Supplementary Materials

Supplementary Figures

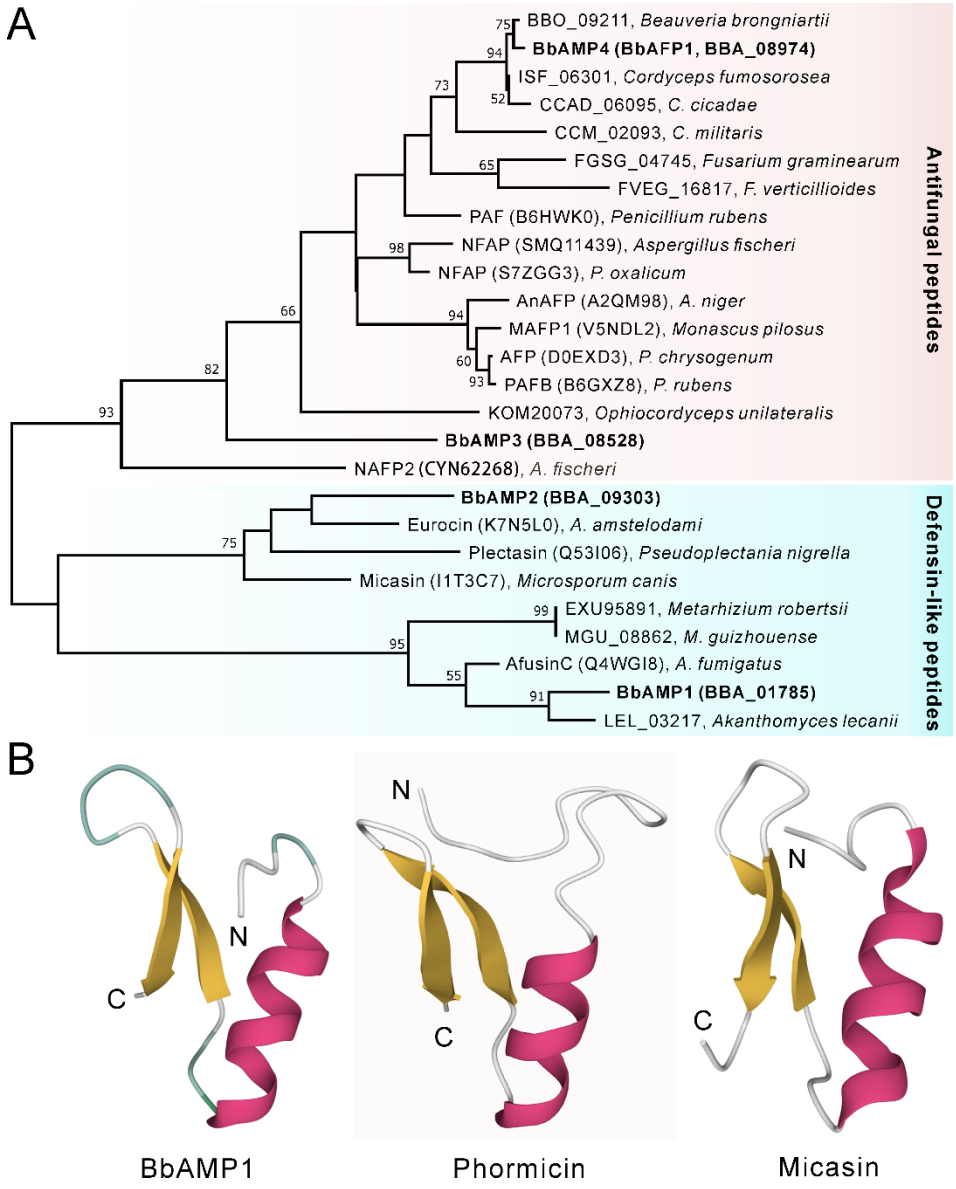

Fig. S1 Phylogenetic and structural analyses of BbAMP1 and its related peptides. (A) Phylogenetic analysis of the putative antimicrobial proteins encoded by *B. bassiana* and the other fungi. The maximum-likelihood tree was inferred using a Jones-Taylor-Thornton matrix-based model with 500 bootstrap replicates. The putative AMPs from *B. bassiana* are highlighted in bold. (B) Structure comparison of the predicted mature BbAMP1 with phormicin and micasin. Phormicin (structure accession: 1ICA) is from the blowfly (*Phormia terranovae*, now named *Protophormia terranovae*) and micasin (I1T3C7) is from the dermatophytic fungal (*Microsporum canis*). N and C represent the N- and C-terminus, respectively.

