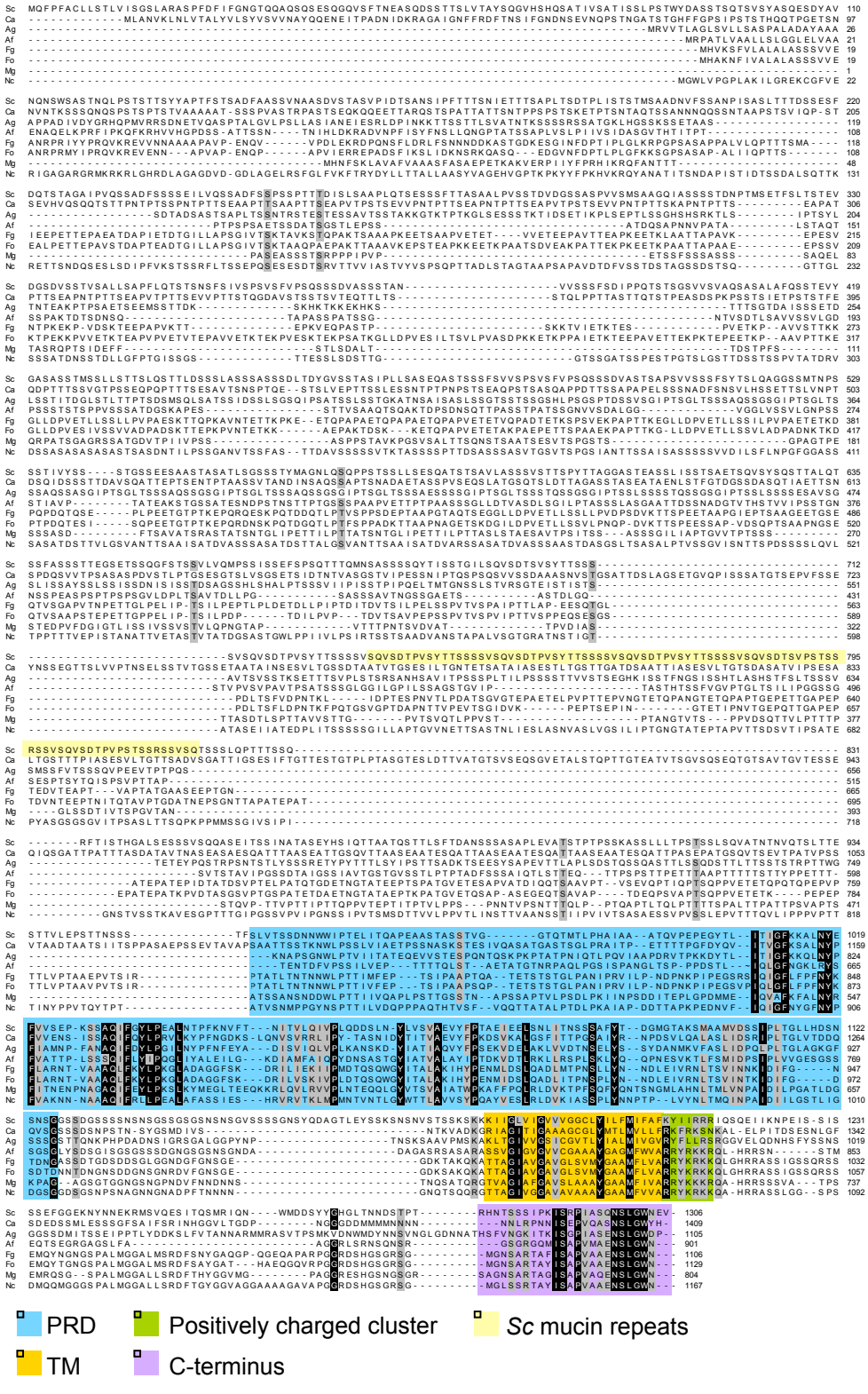
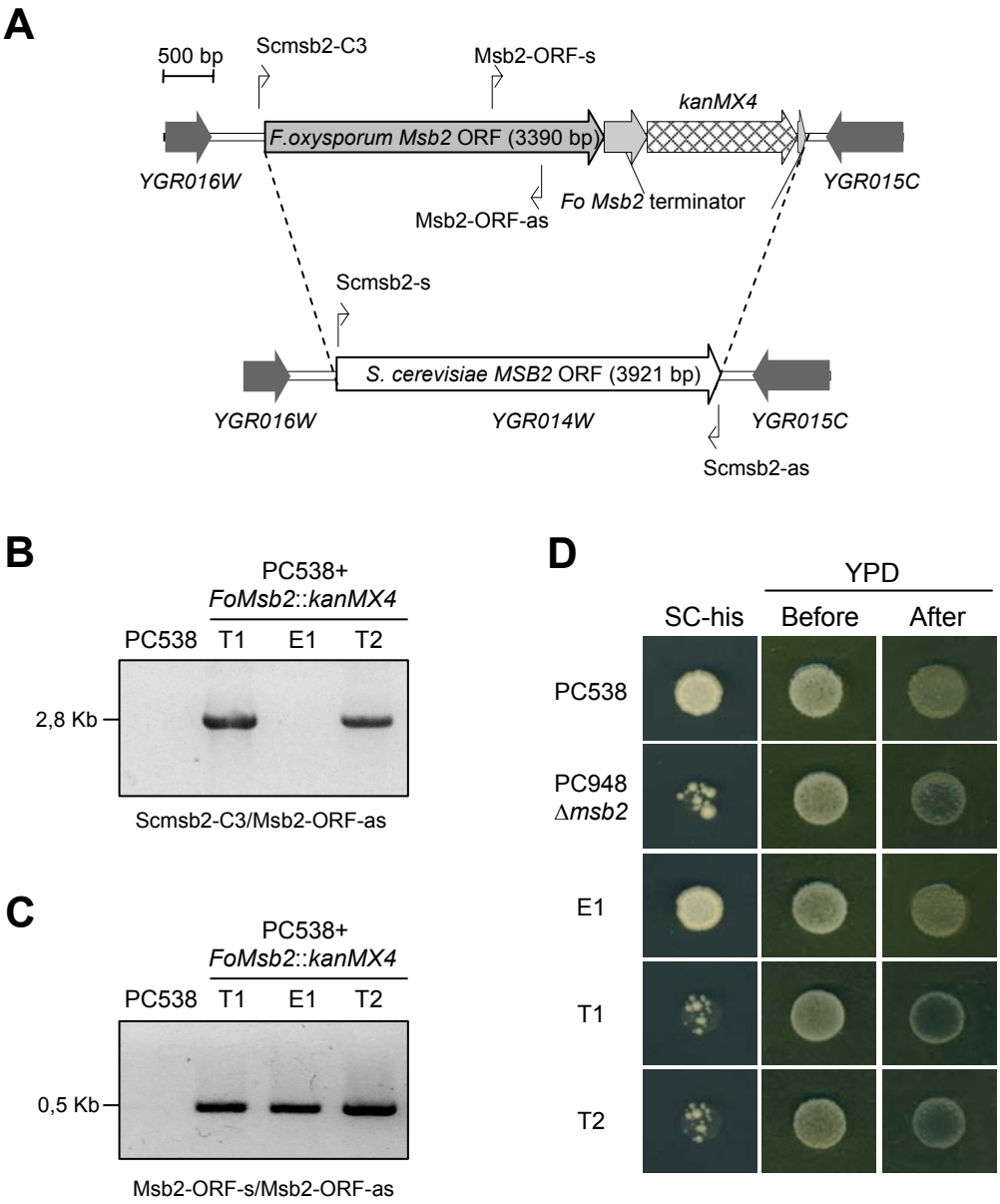
