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Supporting Information for

The ASH1-PEX16 regulatory pathway controls peroxisome biogenesis for appressorium-mediated insect infection by a fungal pathogen

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Supporting Information Text

SI Materials and Methods

Fungal strains and culturing. The WT and mutant strains of *M. robertsii* ARSEF 2575 (obtained from the U.S. Department of Agriculture Entomopathogenic Fungus Collection (ARSEF) in Ithaca, N.Y.) were routinely grown on PDA (BD Difco) at 27 °C. For liquid cultures, fungi were grown in SDB (BD Difco) at 27 °C in a rotary shaker. For insect cuticle exposure, they were grown in a cuticle-containing medium to activate the fungal infection system (1, 2). Fungal conidia (1×10^6 conidia/mL) were first cultured for 40 h in nutrient-rich SDB medium. The mycelia were then harvested and transferred to MM2 medium (0.1% wt/vol KH_2PO_4, 0.1% wt/vol $MgSO_4 \cdot 7H_2O$, and 50% vol/vol tap water) supplemented with 0.8% wt/vol adult locust (*Schistocerca gregaria*) cuticles for 8 h (3). The fatty acid medium was prepared by adding 50 mM NaAc (sodium acetate) and 2.5 mM oleic acid to MM medium (6 g/L $NaNO_3$, 0.52 g/L KCl, 0.52 g/L $MgSO_4 \cdot 7H_2O$, and 0.25 g/L KH_2PO_4).

Mosquito strains and rearing. *A. stephensi* (Dutch strain) mosquitoes were maintained on 10% (wt/vol) sucrose at 26 ± 1 °C with 80 ± 5% relative humidity and a 12-h day/12-h night cycle. The larvae were fed cat food pellets and ground fish food supplements (4).

Construction of *M. robertsii* gene deletion, complementation, and overexpression strains. For targeted deletion of *Mrash1* (X797_010732) or *Mrpex16* (X797_007679) as well as six other lipid metabolism-related genes, the 5′- and 3′-flanking regions of each open reading frame were amplified by PCR from the ARSEF 2575 genomic DNA as a template and then subcloned into the XbaI and SpeI sites of the binary vector pBarGFP, respectively (5). The gene disruption constructs were then used to separately transform *Agrobacterium tumefaciens* AGL-1 for targeted gene disruption by split-marker homologous recombination (6). Replacement-specific PCR amplification of the gene locus was performed using specific primer pairs (listed in *SI Appendix*, Table S1) to amplify either the WT or mutated gene locus. To complement the ΔMrash1 strain, the full-length target gene and its native promoter and terminator sequences were amplified by PCR and cloned into the EcoRV site of pBenGFP (7) to generate the complementation vector pBenGFP-Mrash1. The complemented strain com-Mrash1 was generated by introducing pBenGFP-Mrash1 into the deletion mutant ΔMrash1 using *A. tumefaciens*-mediated transformation.

For construction of the ΔMrash1::pex16 strains, the plasmid pBenGFP-pex16 was constructed as follows. The coding region of *Mrpex16* lacking the 3’ TGA stop codon was PCR-amplified from the ARSEF 2575 cDNA using Mrpex16-GFP_F and Mrpex16-GFP_R and inserted into the SpeI site of the overexpression plasmid pSurGFPOE downstream of the constitutive *B. bassiana gpd* promoter and upstream of a *trpC* terminator to construct the plasmid pSurGFPOE-Mrpex16. The overexpression cassette of *Mrpex16* including the *gpd* promoter and the *trpC* terminator was then amplified by PCR using ovpex16-Ben-F and ovpex16-Ben-R, and the plasmid pSurGFPOE-Mrpex16 was used as the template.

The whole fragment was cloned into the XbaI site of pBenGFP to generate pBenGFP-pex16. pBenGFP-pex16 was used to transform the ΔMrash1 strain.

For expressing RFP-PTS1 in the WT, ΔMrpex16, ΔMrash1, com-Mrash1, and ΔMrash1::pex16 strains, the plasmid pSurRFP-PTS1 was constructed as follows. An approximately 0.7 kb DNA fragment of RFP-PTS1 was amplified by PCR using pSR-SKL-F and pSR-SKL-R, and the plasmid pBar-RFP was used as the template (7). An approximately 140 bp DNA fragment of the *gfp* terminator was amplified by PCR using pSR-SKL-F2 and pSR-SKL-R2, and the plasmid pSurGFP-fusion was used as the template. The RFP-PTS1 fragment with the downstream terminator fragment was cloned into the EcoRV and EcoRI sites of pSurGFP-fusion to generate pSurRFP-PTS1. pSurRFP-PTS1 was used to transform the WT, ΔMrpex16, ΔMrash1, com-Mrash1, and ΔMrash1::pex16 strains.

For construction of the strain WT::MrPEX16-GFP+RFP-PTS1 used for colocalization analysis of MrPEX16 and RFP-PTS1, the vector pBar-MrPEX16-GFP was used. To construct pBar-MrPEX16-GFP, the coding region of *Mrpex16* lacking the 3′ TAG stop codon was amplified by PCR from ARSEF 2575 cDNA and inserted into the EcoRV site of the expression plasmid pBarGFP-fusion downstream of the constitutive *B. bassiana gpd* promoter and upstream of a *GFP* ORF without the 5’ ATG start codon. pBar-MrPEX16-GFP was then transformed into the WT::RFP-PTS1 strain. The primers used for the target genes are listed in *SI Appendix*, Table S1.

Insect bioassays. To assess fungal pathogenicity and virulence, bioassays by topical infection and direct injection using conidial suspension were performed with female adult *A. stephensi* mosquitoes (8). For topical infection, conidial suspensions in 0.01% (vol/vol) Triton X-100 were prepared from 14-d-old fungal PDA cultures. Adult female *A. stephensi* mosquitoes were sprayed with a fungal conidial suspension (6×10^6 conidia/mL). Mosquitoes sprayed with sterile 0.01% (vol/vol) Triton X-100 were used as controls. Each treatment was replicated three times, with 50 mosquitoes per replicate. For direct injection, 138 nL of conidial suspension in PBS with 0.01% (vol/vol) Triton X-100 (1×10^6 conidia/mL) was injected into the haemocoel of each mosquito using Nanoject II microinfector (Drummond). Mosquitoes injected with sterile PBS with 0.01% (vol/vol) Triton X-100 were used as controls. Fifty mosquitoes were included in each treatment. The treated mosquitoes were maintained at 26 ± 1 °C with 80 ± 5% relative humidity and a 12-h day/12-h night cycle until they died. Mortality was recorded every 12 h.

