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Supplementary Information for

Insects defend against fungal infection by employing microRNAs to silence virulence-related genes

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Supplementary Information Text

SI Materials and Methods

Insect rearing and fungal culture. The rearing conditions and infection bioassay of *Anopheles stephensi* (Dutch strain) mosquitoes were as described previously (1). Larvae of silkworm, *Bombyx mori* L. (race: *Nistari*) were reared on fresh mulberry leaves at 25 °C under standard conditions. The wild type fungal strain *Beauveria bassiana* ARSEF252 and its mutants used in this study were cultivated in Sabouraud dextrose agar plus yeast extract (SDAY, 4% glucose, 1% peptone, 1% yeast extract and 2% agar) at 26 °C ± 1 °C.

Insect bioassay. Five-day-old adult female *An. stephensi* mosquitoes were sprayed with fungal conidial suspension (1×10^7-5×10^7 conidia/mL). Mosquitoes sprayed with sterile 0.01% Triton X-100 were used as a negative control. Silkworm larvae at the first day of fifth instar (L5D1) were inoculated by topical immersion in fungal spore suspension (5×10^6 conidia/mL) or 0.01% Triton X-100 (control) for 30 s. Each treatment was replicated three times with 30 silkworm larvae or 50 adult female mosquitoes per replicate, and the bioassays were repeated three times. Mortality was recorded every 12 h.

Mycelia collection and RNA extraction. After the silkworm larvae or *An. stephensi* died, cadavers were surface sterilized for 3 minutes in 1% bleach, rinsed five times with sterile distilled water and placed in sterile petri dishes containing a wet filter paper to encourage fungal emergence (2). Four days later, *B. bassiana* mycelia were harvested carefully from the cadavers free of any insect tissues using a sterilized tweezer. *B. bassiana* mycelia harvested from cadavers were ground into fine powder in liquid nitrogen for total RNA extraction using RNAiso Plus (TaKaRa), according to the manufacturer's instructions. *B. bassiana* mycelia cultured on SDAY medium for 4 days as the uninfected control were also collected and total RNA isolated as described above. Three samples from cadavers were used as biological replicates for sRNA sequencing.

sRNA sequencing and data analysis. sRNA libraries were constructed and sRNAs were sequenced by BGI (http://www.bgitechsolutions.com/). The raw reads were filtered to remove low quality reads, adaptor pollution reads and reads less than 18 nt. sRNA reads that did not map to the *B. bassiana* genome were aligned to the silkworm *B. mori* genome (http://www.ncbi.nlm.nih.gov/genome/genomes/76?). miRNAs were identified by matching the reads to the miRNA database (http://www.mirbase.org). Fungal hyphae miRNA abundance was normalized to the Transcripts Per Million (TPM) value. Three stringent target prediction softwares miRanda (http://www.microna.org), PITA (http://genie.weizmann.ac.il/index.html) and microTar (http://tiger.dbs.nus.edu.sg/microtar/) were jointly used to predict the host miRNA targets in the *B. bassiana* genome using default parameters.

Detection of miRNA entering fungal cell by fluorescence microscopy. To examine the transport and localization of the host miRNA in fungal cells *in vivo*, we injected the synthesized fluorescence (Cy3)-conjugated let-7, miR-100, or scrambled miR-100 control (50 μM, GenePharma) into the hemocoel of adult *An. stephensi* mosquitoes at 60 h post topical infection with *B. bassiana* (10^8 conidia/ml). The resulting hyphal bodies were recovered from the hemolymph of infected mosquitoes after 24 hours, and

washed with PBS, fixed in 4% paraformaldehyde, and then mounted with VECTASHIELD Antifade Mounting Medium containing DAPI (Vector Laboratories, H-1200) for 5 min and fluorescence signals were visualized with an OLYMPUS FV1000 microscope.

Quantitative reverse transcription PCR (qRT-PCR). Total RNA from different samples (mycelia from cadavers, mycelia cultured on SDAY plates, mosquitoes infected with *B. bassiana*, silkworm tissues infected with *B. bassiana*) were extracted using RNAiso Plus (TaKaRa) and were treated with Recombinant DNase I (TaKaRa) to remove DNA contamination. Total RNA was reverse transcribed to synthesize cDNA using a TransScript All-in-One First-Strand cDNA Synthesis SuperMix kit (TransGen). qRT-PCR was performed using the AceQ qPCR SYBR Green MasterMix kit (Vazyme). Gene relative expression was calculated using the $2^{-\Delta Ct}$ method with normalization to *B. bassiana* actin gene.

To synthesize mosquito or *B. bassiana* miRNA cDNAs, 1 μg of total RNA was reverse transcribed using miRcute miRNA First-Strand cDNA Synthesis Kit (Tiangen), and qRT-PCR was performed using miRcute miRNA qPCR detection Kit (Tiangen) according to the manufacturer's protocol. To synthesize silkworm miRNA cDNAs, 1 μg total RNA of the silkworm tissues infected with *B. bassiana* was reverse transcribed to synthesize miRNA cDNA using miRNA 1st Strand cDNA Synthesis Kit (by stem-loop) (Vazyme) according to the manufacturer's protocol. qRT-PCR reactions were performed using the AceQ qPCR SYBR Green MasterMix kit (Vazyme). Primers used for qRT-PCR are listed in Table S3. qRT-PCR was performed in three biological replicates.

Dual luciferase reporter assays. The HEK293T cell line was used for the dual luciferase reporter assay for its high transfection efficiency and low background expression of target genes (3). HEK293T cells were cultured in the DMEM/HIGH GLUCOSE medium (HyClone) supplemented with 10% (vol/vol) of heat-inactivated Fetal Bovine Serum (FBS, Gibco) and 1 × antibiotic–antimycotic (Gibco) at 37 °C under 5% CO_2. A ~600-bp genomic fragment containing *B. mori* let-7 precursor and a ~480-bp genomic fragment containing *B. mori* miR-100 precursor were separately cloned into the *Hind*III and *Eco*RI sites of pmR-mCherry vector (Promega) for the expression of let-7 and miR-100. The ~400-bp sequences of the CDS and the 5′UTR surrounding the predicted let-7 and miR-100 target sites in *sec2p* and *C6TF*, respectively, were separately cloned into the *Xho*I and *Not*I sites of psiCHECK-2 vector (Promega). HEK293T cells were co-transfected with 20 ng of psiCHECK-2-target reporter vector and 200 ng of the miRNA expression vector or empty vector mixed using 1.5 uL Attractene Transfection Reagent (Qiagen). Cells were collected and lysed at 48 h after transfection. The activities of the firefly and Renilla luciferases were measured using the Dual-Luciferase Reporter Assay System (Promega). Results are shown as the ratio Renilla/firefly luciferase activity (mean ± SEM). Each sample was performed in triplicate, and transfections were repeated three times.