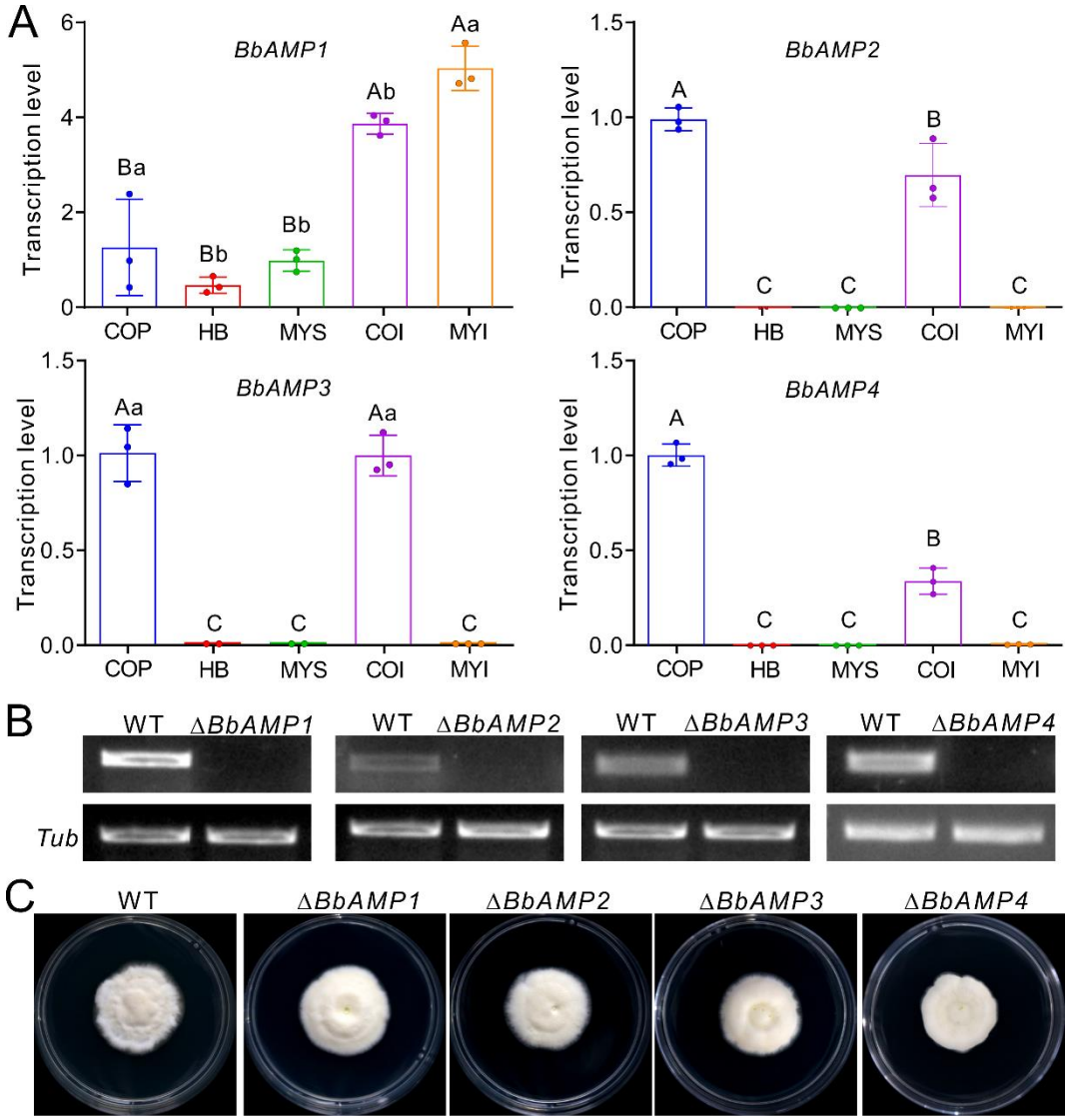

Fig. S2 Gene expression, deletion and mutant phenotyping. (A) Quantitative expression of four putative antimicrobial genes by *B. bassiana* at different stages. COP, conidia harvested from the PDA cultures; HB, hyphal body cells harvested from insect hemocoel; MYS, mycelia harvested from the SDB broth; COI, conidial spores harvested from the cadavers of *G. mellonella*; MYI, mycelial cells collected from the inside of insect cadavers. Different letters labelled above each column indicate $p < 0.01$ (capital) and $p < 0.05$ (lowercase) of different inductive media after one-way ANOVA analysis. (B) RT-PCR verification of gene deletions. Conidia of the WT and putative mutants were harvested from the two-week-old