Appressorium induction. Conidia of the WT and mutant strains were harvested in 0.01% (vol/vol) Triton X-100 from 14 d-old fungal cultures on PDA. For hydrophobic surface induction, assays were conducted using MM (containing 6 g/L $NaNO_3$, 0.52 g/L KCl, 0.52 g/L $MgSO_4 \cdot 7H_2O$, and 0.25 g/L KH_2PO_4) supplemented with 1% (vol/vol) glycerol as the sole carbon source (MM-Gly) on plastic hydrophobic surfaces (9). A 20 μL conidial suspension (5×10^8 conidia/mL) was added to 4 mL of MM-Gly in Thermo plastic plates (Φ = 6 cm) and incubated at 27 °C for 16 to 24 h for appressorium induction. For cicada wing induction, cicada wings were sterilized with 3% NaClO solution for 5 min and washed 3 times with

sterilized deionized water. The wings were placed on 0.7% agar plates. A total of 20 μL of conidial suspension (5×10^6 conidia/mL) was dropped onto a wing, which was then incubated at 27 °C for 16 to 24 h for appressorium induction.

Cuticle penetration assay. Autoclaved cicada wings were lined on MM+1% glucose agar plates. Mycelium blocks of WT and mutant strains were picked from 2 d-old fungal cultures on PDA and inoculated on the wings for 2 d. The wings with cultures were then removed, and the plates were incubated for an additional 5 d. The colony sizes were measured and compared between WT and mutants.

Incipient cytorrhysis assay. Appressorium turgor was measured via an incipient cytorrhysis assay (10). Appressoria of the WT and mutant strains were induced on cicada wings according to the method described above. Individual wings were dipped in PEG8000 solution (6.5 g in 10 mL of distilled water) for 10 min, and the percentage of appressoria undergoing incipient cytorrhysis was determined from 100 appressoria for each strain.

Glycerol assay. Appressoria of the WT and mutant strains induced on hydrophobic plastic surfaces via the methods described above were harvested by scraping with a plastic spatula and then homogenized with liquid nitrogen using a mortar and pestle. The glycerol content of the debris was then measured using a commercial glycerol assay kit (Applygen E1012-50) according to the manufacturer's instructions. Each strain was assayed in triplicate.

Lipid droplet observation. Appressoria of the WT and mutant strains induced on cicada wings were fixed with 4% formaldehyde in PBS for 15 min and then washed twice with PBS. The appressoria were then stained with BODIPY (Invitrogen D3922) to detect neutral lipids (11). BODIPY was used at 10 μg/mL in PBS (stock, 1 mg/mL in DMSO). The stained lipid droplets in the appressoria were observed and photographed with a microscope (Olympus BX53).

Microscopic observation of MrPEX16-GFP and RFP-PTS1 localization. To observe the localization of MrPEX16-GFP and RFP-PTS1, appressoria of the *M. robertsii* strains WT+RFP-PTS1, ΔMrash1+RFP-PTS1, com-Mrash1+RFP-PTS1, ΔMrash1::pex16+RFP-PTS1, ΔMrpex16+RFP-PTS1 and WT::MrPEX16-GFP+RFP-PTS1 on hydrophobic surfaces were induced according to the method described above. Peroxisomes marked with dot-like RFP signals in appressoria were further observed and photographed with a microscope (Olympus BX53) at 20 h post-induction. To evaluate the number of peroxisomes, 50-100 appressoria were detected for each strain.

Western blotting analysis. The mycelia for histone extraction were obtained by inoculating fungal conidia in SDB (1×10^6 conidia/mL) and incubating them with shaking at 180 rpm and 27 °C for 40 h. The mycelia were then transferred into MM2 medium for 24 h. They were then harvested by filtration, frozen in liquid nitrogen, and ground to a fine powder using a mortar and pestle. Histones were acid-extracted as previously described (9). Approximately 10 to 20 μg of total nuclear protein per lane was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Proteins were then transferred to polyvinylidene fluoride membranes and blotted using standard procedures. Primary antibodies specific to the following proteins were used for western blotting: H3K36me1 (abcam ab9048), H3K36me2 (active motif 39255), H3K36me3 (abcam ab9050) and H3 (abmart P30266S). The secondary antibody used was Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L) (Jackson 111-035-003).

ChIP-seq and ChIP quantitative PCR (ChIP–qPCR). ChIP was performed as described previously with some modifications (12, 13). Two separate samples of the WT strain were prepared. Briefly, mycelia were generated by the insect cuticle exposure method as described above. For chromatin fixation, fungal cultures were treated with 1% formaldehyde with gentle shaking (125 rpm) for 15 min at 27 °C. After cell lysis, the chromatin was sheared into 150-300 bp fragments. The soluble chromatin fraction was immunoprecipitated using an antibody specific for H3K36me2 (active motif 39255). DNA fragments were recovered from immunoprecipitated chromatin or total chromatin (input DNA) by treatment with proteinase K.

For ChIP-seq, indexed ChIP-seq libraries were prepared using the NEBNext UltraDNA Library Prep Kit for Illumina (New England Biolabs #E7645) according to the manufacturer's instructions. Selected DNA fragments (200-500 bp) were purified and enriched by PCR. Then, 50-bp paired-end sequencing was performed using an Illumina NovaSeq 6000 genome analyser (Guangzhou RiboBio Co., Ltd. and Shanghai Institute of Nutrition and Health's Biomedical Big Data Center, Chinese Academy of Sciences).

For ChIP–qPCR, ChIP input and IP samples were diluted 10-fold and 100-fold for PCR, respectively. Then, qPCR was performed using a Hieff qPCR SYBR Green Master Mix kit (Yeason 11201ES08) and a PikoReal instrument (Thermo N11471) under the following conditions: denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing and extension at 60 °C for 30 s. The primers used for the target genes are listed in *SI Appendix*, Table S1.