Expression of let-7 or miR-100 in *B. bassiana*. To ectopically express let-7 or miR-100 in *B. bassiana*, a ~600-bp genomic fragment surrounding primary let-7 and a ~480-bp genomic fragment surrounding primary miR-100 were PCR amplified from *B. mori* genomic DNA, and then separately cloned into the *Eco*RI and *Eco*RV sites of the binary vector pBarGFP-PgpdA downstream of the constitutive promoter of the glyceraldehyde-3-phosphate dehydrogenase (gpdA) using the ClonExpress™ MultiS one step cloning

kit (Vazyme, C113-01). The resulting let-7 or miR-100 expression vectors were separately transformed into *B. bassiana* ARSEF252 by *Agrobacterium tumefaciens*-mediated transformation method (4) to generate microRNA overexpression strains. The resulting strains were designated as Bb-gpd::let-7 and Bb-gpd::miR-100. Primers used to construct vectors are listed in Table S3.

Generation of target gene deletion mutants and miRNA-resistant mutated strains. Target gene deletion mutants were constructed using a conventional gene targeting method by homologous recombination. The 5′ and 3′ flanking regions of the *sec2p* and *C6TF* were PCR amplified, and cloned into *Xba*I and *Eco*RV sites of the binary vector pBarGFP, respectively (5). The gene deletion constructs (pBarGFP-*sec2p*, pBarGFP-*C6TF*) were separately transformed into *A. tumefaciens* AGL-1 for targeted gene deletion in *B. bassiana* by homologous recombination (4). For gene deletion mutant screening, replacement-specific PCR was performed with specific primer pairs (Table S3) for each gene.

To generate miRNA-resistant mutated strains, we reverted disruptants Δ*sec2p* and Δ*C6TF* with miRNA-resistant target site mutated gene *sec2p-mu* and *C6TF-mu* that carry the mutated nucleotide sequences in miRNA-binding site without changing the amino acid sequence, respectively. Two pairs of primers containing the mutated sequence corresponding to the miRNA-binding sites were used to amplify *sec2p-mu* and *C6TF-mu* fragments with native promoter from *B. bassiana* genomic DNA. The PCR products and the linearized binary vector pSurGFP digested with *Spe*I and *Eco*RV (NEB) were assembled to generate the pSurGFP-*sec2p-mu* and pSurGFP-*C6TF-mu* constructs using the ClonExpress™ MultiS one step cloning kit (Vazyme, C113-01). Then, the miRNA-resistant mutated strains Δ*sec2p:sec2p-mu* and Δ*C6TF:C6TF-mu* were generated by introduction of pSurGFP-*sec2p-mu* and pSurGFP-*C6TF-mu* into the disruptants Δ*sec2p* and Δ*C6TF* using *A. tumefaciens*-mediated fungal transformation, respectively. Putative transformants were chosen and verified for *sec2p-mu* and *C6TF-mu* gene expression by qRT-PCR. Primers used to construct vectors and screening are listed in Table S3.

Generation of *B. bassiana* strains expressing anti-let-7 and anti-miR-100. To sequester the translocated host miRNAs and inhibit their activity in the fungus, we generated Bb-anti-let7 and Bb-anti-mir100 and Bb-anti-mir100-let7 strains that express specific miRNA sponge(s). We used the miRNAsong web site (https://www2.med.muni.cz/histology/miRNAsong/) to design the let-7 sponge and miR-100 sponge that contain binding sites complementary to the seed region of the target miRNAs (6). The let-7 sponge sequence contained 3 repetitive sequences (ACTATACTTATACTACCTCA) with a 5-nt linker (TGCAC) between each repeat. The miR-100 sponge sequence contained 3 repetitive sequences (CACAAGTTATCTCTACGGGTT) with a 5-nt linker (TGCAC) between each repeat. Moreover, the let-7 sponge or the miR-100 sponge repeat sequence contained bulged sites that were mispaired to the miRNA at positions 8~10. A pair of primers were annealed to produce sponge sequences and then subcloned into the *Eco*RI and *Eco*RV of the binary vector pBarGFP-PgpdA using the ClonExpress™ MultiS one step cloning kit (Vazyme, C113-01). The pBarGFP-PgpdA-let7-sponge and pBarGFP-PgpdA-miR100-sponge plasmids were separately transformed into *B. bassiana* spores by an *A. tumefaciens*-mediated transformation method (4). The resulting strains were designated as Bb-anti-let7 and Bb-anti-mir100. To generated Bb-anti-mir100-let7 strain that expresses both let-7 and miR-100 sponges, we subcloned the

let-7 sponge sequence into the *Spe*I and *Eco*RV of the binary vector pSurGFP-PgpdA using the ClonExpress™ MultiS one step cloning kit (Vazyme, C113-01) and transformed the pSurGFP-PgpdA-let7-sponge plasmid into Bb-anti-mir100 strain. Primers used to construct vectors were listed in Table S3.

Determination of fungal invasive growth and hyphal body formation. The cicada hind wings were surface sterilized in 3% bleach for 5mins, rinsed three times with sterile water, and placed on 0.7% water agar and dropped 20 μL of 10^7 conidia/mL suspension. 12 hours after incubation at 27°C, conidial germination and fungal growth were determined by microscopic observation. To monitor hyphal body differentiation in the insect hemocoel, the fifth instar larvae of *B. mori* (Nistari) were inoculated by topical immersion in fungal spore suspension (5×10^6 conidia/mL) for 30 s. The hemolymph was collected 60 hours post topical infection. The hyphal bodies induced in the hemolymph were counted by microscopic observation using a hemocytometer. Each treatment involved 10 larvae. The experiment was repeated three times.