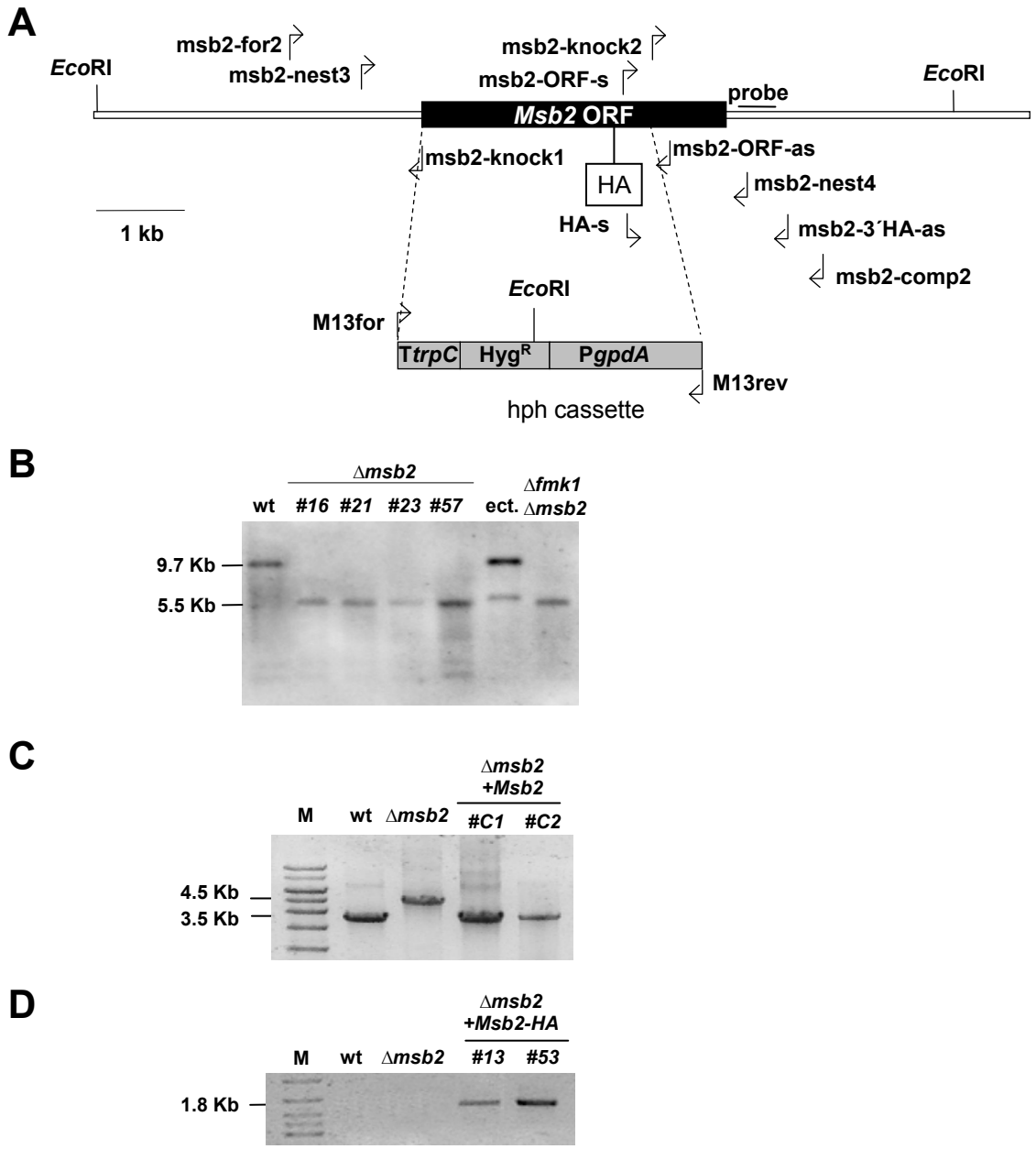