RNA extraction, RT–qPCR and RNA-seq. To monitor the transcription of target genes, fungal mycelia were generated in SDB or by insect cuticle exposure as described above. Samples were also prepared during fungal topical infection of female adult *A. stephensi* mosquitoes. The mosquitoes were sprayed with conidial suspension (5×10^7 conidia/mL), and samples were collected at 12, 24, 36, 60, and 84 h post-topical infection by removing the head, wings and legs. The samples were lyophilized and ground in liquid nitrogen. Total RNA was extracted using an RNAiso Plus Kit (Takara D9108A), and cDNA was synthesized using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara DRR047A) according to the

manufacturer's instructions. Then, qPCR was performed using a Hieff qPCR SYBR Green Master Mix kit (Yeason 11201ES08) and a PikoReal instrument (Thermo N11471) under the following conditions: denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing and extension at 60 °C for 30 s. The primers used for the target genes and the reference gene actin are listed in *SI Appendix*, Table S1.

For RNA-seq, fungal mycelia were generated by insect cuticle exposure. Samples were ground in liquid nitrogen, and total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen 74904) with RNase-free DNase. RNA-seq libraries were constructed and sequenced on an Illumina HiSeq X Ten genome analyser (Shanghai OE Biotech. Co., Ltd.).

Next-generation sequencing data analysis. ChIP-seq reads were mapped to the *M. robertsii* ARSEF 2575 genome (http://ncbi.nlm.nih.gov/bioproject/PRJNA230500) by using Bowtie (v1.1.1) with default parameters (14). Model-based Analysis for ChIP-seq (MACS) (v1.4.2) was used to call peaks (15). BedGraph files were normalized for the total mapped read counts, and the signal was calculated as per million per kilo-base (RPM) values. TopHat (v2.1.1) was used to align RNA-seq reads to the *M. robertsii* ARSEF 2575 genome with the parameters -N 1 -g 1. Cufflinks (v2.2.1) was used to assemble the reads with default parameters (16). The Integrative Genomics Viewer genome browser was used to visualize the ChIP-seq and RNA-seq data (17). Gene ontology analysis of DEGs generated from RNA-seq data was conducted by using the GO Analysis Toolkit and Database for Agricultural Community (agriGO, http://bioinfo.cau.edu.cn/agriGO/) (18).

Statistical analysis. The statistical significance of the survival data from the fungal bioassays was analysed using the log-rank (Mantel–Cox) test. The statistical significance of other variables was determined using Student's *t* test for unpaired comparisons between two treatments. A value of $P < 0.05$ was regarded as statistically significant. All statistical analyses were performed using GraphPad Prism version 6.0 for Windows (GraphPad Software).