Quantification of fungal biomass in the hemocoel. To quantify the load of *B*. *bassiana* in the mosquito hemocoel, 20 mosquitoes were collected at 60 hours after infection with WT or mutants. The total DNA was extracted by DNeasy Blood & Tissue Kits (Qiagen) according to the manufacturer's instruction. The fungal housekeeping gene 18S rRNA was used to quantify fungal load by qPCR analysis.

miRNA antagomir injection. The miRNA antogomir was used to down-regulate the corresponding endogenous miRNA levels. The miRNA antagomir is a chemically modified, single-stranded RNA molecule complementary to the corresponding miRNA. 69 nL let-7 antagomir (200 nM) or miR-100 antagomir (200 nM) was microinjected into the thorax of 3-day-old female mosquitoes using Nanoject II microinfector (Drummond). Control mosquitoes were injected with negative control antagomir (5′-CAGUACUUUUGUGUAGUACAA-3′) (200 nM). The miRNA antagomirs and negative control antagomirs were synthesized by Sangon Biotech (Shanghai, China). Mosquitoes were allowed to recover for 3 d before conducting fungal infection.

Statistical analysis. The statistical significance for survival data from fungal infection bioassays was analyzed with a log-rank (Mantel-Cox) test. Other statistical significance was determined by Student's t-test or one-way ANOVA. A value of $P < 0.05$ was regarded as statistically significant. Statistical analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software).

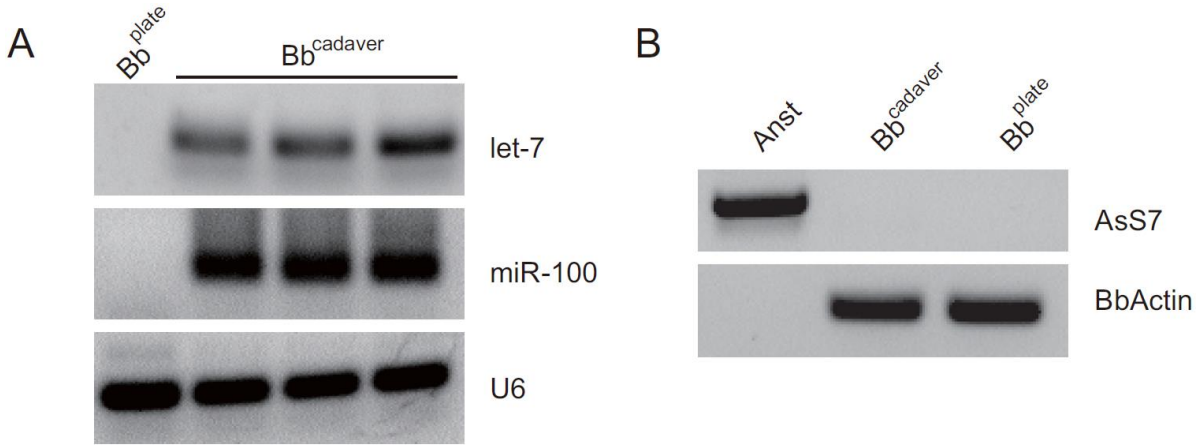

Fig. S1. Detection of insect host miRNAs in fungal hyphae recovered from infected insect cadavers but not in cultured hyphae. (*A*) RT-PCR detection of host miRNAs let-7 and miR-100 in fungal hyphae recovered from infected silkworm larva cadavers ($Bb^{cadaver}$) and cultured *B*. *bassiana* hyphae (Bb^{plate}). The *B*. *bassiana* snRNA U6 was used as an internal reference. (*B*) RT-PCR detection showing that fungal mycelia recovered from infected *Anopheles stephensi* mosquito cadavers ($Bb^{cadaver}$) were not contaminated with mosquito tissues. *An. stephensi* ribosomal protein S7 (*AsS7*) primers were used to detect mosquito nucleic acids. Anst, non-infected mosquito adults; $Bb^{cadaver}$, fungal mycelia recovered from infected *An. stephensi* mosquito cadavers; Bb^{plate}, fungal mycelia grown on plates. *B*. *bassiana actin* (*Bb-actin*) primers were used to detect *B*. *bassiana* nucleic acids.

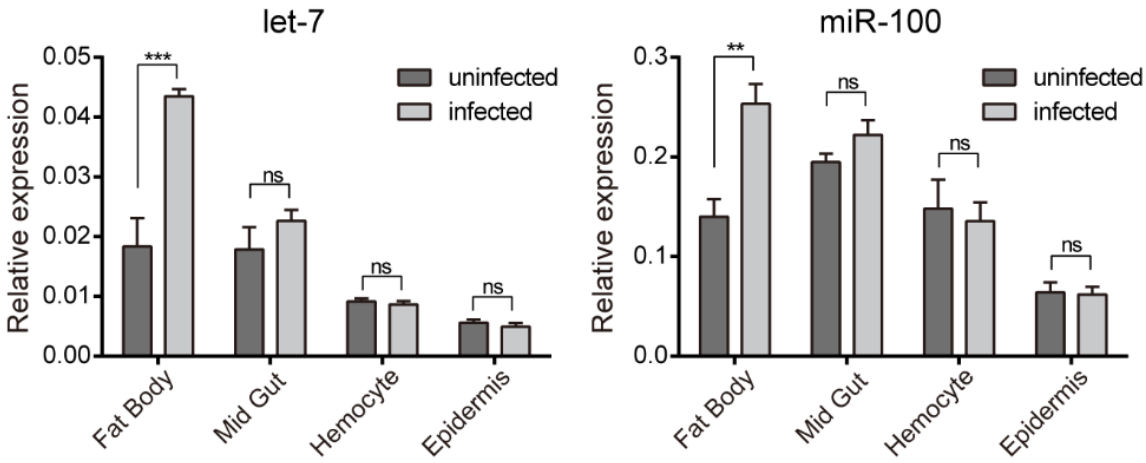

Fig. S2. Expression of let-7 and miR-100 is induced in the fat body of silkworms infected with *B. bassiana*. Stem-loop qRT-PCR showed that the expression of let-7 and miR-100 was significantly increased in the silkworm fat body at 3 d post topical infection with *B. bassiana* ARSEF252, but not in the midgut, hemocytes or epidermis. The *B. mori* U6 snRNA was used as an internal reference. Error bars indicate the SEM of three technical repeats. $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant (Student's *t*-test)