Supplemental Figure 1. Sequence alignment of fungal Msb2 proteins.
The alignment shows the predicted amino acid sequences of Msb2 orthologues from *S. cerevisiae* (*Sc*), *C. albicans* (*Ca*), *A. gossypii* (*Ag*), *A. fumigatus* (Af), *F. graminearum* (*Fg*), *F. oxysporum* (*Fo*), *M. oryzae* (*Mg*) and *N. crassa* (*Nc*). Highly conserved residues are shaded in black, moderately conserved residues are shaded in grey.

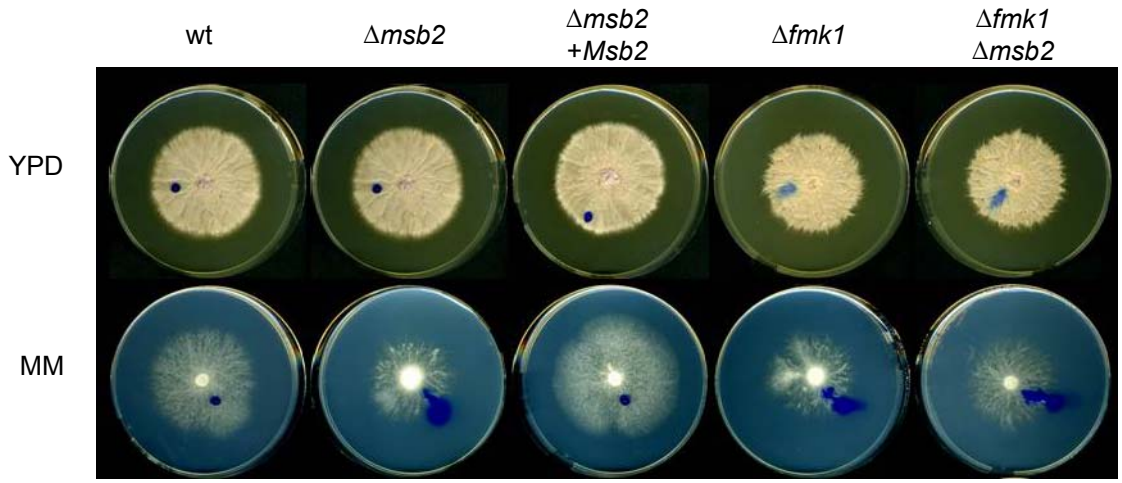


Supplemental Figure 2. *F. oxysporum* Msb2 does not complement signalling function in *S. cerevisiae*.

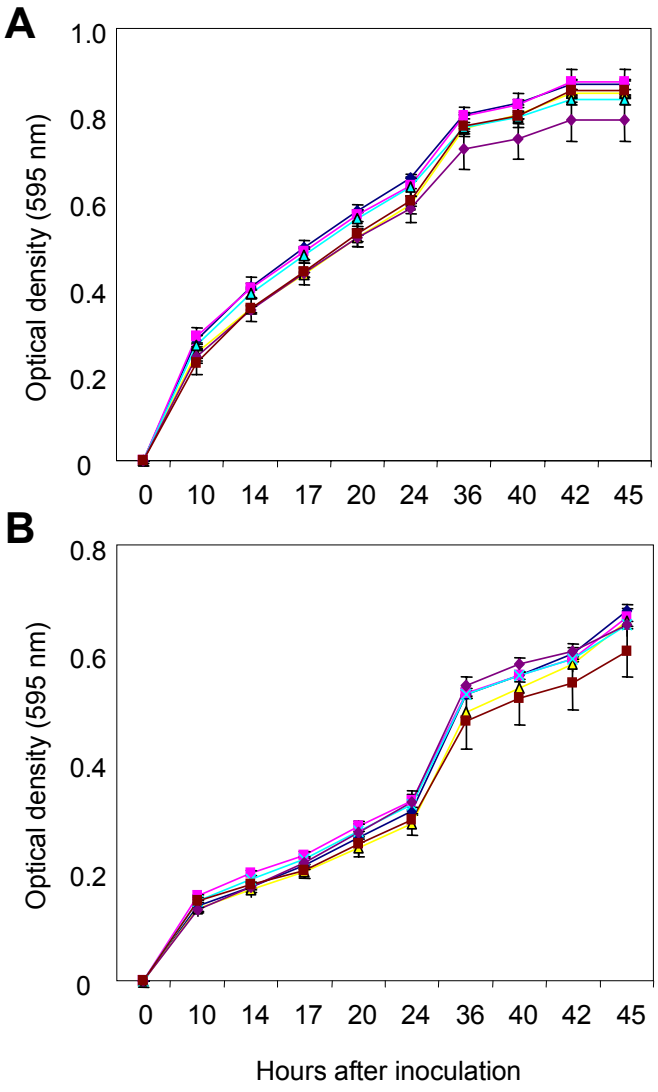
(**A**) Physical maps of the gene replacement construct containing the *F. oxysporum Msb2* ORF and terminator with the kanamycin resistance cassette, and of the *S. cerevisiae MSB2* gene locus. The relative positions of the primers used for PCR analysis of transformants are indicated. (**B**) Amplification of genomic DNA of *S. cerevisiae* strain PC538 and three derived transformants using the indicated combination of primers. An amplification product indicative of homologous insertion of the construct at the *MSB2* locus is detected in transformants T1 and T2, but not in the ectopic transformant E1. (**C**) RT-PCR of cDNA obtained from *S. cerevisiae* strain PC538 and three derived transformants using the indicated combination of primers. An amplification product indicative of the presence of the *F. oxysporum Msb2* transcript is detected in transformants T1, T2 and E1. (**D**) Cells of the indicated *S. cerevisiae* strains were spotted onto SC medium lacking histidine to visualize activation of the FG pathway-dependent *FUS1:HIS3* reporter gene (left panel), or onto YPD medium (middle panels) and washed after 48 h (right panels) to determine agar invasion.