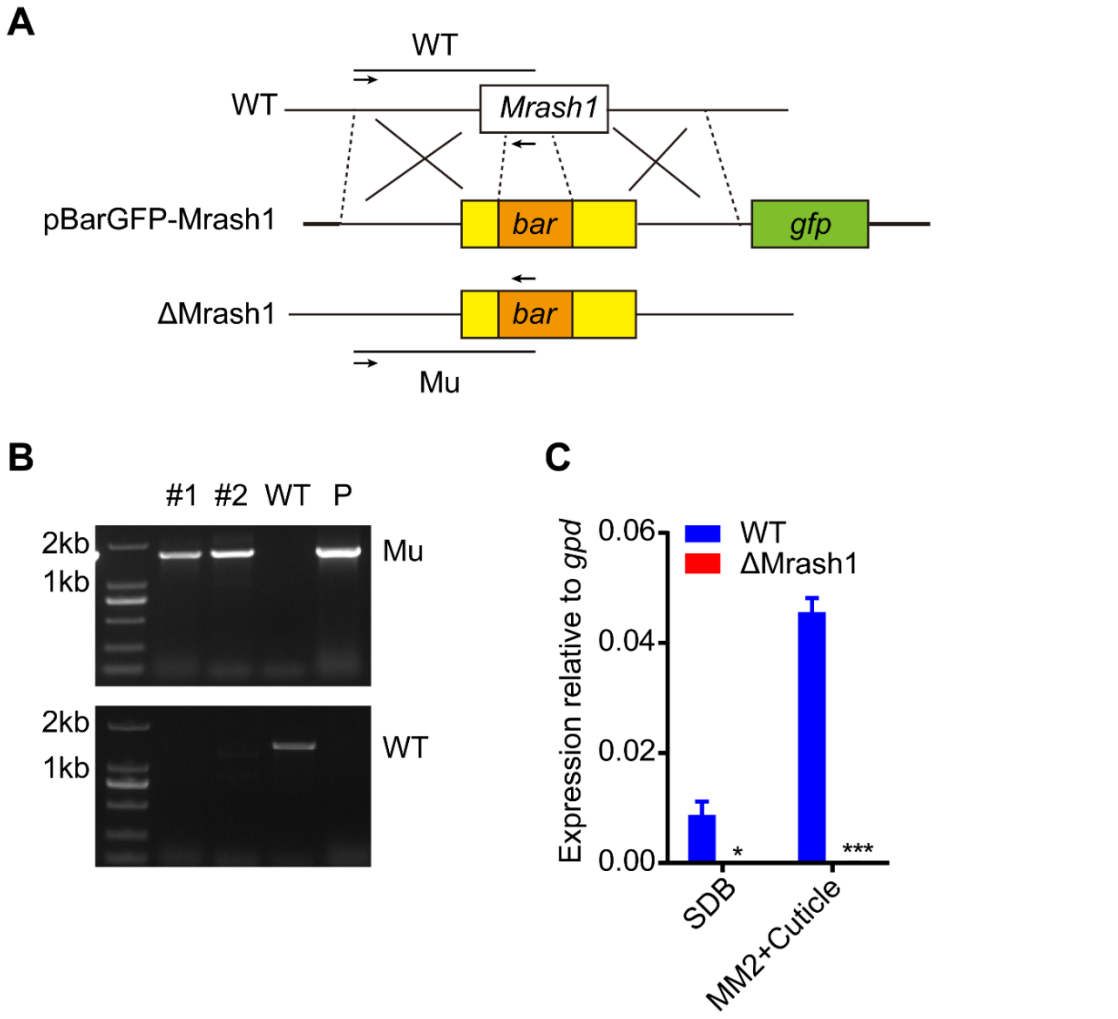

Fig. S1. Disruption of the *Mrash1* gene in *M. robertsii* ARSEF 2575. (A) Schematic representation of the gene *Mrash1* locus in the WT strain, the vector pBarGFP-Mrash1 (with two regions identical to the *Mrash1* reading frame) used for disruption of *Mrash1* via double-crossover recombination, and the *Mrash1* locus replaced by the antibiotic resistance gene *bar* in the ΔMrash1 strain. Replacement and WT-specific primer combinations (shown by arrows) and the expected products are shown as black lines between arrows. (B) Replacement-specific PCR analysis. The gene targeting was confirmed using primer combinations that amplify a signal only from the recombinant locus (Mu). The absence of a WT-specific signal in the two Mrash1 mutants (#1 and #2) and plasmid pBarGFPMrash1 (P) in the lower image confirms the genetic homogeneity of the mutants. (C) *Mrash1* expression was verified by quantitative PCR (qPCR) using cDNA from WT and ΔMrash1 cultures in SDB and MM supplemented with adult locust cuticle. Data are shown as the mean ± SD of three technical replicates. Significant differences were determined with Student's *t* test. **P* < 0.05 and ****P* < 0.001. *gpd* was used as a reference gene. The experiments were repeated twice with similar results.

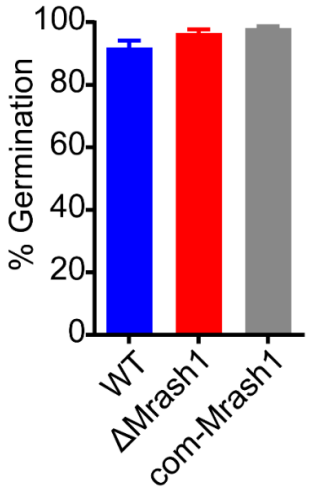

Fig. S2. *Mrash1* does not impact conidial germination. Germination rates of WT, ΔMrash1 and com-Mrash1 at 16 h post-induction in MM-Gly medium on hydrophobic plates. Data are shown as the mean ± SD of three biological replicates. The experiments were repeated twice with similar results.

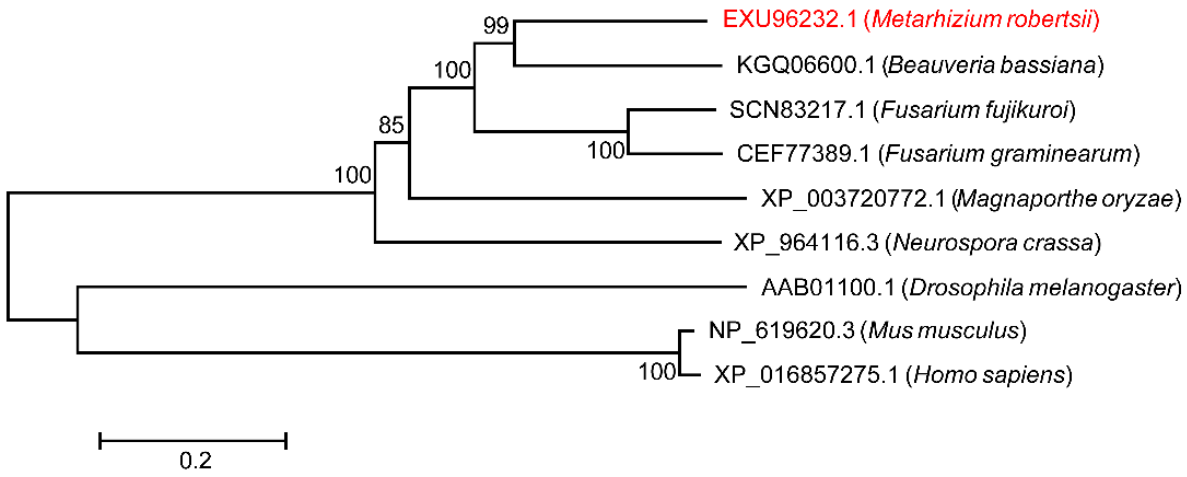

Fig. S3. Phylogenetic relationships between MrASH1 (EXU96232.1) and its homologues in other species. The amino acid sequences were aligned, and a neighbour-joining tree was generated with 1,000 bootstrap replicates by using Mega 6.0 software.

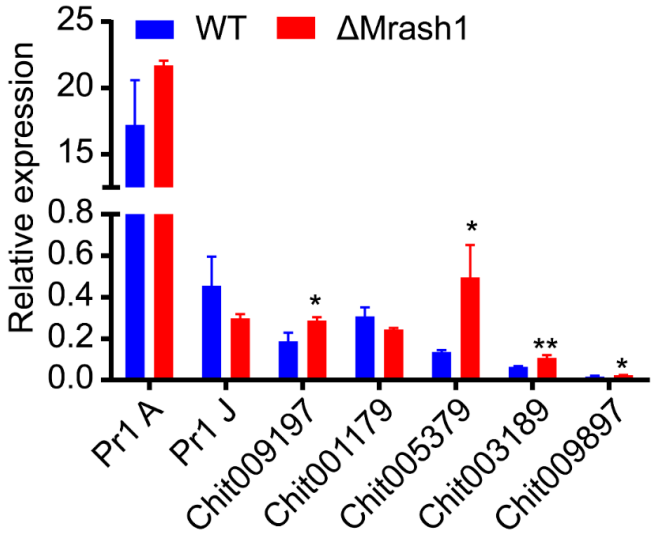

Fig. S4. MrASH1 does not downregulate the transcription of protease and chitinase genes in *M. robertsii*. Mycelia of WT and ΔMrash1 inoculated in MM2 medium supplemented with adult locust cuticle for 8 h were analysed by qPCR. *gpd* was used as a reference gene. Data are shown as the mean ± SD of three technical replicates. Statistics were performed with Student's *t* test. **P* < 0.05 and ***P* < 0.01. *gpd* was used as a reference gene.