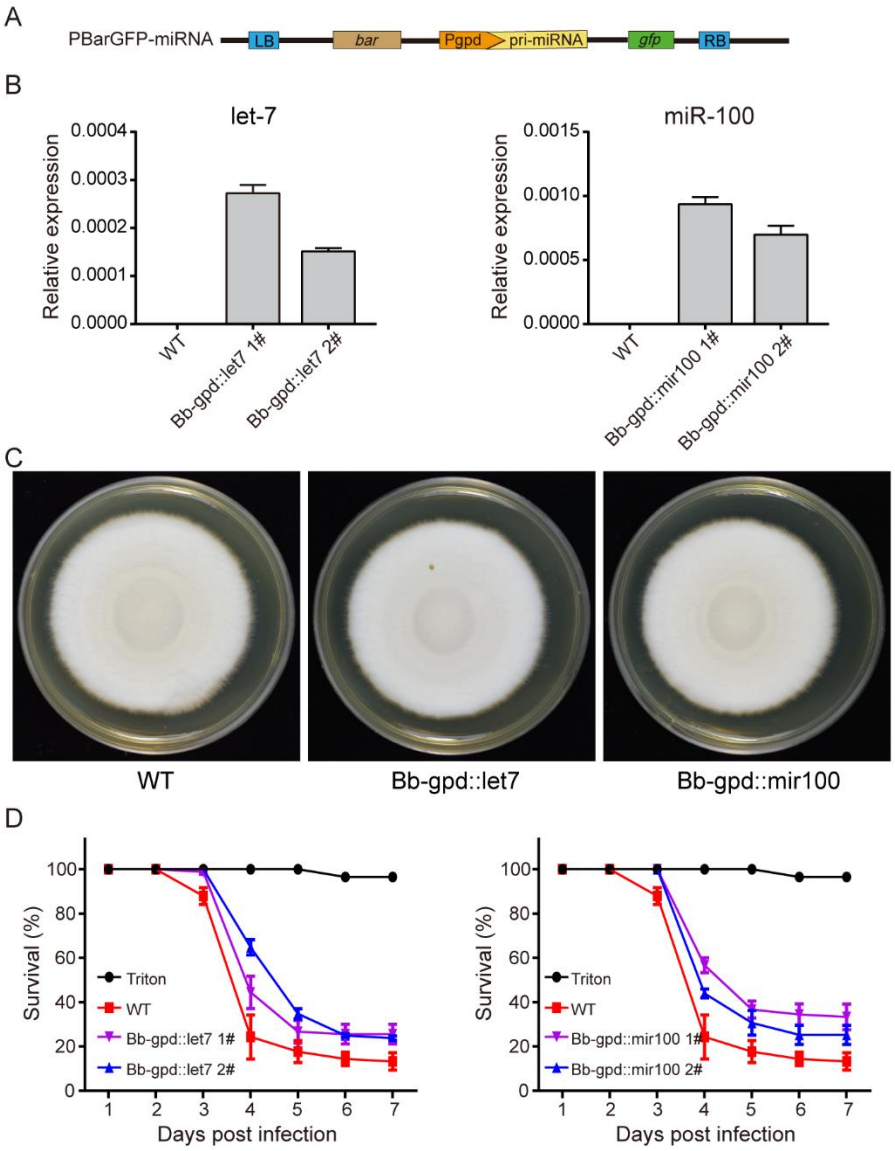

Fig. S3. Transgenic *B. bassiana* strains expressing host let-7 or miR-100 exhibit decreased virulence toward silkworm larvae. (*A*) Schematic diagram of the vector pBarGFP-miRNA that was used for fungal miRNA expression. Pgpd, *Aspergillus nidulans gpdA* promoter; bar, the Ignite/basta-resistance (bar) gene; pri-miRNA, primary miRNA; LB, left border; RB, right border. (*B*) Relative expression levels of let-7 and miR-100 in independent *B. bassiana* transformants. The *B. bassiana* U6 snRNA was used as an internal reference in qRT-PCR analysis. Error bars indicate the SEM of three repeats. (*C*) Representative colony morphology for WT, Bb-gpd::let7 and Bb-gpd::mir100 strains grown on SDAY plates for 12 days. (*D*) Silkworm larval survival after topical application of WT or transgenic spore suspension (5×10^6 conidia/mL), as indicated. Larvae immersed in sterile 0.01% Triton X-100 were used as a negative control. Transgenic *B. bassiana* strains were less virulent compared with WT ($P < 0.05$, Log-rank test). Data are presented as means ± SEM (n = 3).