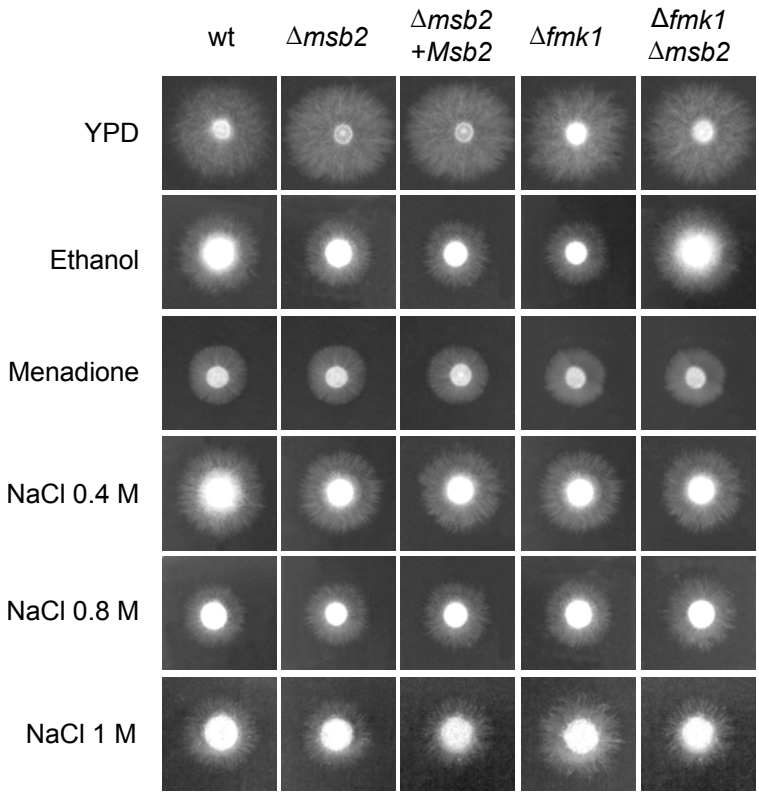
Supplemental Figure 3. Targeted disruption of *F. oxysporum Msb2*.
(**A**) Physical maps of the *Msb2* locus and the gene replacement construct obtained by PCR fusion (Δ*msb2* allele). Relative positions of the primers used for generation of the gene disruption construct and PCR analysis of transformants and complemented strains, the probe used for Southern analysis, and the HA epitope in the Δ*msb2*+*Msb2HA* strains are indicated. (**B**) Southern blot hybridization analysis of the wild-type strain 4287, different Δ*msb2* mutants and an ectopic transformant. Genomic DNA treated with *EcoRI* was hybridized with the probe indicated in (A). Molecular sizes of the hybridizing fragments are indicated on the left. (**C**) Amplification of genomic DNA of the indicated strains using forward primer nest3 and msb2-ORF-as indicated in (A), to differentiate the wild-type allele from that corresponding to the Δ*msb2* allele. (**D**) PCR amplification of genomic DNA of the indicated strains using forward primer HA-s, specific for the HA epitope nucleotide sequence, and reverse primer msb2-3´HA-as depicted in (A).



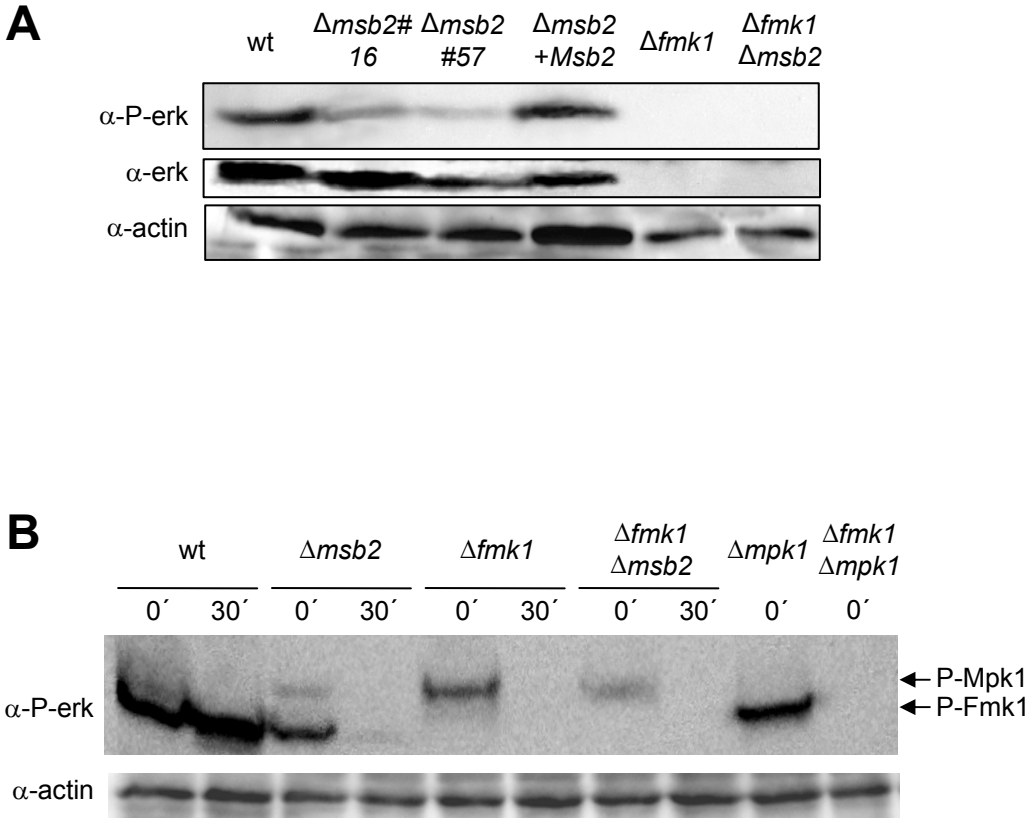
Supplemental Figure 4. Msb2 contributes to growth of aerial mycelium and colony hydrophobicity under conditions of nutrient limitation.
The indicated strains were grown for five days on yeast peptone glucose (YPD) or Minimal Medium (MM). A 100-μl drop of water stained with Bromophenol blue was deposited at a distance of 5 mm from the edge of the fungal colony. Plates were incubated for 2 h at room temperature and photographed.