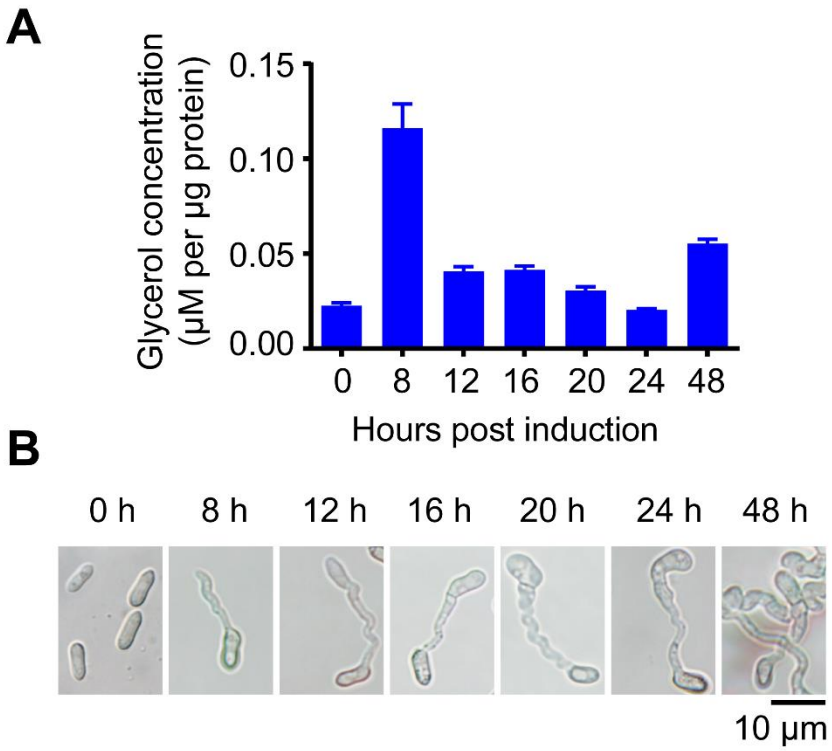

Fig. S5. Glycerol concentration in conidia and developing appressoria at different time points after induction in MM-Gly on hydrophobic surfaces in plastic plates. (A) Intracellular glycerol levels in germinating conidia and developing appressoria of WT induced on hydrophobic plates for up to 48 h, determined by a glycerol-specific enzymatic assay. Data are shown as the mean ± SD of three technical replicates. (B) Morphology of germinating conidia and developing appressoria of WT induced on hydrophobic plates for 48 h.

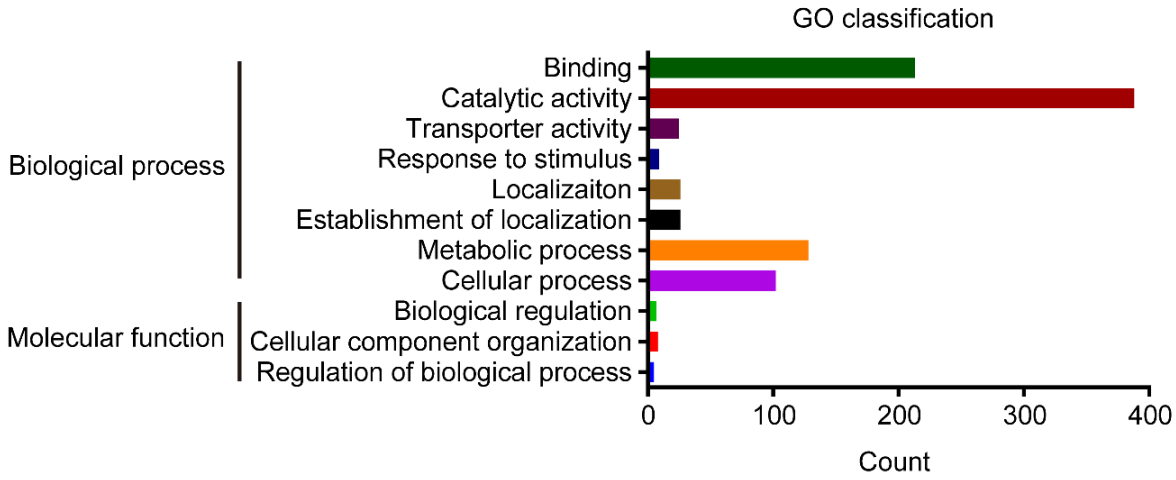

Fig. S6. Classification of differentially expressed genes (DEGs) according to gene ontology annotation. The number of DEGs in each category is represented by a horizontal column.