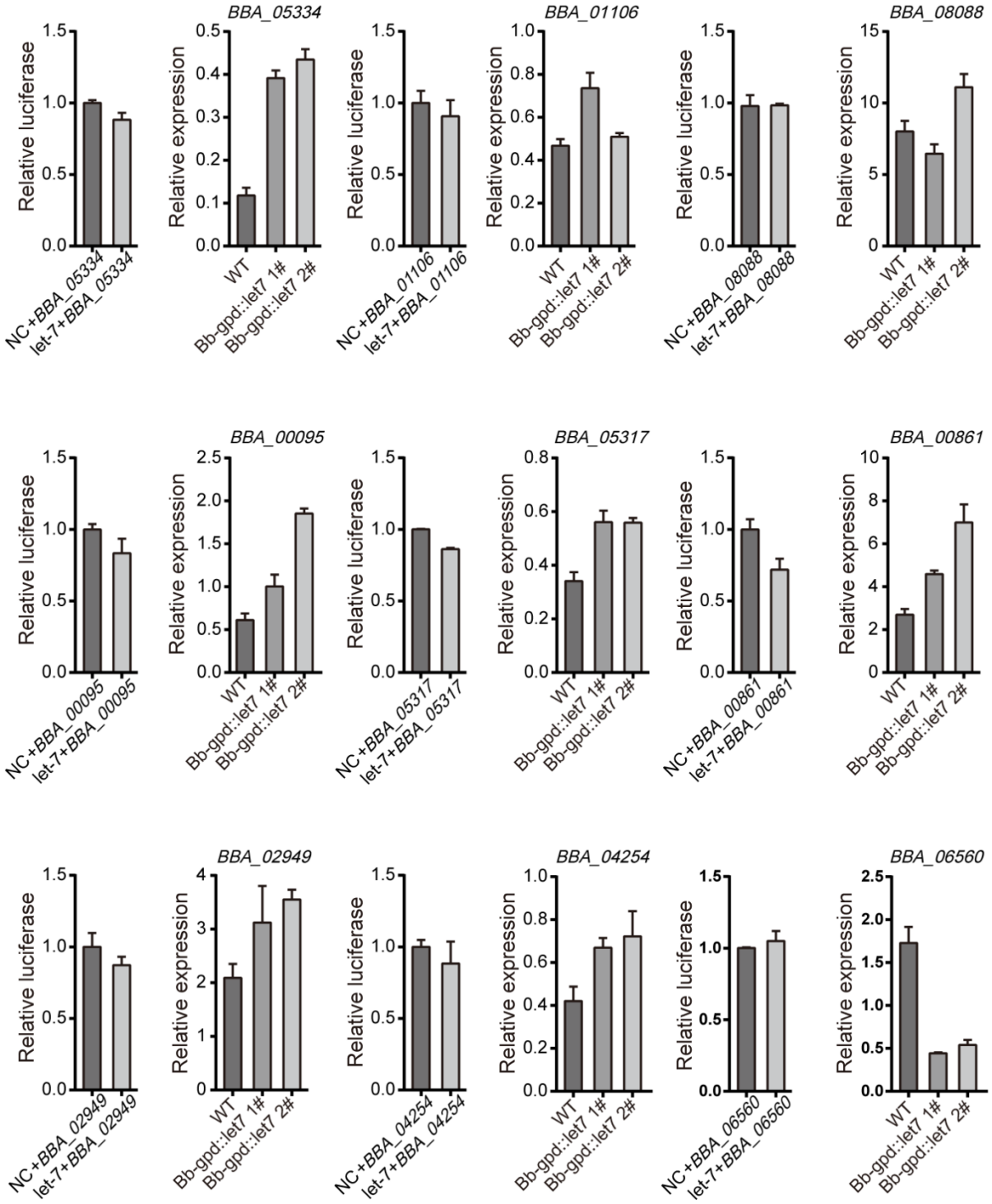

Fig. S4. Testing of the predicted let-7 targets. Candidate target genes were tested by luciferase reporter assays in cultured cells (left panel of each group) and *in vivo* by qRT-PCR in the transgenic fungal strains overexpressing let-7 (right panel of each group). Data are presented as means ± SEM (n = 3).

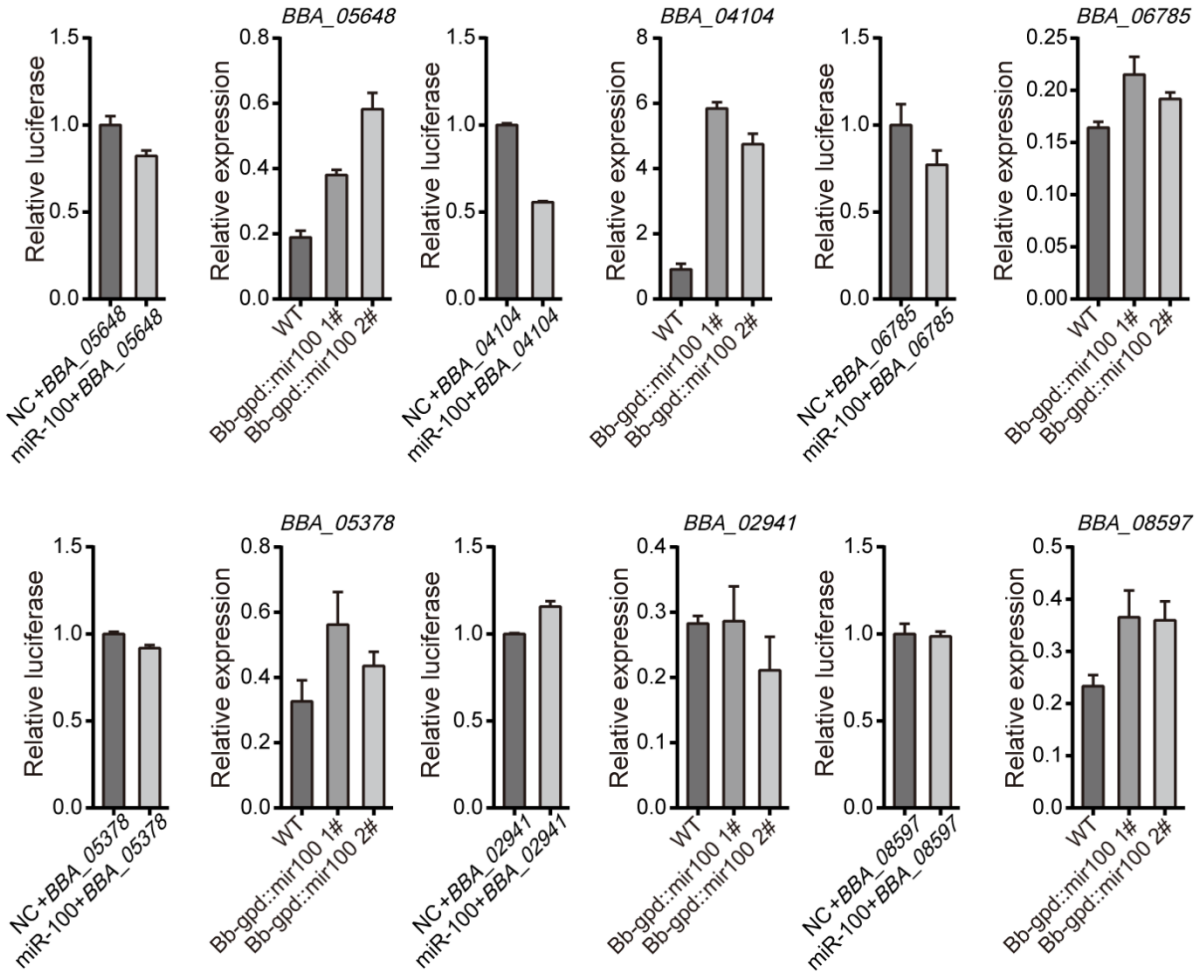

Fig. S5. Testing of the predicted miR-100 targets. Candidate target genes were tested by luciferase reporter assays in cultured cells (left panel of each group) and *in vivo* by qRT-PCR in the transgenic fungal strains overexpressing miR-100 (right panel of each group). Data are presented as means ± SEM (n = 3).