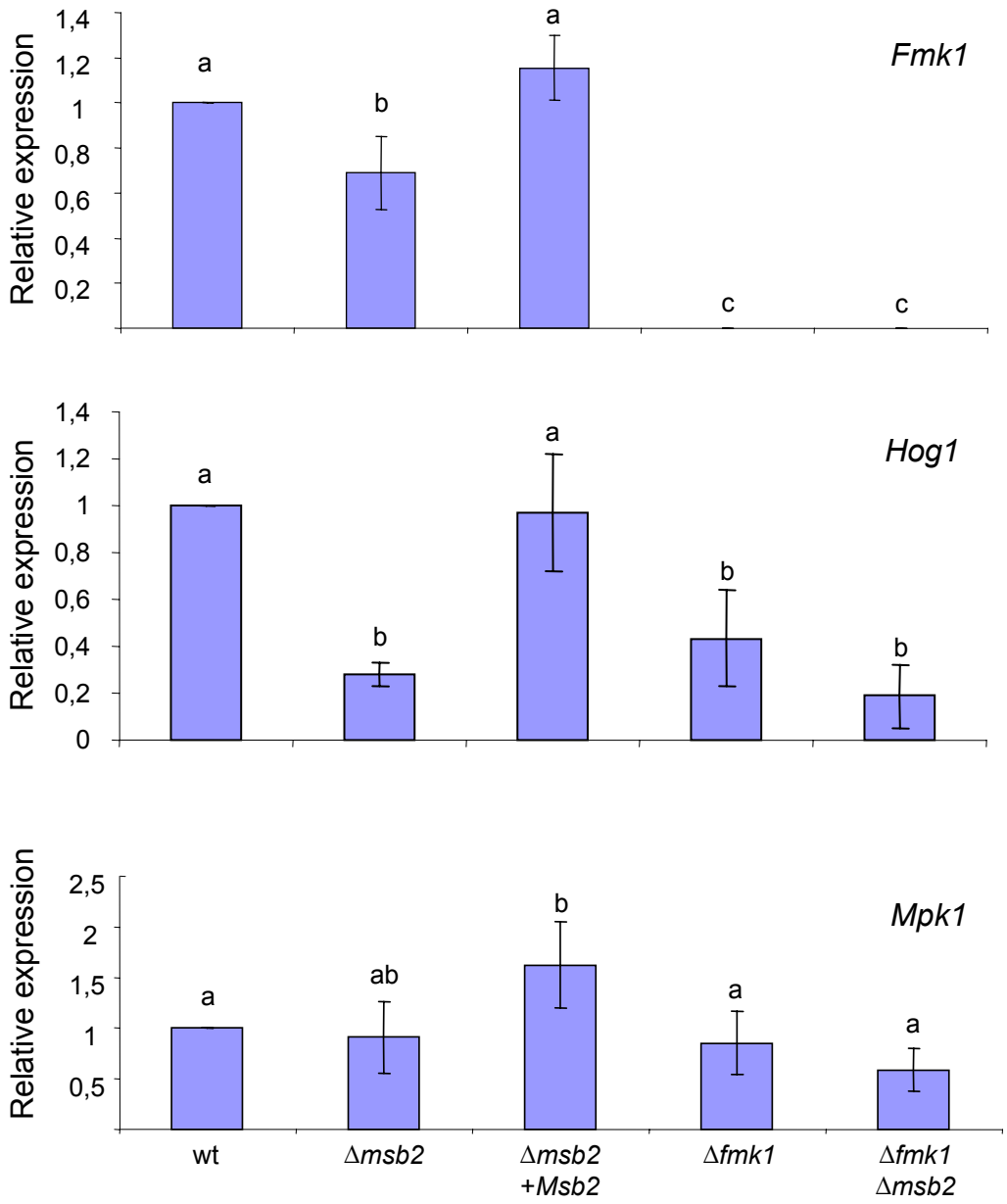
Supplemental Figure 5. Growth of different strains in submerged culture.
Growth of the wild-type strain (blue diamonds), Δ*msb2#16* (pink squares), Δ*msb2#57* (yellow triangles), Δ*msb2*+*Msb2* (blue triangles), Δ*fmk1* (purple diamonds) and Δ*fmk1* Δ*msb2* (brown squares) was measured in microtiter wells containing liquid yeast peptone glucose (YPD) (**A**) or Minimal Medium (MM) (**B**), by determining the optical density at 595 nm at different time points after inoculation. Error bars represent standard errors calculated from 3 wells.