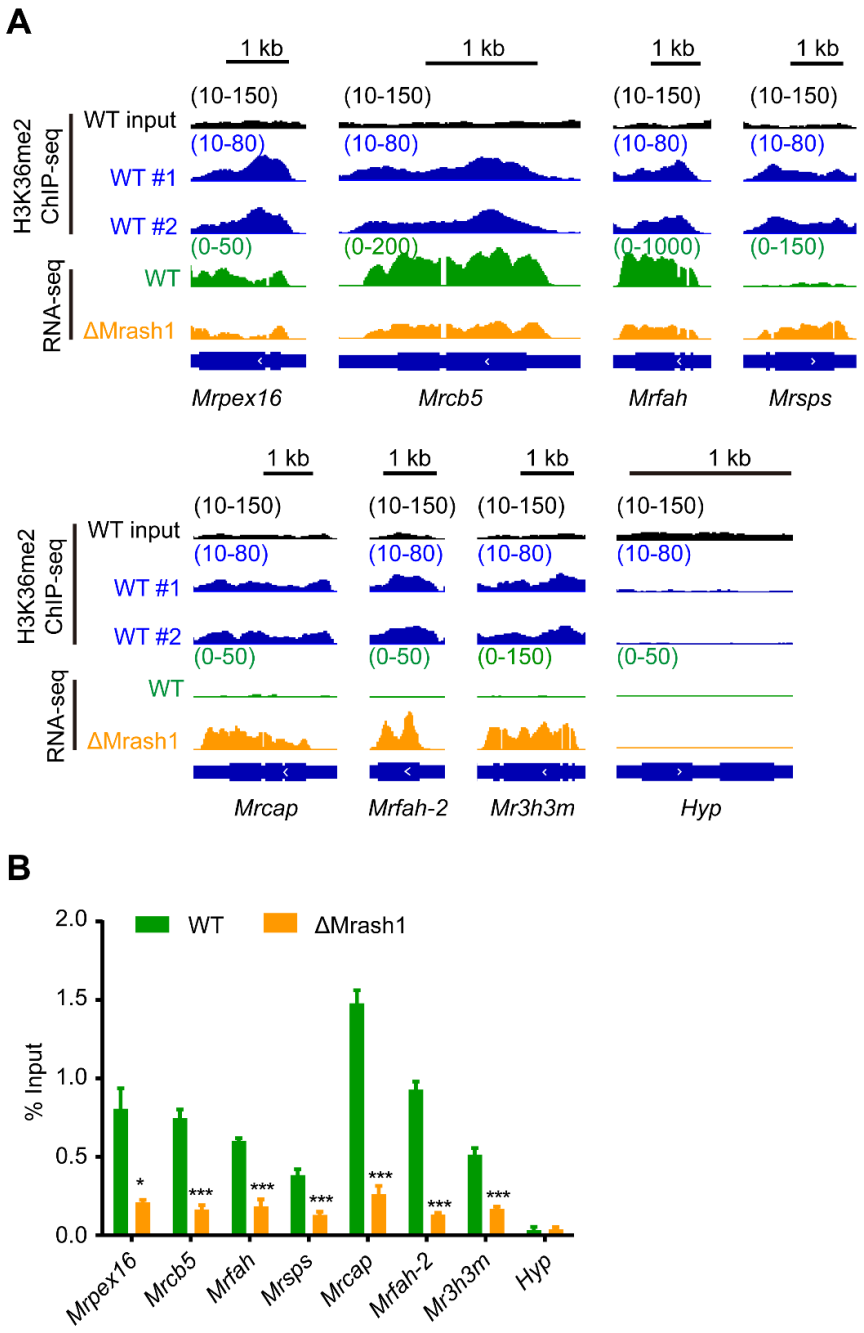

Fig. S7. Seven lipid metabolism-related genes are direct targets of H3K36me2. (A) Representative genome browser views of the enrichment of H3K36me2 in two WT replicates and mRNA signals in WT and ΔMrash1 at seven target gene and the reference gene *Hyp* (hypothetical protein) loci. (B) ChIP–qPCR analysis of H3K36me2 levels at seven target gene and the reference gene *Hyp* loci in WT and ΔMrash1. The vertical axis represents the percentage of the enriched samples (precipitated by the antibody) relative to the corresponding input samples. Data are shown as the mean ± SD of three technical replicates. Statistical significance was determined with Student's *t* test. **P* < 0.05 and ****P* < 0.001.

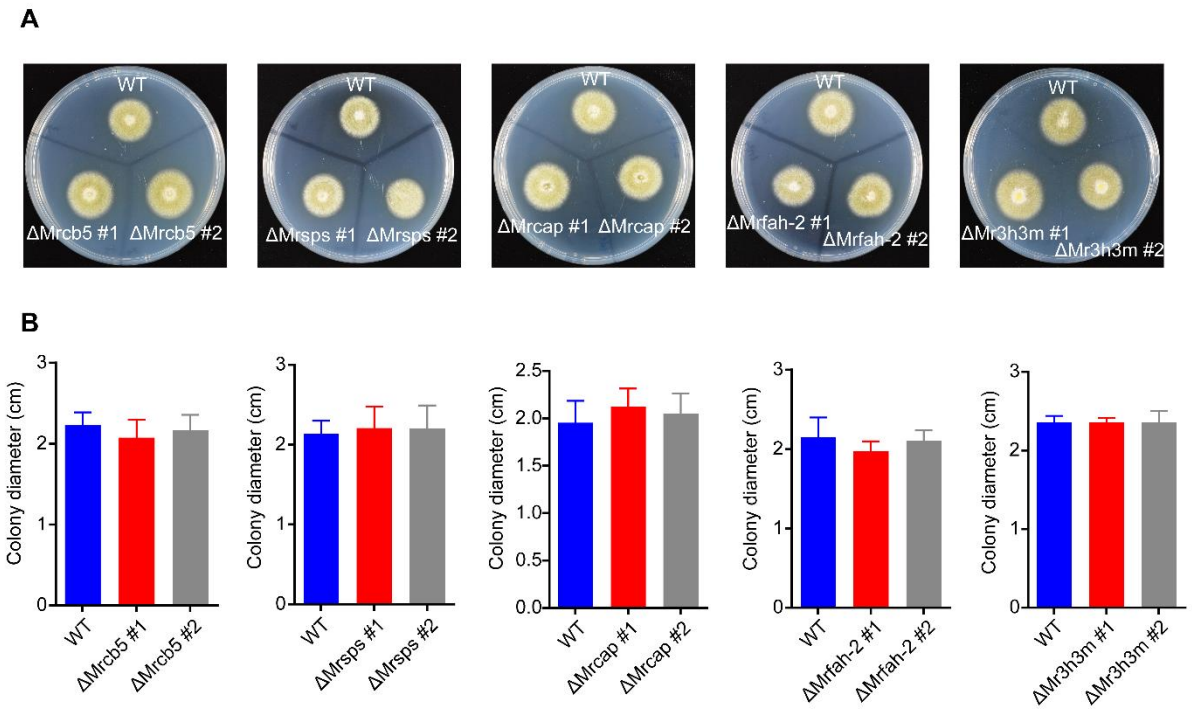

Fig. S8. Six lipid metabolism-related genes do not affect fungal pathogenicity. (A) The penetration capacity of WT and mutants of six lipid metabolism-related genes was assayed using cicada wings. Mycelial blocks inoculated on the cicada wings were incubated for 2 d. Then, the cicada wings were removed, and the penetrating mycelia were incubated for an additional 5 d. (B) Colony diameters of fungal strains in the cuticle penetration assay. Data are shown as the mean ± SD of three biological replicates.