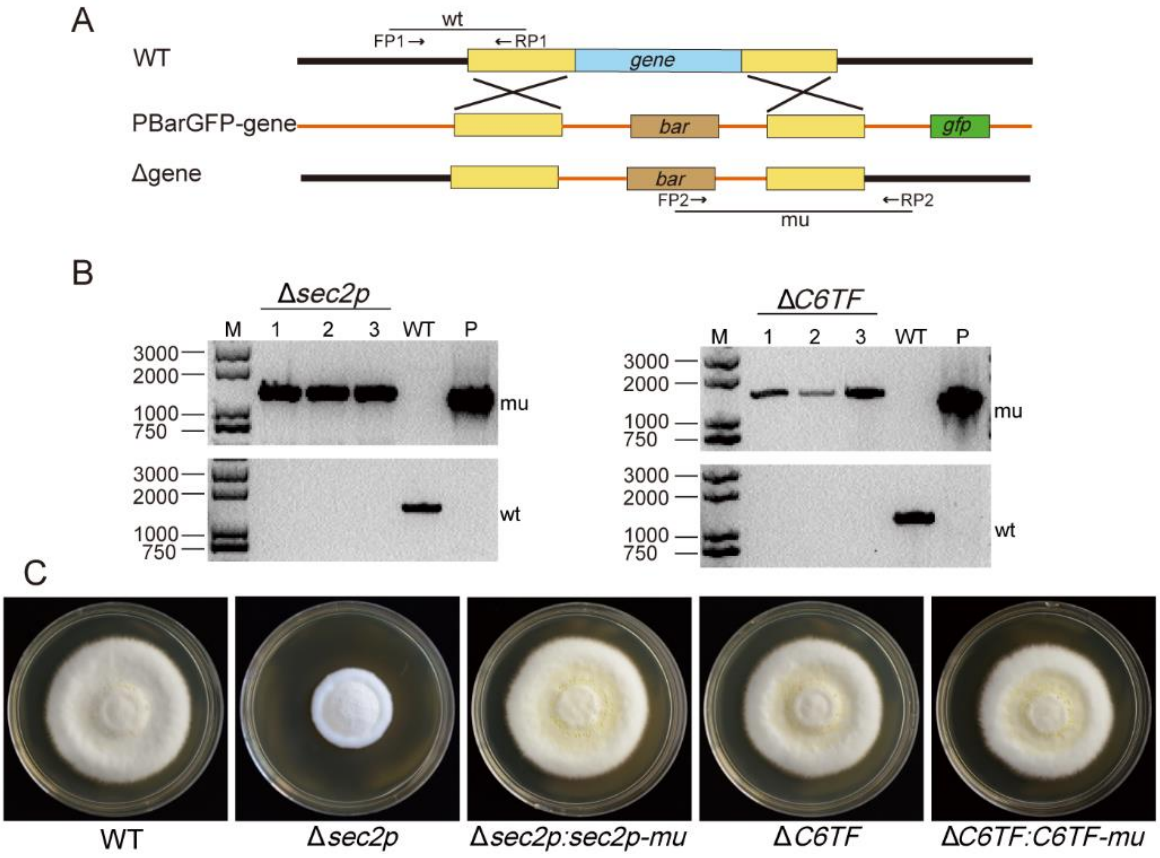

Fig. S6. Deletion of *B. bassiana* target genes. (*A*) Schematic representation of a target *B. bassiana* gene and the plasmid pBarGFP that was used for gene disruption via homologous recombination. The primer pairs FP1 and RP1 were used to amplify WT genome DNA fragment, FP2 and RP2 were used to verify the deletion mutants. (*B*) Verification of Δ*sec2p* and Δ*C6TF* mutant strains by PCR using primer pairs shown in panel A. Plasmid pBarGFP-genes (P) was used as a positive control. 1, 2 and 3 are three mutant strains. (*C*) Representative colony morphology for WT, *sec2p* deletion mutants Δ*sec2p*, let-7-resistant and *sec2p*-complemented strain Δ*sec2p:sec2p-mu*, *C6TF* deletion mutants Δ*C6TF*, miR100-resistant and *C6TF*-complemented strain Δ*C6TF:C6TF-mu* on SDAY plates for 12 days.

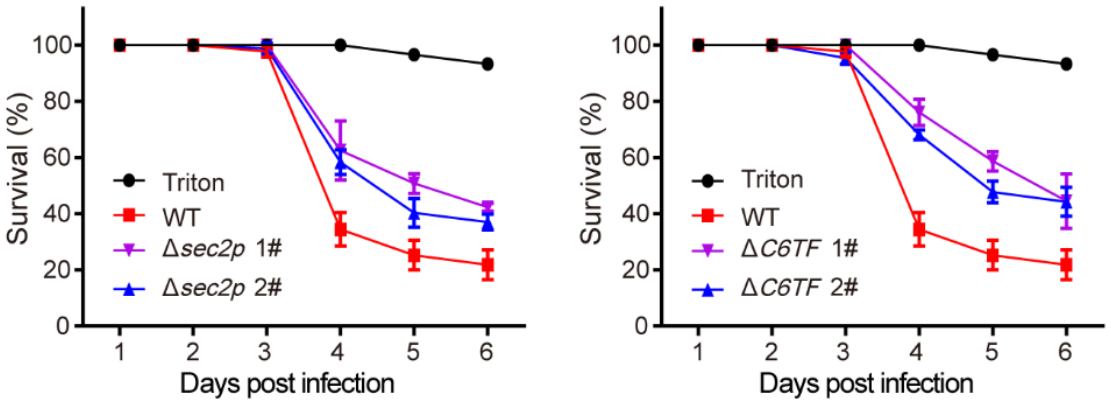

Fig. S7. Δ*sec2p* and Δ*C6TF* mutants exhibit decreased virulence in silkworm larvae. Survival of L5D1 silkworm larvae infected with WT, Δ*sec2p* or Δ*C6TF* mutant strains after immersed in a spore suspension (5×10^6 conidia/mL). Silkworm larvae immersed in sterile 0.01% Triton X-100 were used as a negative control. Δ*sec2p* and Δ*C6TF* mutants exhibited reduced virulence compared to WT ($P < 0.05$, Log-rank test). Data are presented as means ± SEM (n = 3).