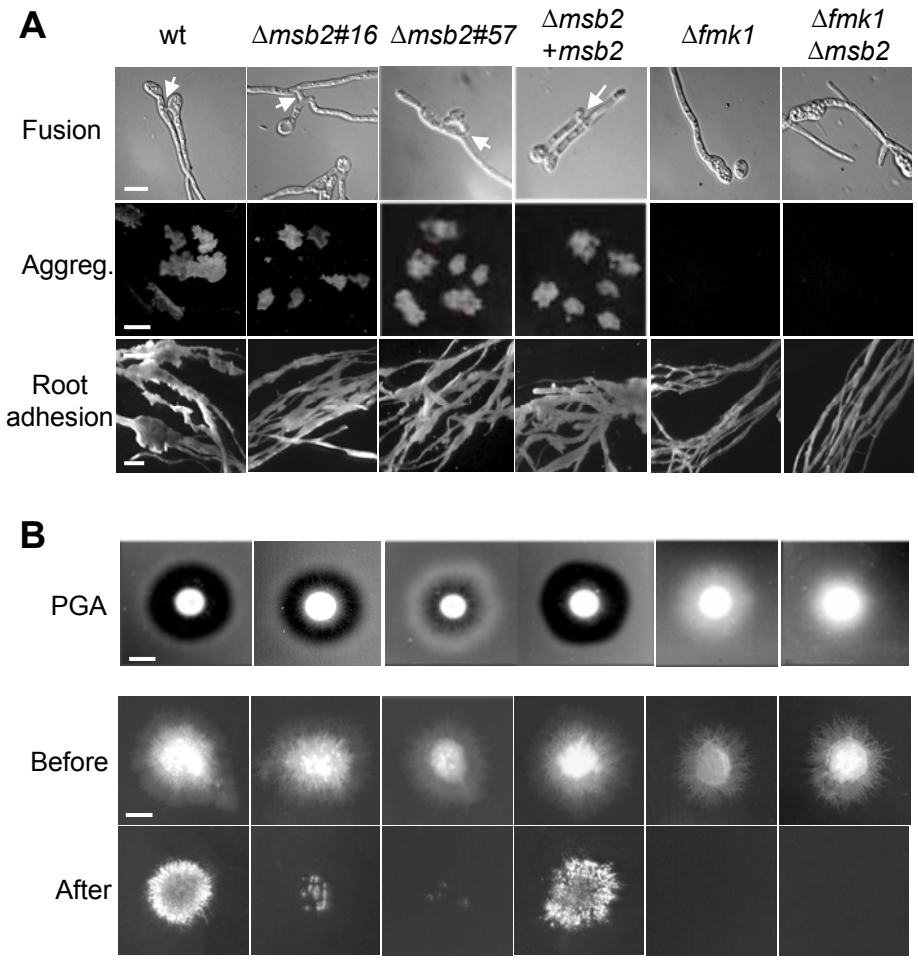
Supplemental Figure 6. Msb2 is not required for the oxidative and osmotic (salt) stress response.
Colony phenotype of the indicated strains grown on yeast peptone glucose (YPD) or YPD supplemented with 10 µg/ml menadione or with the indicated concentrations of NaCl. Plates were spot-inoculated with 10^5 microconidia, incubated three days at 28°C and scanned.



Supplemental Figure 7. Phosphorylation of the Fmk1 and Mpk1 MAPKs in different mutants. **(A)** Msb2 is required for full levels of Fmk1 phosphorylation. Total protein extracts from the indicated strains germinated for 14 h in PDB and subsequently transferred for 30 min onto plates containing MM were submitted to immunoblot analysis as described in Fig. 5. A monoclonal α-actin antibody was used as an additional loading control. **(B)** Phosphorylation of the Fmk1 and Mpk1 MAPKs in different mutants. Total protein extracts from the indicated strains germinated for 14 h in PDB (0´) and subsequently transferred for 30 min onto plates containing MM (30´) were submitted to immunoblot analysis with anti-phospho-p44/42 MAPK antibody (α-P-erk), using an extended exposure time that allows simultaneous detection of P-Fmk1 (lower band) and P-Mpk1 (upper band). A monoclonal α-actin antibody was used as loading control. Relative positions of P-Fmk1 and P-Mpk1 are indicated to the right.