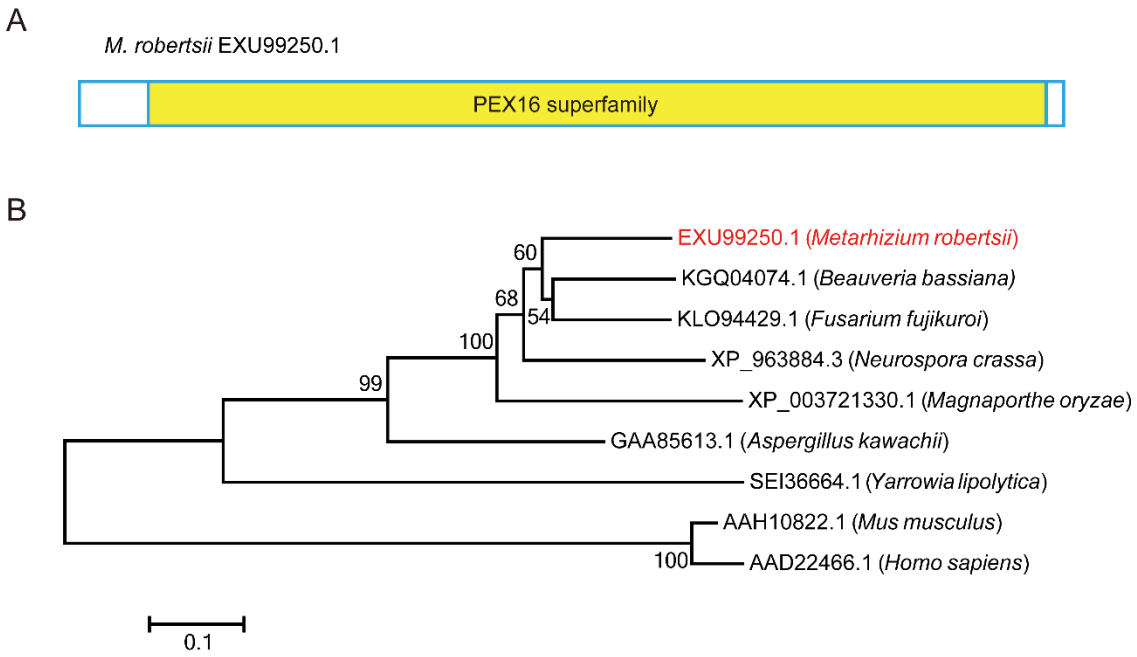

Fig. S9. Characterization of MrPEX16 (EXU99250.1). (A) Domain structures of MrPEX16. MrPEX16 was predicted to contain a PEX16 superfamily domain (marked in yellow). (B) Phylogenetic relationships between MrPEX16 and its homologues in other species. The amino acid sequences were aligned, and a neighbour-joining tree was generated with 1,000 bootstrap replicates by using Mega 6.0 software.

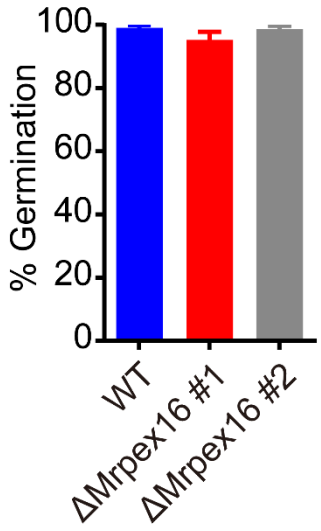

Fig. S10. *Mrpex16* does not affect conidial germination. Germination rate of the WT and two ΔMrpex16 strains at 16 h post-induction in MM-Gly medium on hydrophobic plates. Data are shown as the mean ± SD of three biological replicates. The experiments were repeated three times with similar results.

Table S1. Primers used in this study.

Primers	5'-3' sequences	Application
Mrash1-LF	CAGTACACGAGGACTTCTAGAGTGGTCTTGTGGAGGTGCGT	construction of plasmids for gene deletion
Mrash1-LR	CGAGTCTAGACGGTGATTACGTCGTGTTTAGG	
Mrash1-RF	GAGAAACTAGCTCCATCGCTACCACTCTGTCC	
Mrash1-RR	GCAGGTCGACGGATCAGATCTAGAGTTAGCGGCATTGTCAGAGC	
Mrpex16-LF	CAGTACACGAGGACTTCTAGATAGGTCACAACTTTACCCCGGC	
Mrpex16-LR	CGAGTCTAGAGCTGCTTTTGCTTGGGGTT	
Mrpex16-RF	ATTTCCGGATATCGGCTTGCCCGACCTGCCG	
Mrpex16-RR	ACCGACGGAATTGAGGATATCAACTCCCTTTCTTTTGTCCCTCTC	
Mr008851-LF	CAGTACACGAGGACTTCTAGAGCGGCGTTGGCGAAGAGATAA	
Mr008851-LR	GGCACAGATCTAGTCTCTAGATTAGCGGTGCGAGGCATTGAC	
Mr008851-RF	CTCACACTAGTGACTGATATCGTAGCACTTGGACACGGCAGAT	
Mr008851-RR	ACCGACGGAATTGAGGATATCAAGGCGGTGGAAAGGGACAGA	
Mr002674-LF	CAGTACACGAGGACTTCTAGATGCCAGACTGACAGAGCCTCAA	
Mr002674-LR	GGCACAGATCTAGTCTCTAGAAGTGTCTCGTCGGAAGCAGGAT	
Mr002674-RF	CTCACACTAGTGACTGATATCACACGAAGCTCGGACGGCATAA	
Mr002674-RR	ACCGACGGAATTGAGGATATCGCCACGGTGATGGAAGGACAGT	
Mr006414-LF	CAGTACACGAGGACTTCTAGAAGTAATTGAGTCGCCGCCTAACC	
Mr006414-LR	GGCACAGATCTAGTCTCTAGAGGTGTTGGTCCTGGAACCTTGG	
Mr006414-RF	CTCACACTAGTGACTGATATCCATTCGCCAGTGTCGCAATCA	
Mr006414-RR	ACCGACGGAATTGAGGATATCCGTCCATCACTCTGCTTCTCCT	
Mr002723-LF	CAGTACACGAGGACTTCTAGAGCTTCTTGTGCTGCCTTCTTGC	
Mr002723-LR	GGCACAGATCTAGTCTCTAGATCGTGACCGTATCCCGTGTGTA	
Mr002723-RF	CTCACACTAGTGACTGATATCGGAGAGGAGGTCTGTGGCAACA	
Mr002723-RR	ACCGACGGAATTGAGGATATCCGAGACAGCAGGCGAGATTAGC	
Mr006379-LF	CAGTACACGAGGACTTCTAGAATGAGAGCAGCCGCAAGTAACT	
Mr006379-LR	GGCACAGATCTAGTCTCTAGATGACGATGTGACCGCAAGGAG	
Mr006379-RF	CTCACACTAGTGACTGATATCAGTCACCTACACCAGTCGCAGTT	
Mr006379-RR	ACCGACGGAATTGAGGATATCGCTCTGGATTGTCGTCACACTCA	
Mr006417-LF	CAGTACACGAGGACTTCTAGATTCACAGCCGAGCAGAGGTATG	
Mr006417-LR	GGCACAGATCTAGTCTCTAGAGCATCTTCACTCCGCCTGGTAT	
Mr006417-RF	CTCACACTAGTGACTGATATCGCAGGAACAGGCTGAGCAGATT	
Mr006417-RR	ACCGACGGAATTGAGGATATCACGCCACATCAGTCGCCAAAC	
com-Mrash1-F	GCAAGGCGATTGATTGTA	
com-Mrash1-R	GCTCTAGACAGCAATTTGTGAATAG	