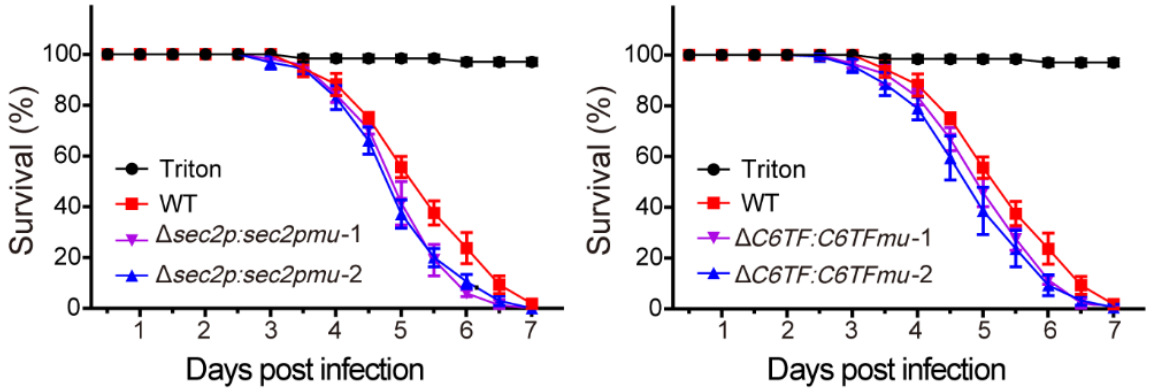

Fig. S8. The miRNA-resistant complemented strains are more virulent to mosquitoes. Survival of adult female mosquitoes infected with WT and miRNA-resistant complementary strains Δ*sec2p:sec2p-mu* and Δ*C6TF:C6TF-mu* following topical application of a spore suspension (10^7 conidia/mL). Mosquitoes sprayed with sterile 0.01% Triton X-100 were used as a negative control (Triton). The miRNA-resistant complemented strains Δ*sec2p:sec2p-mu* and Δ*C6TF:C6TF-mu* showed stronger virulence compared to WT ($P < 0.05$, Log-rank test). The experiments were repeated three times with similar results. Data are presented as means ± SEM (n = 3).

Table S1. let-7 target prediction in *B. bassiana* using the multi-algorithm and multi-model strategy.

Gene ID	Gene Description	mfe (kcal/mol)	Target site
BBA_07763	GDP/GTP exchange factor Sec2p	-18.2	3' UGAUA-UGUUGGAUGAUGGAGU 5' ||| | ||\:|||||\: 5' AAUAUCCCUUCCUGCUACCUUC 3'
BBA_06560	ABC transporter	-26.5	3' UGAUAUGUUGGAUGAUGGAGU 5' |:||| | |||||||| 5' GCUGUAC-UCGUACUACCUCA 3'
BBA_05334	Fungal specific transcription factor	-23.7	3' UGAUAUGUUGGA--UGAUGGAGU 5' |:|||| |||||||| 5' CCACCUCGACCUCGACUACCUCG 3'
BBA_00095	Oxidoreductase molybdopterin binging domain-containing protein	-20.3	3' UGAUAUGU----UGGAUGAUGGAGU 5' ||||| | |||||||:|| 5' UACAUACACCCCAGCUACUACUUCA 3'
BBA_01160	Ser/Thr protein phosphatase superfamily	-19.7	3' UGAUAUGUUGGAUGAUGGAGU 5' ||||:| ||||||| 5' AUCCCACAAUC-CCUACCUCG 3'
BBA_08088	Oxoglutarate dehydrogenase	-26.3	3' UGAUAU-GUUGGAUGAUGGAGU 5' |:|| ||:||| ||:|||| 5' GUUGUAGCAGCCU-CUGCCUCA 3'
BBA_00861	C6 transcription factor	-23.0	3' UGAUAUGUUGGAUGAUGGAGU 5' |||:|:| :||||||| 5' CACUUACGAUC-GCUACCUCU 3'
BBA_05317	Cutinase transcription factor 1 alpha	-18.7	3' UGAUAUGUUGGAUGAUGGAGU 5' ::|:||||||| 5' CGCAGUUUUUUUGCUACCUCG 3'
BBA_02949	ATP synthase F0	-24.2	3' UGAUAUGUUGGAUGAUGGAGU 5' :||||:||:|||| 5' UCCGCCAGACCUGCUGCCUCG 3'
BBA_04254	MAP kinase kinase	-22.3	3' UGAUAUGUUGGAUGAUGGAGU 5' :||||||||||| 5' CUCCAAGCGCCUACUACCUCU 3'

Table S2. miR-100 target prediction in *B. bassiana* using the multi-algorithm and multi-model strategy.

Gene ID	Gene Description	mfe (kcal/mol)	Target site
BBA_02941	Chitinase A1	-26.0	3' GUGUUCAAG-CCUAGAUGCCCAA 5' ||||| | |||||||||| 5' GUCAAGUACAUGAUCUACGGGUC 3'
BBA_08597	Meiotic recombination protein recl4	-21.1	3' GUGUUCAAGC-CU-AGAUGCCCAA 5' ||| || |||||||| 5' CGUUCUAUCGUGAGGCUACGGGUG 3'
BBA_03208	Fungal specific transcription factor	-24.5	3' GUGUUCA-AGCCUAGAUGCCCAA 5' :|| |:||||:|:|||| 5' AUUUGGUGUUGGAUUUGCGGGUG 3'
BBA_06785	Putative DNA-directed RNA polymerase III subunit C11	-19.5	3' GUGUUCAAGCCUAGAUGCCCAA 5' || | || :||||||| 5' AUCACUUGCGUUGUUACGGGUG 3'
BBA_05378	Nicotinamide N-methyltransferase	-20.0	3' GUGUUCAAGCCUAGAUGCCCAA 5' |:|| ||| ||||||| 5' GACGAGAAGAGAU-UACGGGUC 3'
BBA_04104	DNA-binding protein	-23.6	3' GUGUUCAAGCCUAG-AUGCCCAA 5' |||||:|: ||||||| 5' CGUCUAUUCGGGUUGUACGGGUA 3'
BBA_05648	Glutathione S-transferase	-21.6	3' GUGUUCAAGCCUAG-AUGCCCAA 5' |:| ||| |:|| ||||||| 5' AGCGA-UUCUGGUCAUACGGGUC 3'

Table S3. Primers used in this study.