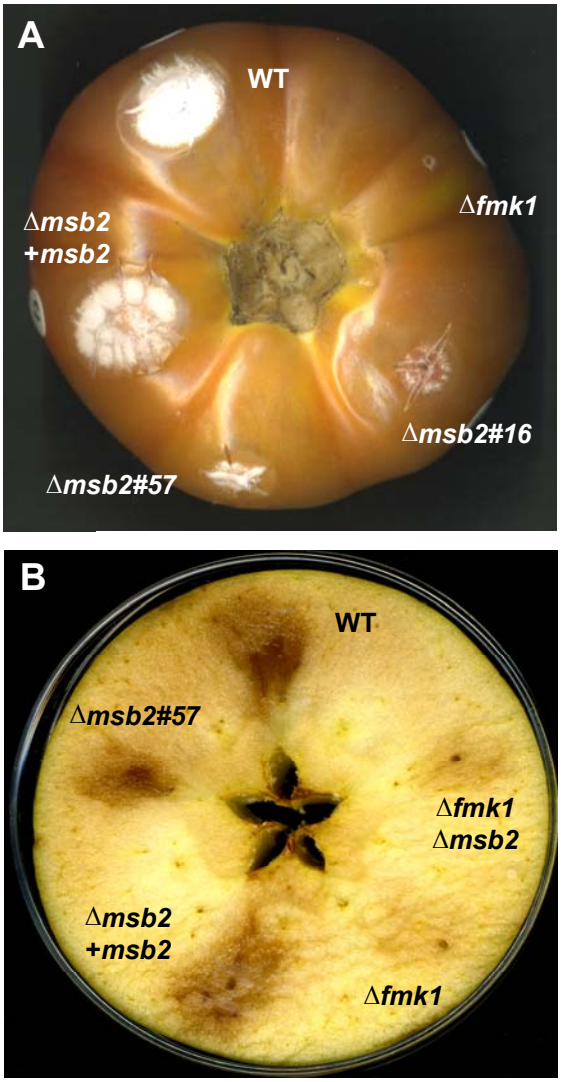


Supplemental Figure 8. Expression of MAPK genes in different fungal strains.
mRNA abundance of the indicated genes was measured 6 h after transfer of the strains to MM plates using quantitative real time PCR. Relative expression levels represent mean ΔCt values normalized to actin gene expression levels and relative to expression values in the wild type strain. Bars represent standard errors calculated from four biological replicates. Values with the same letter are not significantly different according to the Mann-Whitney test ($p \leq 0.05$).

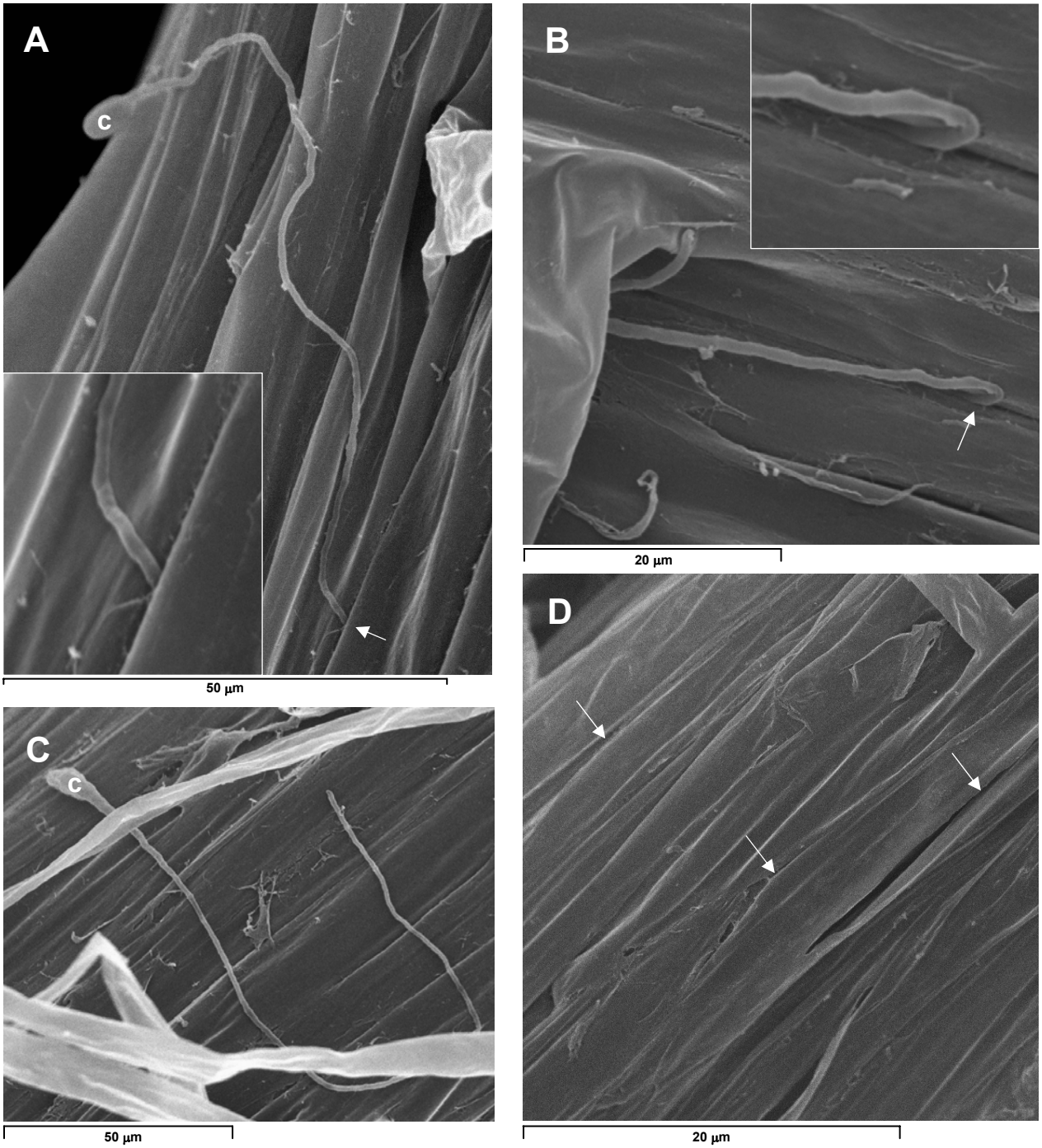


Supplemental Figure 9. Msb2 regulates a subset of Fmk1-dependent functions related to invasive growth.