qMrash1-F	GAAGACTACCACGAAGCTGGT	qPCR
qMrash1-R	GCGCGAGACCTTGTTAGACT	
qMrpex16-F	AGGGCGGACTGCTGATGG	
qMrpex16-R	CGACGGCAGGCTCATCC	
qMrPr1A-F	GCATGGACTACGTTGCACAGG	
qMrPr1A-R	GACGGCAAGGAAGACACCAG	
qMrPr1J-F	ACTGGGTTCGGGATGTTCAA	
qMrPr1J-R	CAGGGTATGCTCGTCAAAGTG	
qChit009197-F	GGCCGTCCAGTATGTATGCG	
qChit009197-R	AGTATTGCTGGTCGTGGGCT	
qChit001179-F	GCCAAGACGAAGGCAGAGTATG	
qChit001179-R	CGTATTTAGTCTCGGGGTATGTGATG	
qChit005379-F	CGTGGCAAAGGGTTACTACAGC	
qChit005379-R	TGTTGCTACTAGTCCCAATGAGTGAG	
qChit003189-F	TGCAGAATGGCAAGGAGGAC	
qChit003189-R	TTGAAATGGCTCCAGTCGTG	
qChit009897-F	ACAGCTACGACAACCGCACGC	
qChit009897-R	GCTCGTGCCGATGAGGGATT	
qMr008851-F	GAGGTCGAGGCGCACAACT	
qMr008851-R	ACATCCTTGCCAGCATAGTCA	
qMr002674-F	CTGGAGCTTGCGCCACAT	
qMr002674-R	GGAGGCGTAGTTTCCAATGAAC	
qMr006414-F	GCGACGCAAGATTGGTAGA	
qMr006414-R	TCGAAATCCTCACGCACA	
qMr002723-F	GCAACGTGGGACTTTGAAGA	
qMr002723-R	GTCCAGGTTTGACGATGTGATGTA	
qMr006379-F	ACGCCTTCACAAGATACATCACA	
qMr006379-R	ACACCACCAGCGGCAGAGT	
qMr006417-F	CGAGCTACGCAACTTGACTTA	
qMr006417-R	GGCAACGTATCGCTTCTCAC	
qgpd-F	ACTCCTCCATCTTTGACGCC	
qgpd-R	GGAATAGCCCCACTCGTTGT	
ChIPqMrpex16-F1	CAAAGAATGCCGGACAAG	ChIP qPCR
ChIPqMrpex16-R1	AAGGAGACACTGGGAATCAAC	
ChIPqMrpex16-F2	AGGGAGGCCATTCCCGAC	
ChIPqMrpex16-R2	CCCTCCATCAGCAGTCCG	

ChIPqMrpex16-F3	GCCCGCTACACGCGGTTCT	
ChIPqMrpex16-R3	GCCGCCATCTCGCACAGGA	
ChIPqMrpex16-F4	CGCCTTTTACGACAATGTCACCG	
ChIPqMrpex16-R4	TTCTCCCACAGGTACTCGTAGTCCTC	
ChIPqMrpex16-F5	GATACCAGGTTGAATGGGTTTAG	
ChIPqMrpex16-R5	AATTGCTCGGCGGTGCTG	
ChIPqMrpex16-F6	CAACCCCAAGCAAAAGCAGC	
ChIPqMrpex16-R6	CGTCGTACATGGCCAGCCA	
ChIPqMr008851-F	GAGGTCGAGGCGCACAACT	
ChIPqMr008851-R	ACATCCTTGCCAGCATAGTCA	
ChIPqMr002674-F	ACAAACCACGGCAATGCTA	
ChIPqMr002674-R	ACCTCCTCGAAGACACTGAAAT	
ChIPqMr006414-F	GAGCCTGCTGACCTGTGATT	
ChIPqMr006414-R	TGTCGGTTCATTGTCTTCGTC	
ChIPqMr002723-F	CTCAGTATTGCTCATCAGCGTCTC	
ChIPqMr002723-R	TGGGTCCTGTATGATTGTTTGC	
ChIPqMr006379-F	ACGCCTTCACAAGATACATCACA	
ChIPqMr006379-R	ACACCACCAGCGGCAGAGT	
ChIPqMr006417-F	CGACAGGGAAGGTGAGTTG	
ChIPqMr006417-R	CCGTTAAAGCAAGGGAGC	
ChIPqMrHyp-F	CCAAGGACCAGACGAATGAGC	
ChIPqMrHyp-R	TTGATCCATCGAGTCTCCACC	
pSR-SKL-F	TTTTAATCAATAACAGATATCATGGTGAGCAAGGGCGAGG	construction of the plasmid pSurRFP-PTS1
pSR-SKL-R	TTACAGCTTCGAGTTTCCGGA	
pSR-SKL_F2	CCGGAAACTCGAAGCTGTAAACAATCAATCCATTTCGCTATAGTTAA	
pSR-SKL-R2	CTATGACATGATTACGAATTCCTCAATTCCGTCGGTCGATTA	
Mrpex16-GFP_F	TTTTAATCAATAACAGATATCATGCCTGCACCCGCCGCG	construction of pBar-Mrpex16-GFP and pSurGFPOE-Mrpex16
Mrpex16-GFP_R	CACACCAGGAGAACCGATATCCGCACTGGTGCTAAAGTAGTAGTTC	
ovpex16-Ben-F	CGGGGATCCACTAGTTCTAGAGGTTGGGTATGCTCCGGC	construction of pBenGFP-pex16
ovpex16-Ben-R	CGTAGTAAATGTAGGTCTAGACAGGGCTGGTGACGGAATT	

F, forward; R, reverse.

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