Primer	Sequence
qBmlet7-F	TGAGGTAGTAGGTTGTATAGT
qAslet7-F	TGAGGTAGTTGGTTGTATAGT
qmiR100-F	AACCCGTAGATCCGAACTTGTG
qBbU6-F	TCGGCTCATTTGGTCTTTTGA
qBbU6-R	TGCGTGTCATCCTTAGTGCA
qsec2p-F	TGAGCCAGTTCCCACCAAT
qsec2p-R	TCCCAAGCCGTCTTTTCG
qC6TF-F	CGTTTGTTCCCTACGCTGTG
qC6TF-R	CCCGCTCCATTTCCTTGA
qactin-F	ACGACGCAGGAAGGGGATA
qactin-R	GGTGGGTACTCACGGAAAACA
qBmorp49F	TCAATCGGATCGCTATGACA
qBmorp49R	ATGACGGGTCTTCTTGTTGG
let7-stemloopRT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTATA
let7-FP	TCGGTCGTGAGGTAGTAGGTTG
miR100-stemloopRT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACAAG
miR100-FP	TCGGTCGAACCCGTAGATCCGAA
BmoU6stemloopRT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACGATTTTGCGT
BmoU6FP	ACGGCTGCGCAAGGATGACACG
common reverse primer	GCAGGGTCCGAGGTATTC
OVlet7-F	TCCACCTCACCCTTCCGGAATTCTTGAGGTGCCATCTGTCATG
OVlet7-R	GCTTAGCTGAGGAATCCTCATGATATCCCGTTTTATTTGGTATCAGCA
OVmiR100-F	TCCACCTCACCCTTCCGGAATTCAGGTTTACACTGACGGTCTTCG
OVmiR100-R	GCTTAGCTGAGGAATCCTCATGATATCGCCACGCAACTCAACTCTTC
pmRmiR100F	CTCAGATCTCGAGCTCAAGCTTAGGTTTACACTGACGGTCTTCG
pmRmiR100R	CGGTACCGTCGACTGCAGAATTCGCCACGCAACTCAACTCTTC
pmRlet7F	CTCAGATCTCGAGCTCAAGCTTTTGAGGTGCCATCTGTCATG
pmRlet7R	CGGTACCGTCGACTGCAGAATTCCCGTTTTATTTGGTATCAGCA
psisec2p-F	AATTCTAGGCGATCGCTCGAGCATCAATCTGGCGCATCAGT
psisec2p-R	ATTTTATTGCGGCCAGCGGCCGCCCGTCCGTAACAGCAGTCAA
psiC6TF-F	AATTCTAGGCGATCGCTCGAGGAAGCGGGGTTACGGAAAT
psiC6TF-R	ATTTTATTGCGGCCAGCGGCCGCGGAACGACAATCGGCTCAAT
KOsec2pLF	CACAGTACACGAGGACTTCTATACCAGACGGGATGAAGCG
KOsec2pLR	AATATCATCTTCTGTCGAGTCGGAAGGACCAAGGATAGGAGAAT

KOsec2pRF	CGGTGTCTGTATTTCCGGATTCTAGCGAACAAGGCGATTTC
KOsec2pRR	CGACCGACGGAATTGAGGATTCTGGTGGTCCGTCACTCTG
KOC6TFLF	CACAGTACACGAGGACTTCTATGGTTCCTTGAAACTTGGCTCT
KOC6TFLR	AATATCATCTTCTGTCGAGTCATTTACCTGGGGCGACTGG
KOC6TFRF	CGGTGTCTGTATTTCCGGATCCCTCAACTCCAGCACCTCT
KOC6TFRR	CGACCGACGGAATTGAGGATTGGCGAGAACAGTCAAGGATG
sec2p-muF1	ATTGCATGCTCTCACACTAGTCTTGATGCGGTAAGCAGGAG
sec2p-muR1	CGACGTGCTTCCAGCCAGTCCGAGACCAAGCGCGGTCAG
sec2p-muF2	GACTGGCTGGAAGCACGTCGAGCGGTCACATTTCGAACGC
sec2p-muR2	GACCGACGGAATTGAGGATATCCTCCACACCCCGATGTCGAG
C6TF-muF1	CGATGGTCTAGGTTAGCGACAAATGGCTGGTTAGGGCTCGTCGC
C6TF-muR1	ATTGCATGCTCTCACACTAGTGTCCAACGACCATCACCGA
C6TF-muF2	GACCGACGGAATTGAGGATATCGTTTTTTCTTGTCGGGACTTGCG
C6TF-muR2	ATTTGTCGCTAACCTAGACCATCGATCATTTTAGTGAGGAAGCT
let7-spongeF	TCTCTCTTCCACCTCACCCTTCCGACTATACTTATACTACCTCATGCACACTATACTTATACTA
let7-spongeR	GCTTAGCTGAGGAATCCTCATTGAGGTAGTATAAGTATAGTGTGCATGAGGTAGTATAAGTA
miR100-spongeF	TCTCTCTTCCACCTCACCCTTCCGCACAAGTTATCTCTACGGGTTTGCACCACAAGTTATCTCT
miR100-spongeR	GCTTAGCTGAGGAATCCTCATAACCCGTAGAGATAACTTGTGGTGCAAACCCGTAGAGATAACT
Bb18S-F	TGGTTTCTAGGACCGCCGTAA
Bb18S-F	CCTTGGCAAATGCTTTCGC
qS7-F	TGATCTGTACATCACCCGCG
qS7-R	AGAATACGACGCTCCGCAAT

F, forward; R, reverse.

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