(**A**) Msb2 is not required for vegetative hyphal fusion, mycelial aggregation and root adhesion. Strains were grown overnight in PDB diluted 1:50 with water and supplemented with 20 mM glutamic acid. Upper: Fungal cultures were observed with a Leica DMR microscope using the Nomarsky technique. Arrows point to fusion events between hyphae and conidial germ tubes of the wild-type and Δ*msb2* strains. Bar, 10 μm. Middle: Fungal cultures were transferred to a Petri dish and photographed. Note the presence of mycelial aggregates in the wild type and Δ*msb2* strains. Bar, 1 cm. Lower: Roots of tomato seedlings were immersed for 36 h in microconidial suspensions, washed by vigorous shaking in water and observed with a binocular microscope. Adhering mycelium is visible as a white mass covering the roots. Bar, 1 cm. (**B**) Msb2 contributes to pectinolytic activity and penetration of cellophane membranes. Extracellular pectinolytic activity on plates containing polygalacturonic acid (PGA) was visualized as a contrasting halo underneath the fungal colony. Penetration of cellophane membranes was determined by growing colonies for four days on a MM plate covered by a cellophane membrane (before). The cellophane with the fungal colony was removed and plates were incubated for an additional day (after). Bar, 1 cm.



Supplemental Figure 10. Msb2 contributes to invasive growth on living fruit tissue.
Tomato (**A**) and apple (**B**) fruits were inoculated with 5 × 10^4 microconidia of the indicated strains, incubated at 28ºC for four days and photographed.



Supplemental Figure 11. *F. oxysporum* penetrates roots preferentially through openings at the junctions of epidermal cells.

Scanning electron microscopy analysis of tomato roots, 24 h after inoculation with microconidia of the *F. oxysporum* f. sp. *lycopersici* wild-type strain (**A,B**), Δ*fmk1* mutant (**C**), or of uninoculated roots (**D**). (**A,B**)Fungal hyphae scanning the root surface and entering roots through openings at intercellular junctions. Insets show magnifications of penetration sites. Arrows point to penetration events (**A,B**) or openings visible in uninoculated tomato roots. c, conidium.

Supplemental Table 1. Primers used in this study.

	Name	Sequence 5'-3'
pGemT	M13for	CGCCAGGGTTTTCCCAGTCACGAC
	M13rev	AGCGGATAACAATTTCACACAGGA
Fo*msb2*	msb2-5'-HA-s	GCAGATGGAGGCGCTGAAGCAT
		GGCATAGTCAGGAACGTCATATGGATA-
	msb2-5'-HA(722)-as	AGTCTCGGTGGCAGGGCTGCC
		TATCCATATGACGTTCCTGACTATGCC-
	msb2-3'-HA-s	ACTGATGCGGAGACCAACGGCAC
	msb2-3'-HA-as	CTGAACAACACCACCGCTTCCC
	msb2-for2	GAGATTCCAACAATAGCAGATG
	msb2-comp2	GCAATCCGCGCCCAATAGAC
	msb2-rev	CAGGTCGCCAGGATGGAGCC
	msb2-HA-s	CCATATGACGTTCCTGACTATG
	msb2-nest3	CAAGCATCAAAGGCGTCGTC
	msb2-nest4	CTCACGCCTAACGCCTCCAA
		GTGACTGGGAAAACCCTGGCG-
	msb2-knock1	AGTTGGATACTGTTTGGTGATTG
		TCCTGTGTGAAATTGTTATCCGCT-
	msb2-knock2	ACTCGTTAGCAAGATTGTTCCTC
	msb2-ORF-s	TGCCCCCACAGATGAGCAAC
	msb2-ORF-as	GGATCTTGGCGAGAGCAGTG
qPCR	09795-for (fpr1)	CCCAAGAAGAACCCTGCTCC
	09795-rev (fpr1)	GAGTAGGGGTTGGAGCCGC
	Chs3-12	GTGTCATGGGGAACAAAGGG
	Chs3-18	CCTGTAACCCCAAAAGTATGT
	ChsV-20	GCACAATTTGGCTGAGCTTAT
	ChsV-36B	GGATCCCTACAATTGGCCAGAAAGCA
	act-2	GAGGGACCGCTCTCGTCGT
	act-q6	GGAGATCCAGACTGCCGCTCAG
	Fmk1-F1	CCGGTTTCATGACTGAATACGT
	Fmk1-R1	ATCGTGGTAAGGCTCAAGGTAT
	FoHog1-F1	GTCACTTATGGGATCGCGATC
	FoHog1-R1	TAGTGGCGGTTGTATCAGGCT
	FoMpk1-F1	CCATCAAGAAGGTCACCAATGT
	FoMpk1-R1	AGCAACGTACTCGGTCATGTAT
Yeast compl.	Msb2-PstI-F1	CTGCAGATCCCTCGCGAGACCCGTTT
	Msb2-Sac2-R1	CCGCGGAACACCACCGCTTCCCATCG
	Fomsb2-S1	ACCTGGCCAACCTTTTTTAATGCTCTTGACCTTTCTT
		TTCTCTTGCGTACGCTGCAGGTCGAC
	Fomsb2-S2	TACAAGTTGACATGTATCCTATCATCCAGAACCTGC
		TCTCATTGTATCGATGAATTCGAGCTCG
	Fomsb2-C1	CTATTTTTATTGACTTTTCATTAGGCTTCCTAATTAT
		ACCCATCTATGCACGCAAAGAACTTCAT
	Fomsb2-C2	ATGAAATAAGTAGGTATCGTTATAAGTTTATAAGGTT
		ATGCAAGCTGGACCAAACACACCTAAAA