PDA plates for RNA extraction and RT-PCR analysis. *Tub*, the β-tubulin gene was used as a reference. (C) No obvious phenotype differences between WT and mutants after growth on PDA for two weeks.

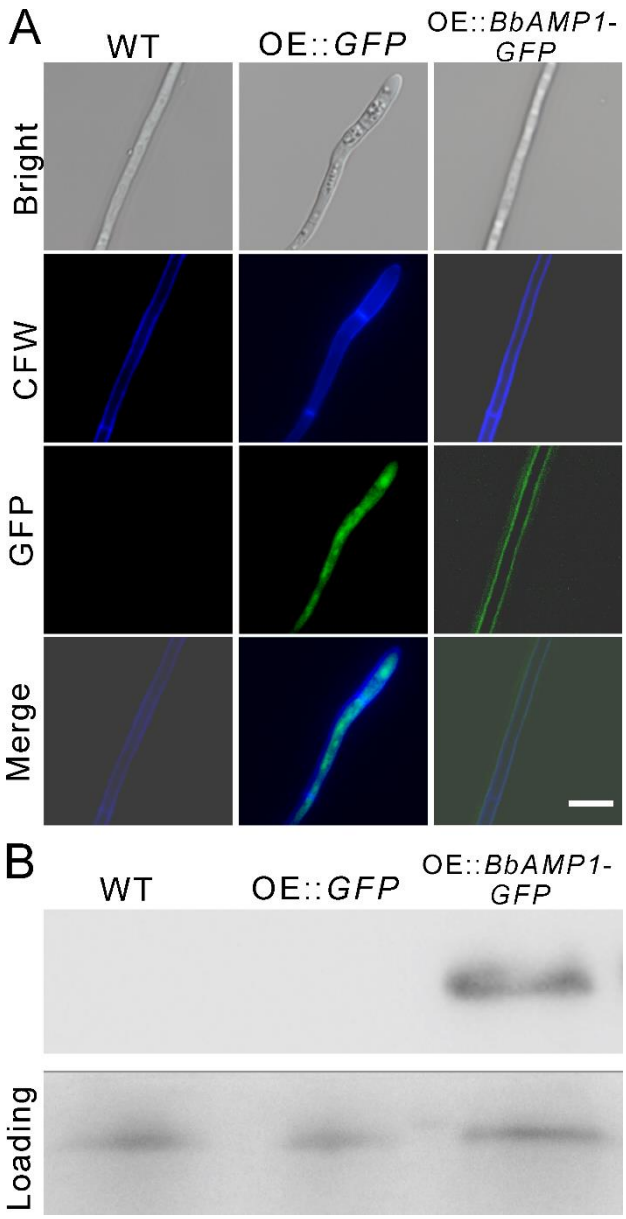

Fig. S3 BbAMP1 localization and secretion analysis. (A) Localization of BbAMP1 on the cell wall of mycelia. CFW, Calcofluor white for staining cell wall chitin component. Bar, 10 μm. (B) Detection of the fused BbAMP1-GFP in culture filtrate. The spores of the WT and mutants were inoculated in SDB for three days, and the culture filtrates of each strain were concentrated for Western blot analysis with an anti-GFP antibody. The SDS-PAGE analysis of the concentrated samples was included as the loading control.

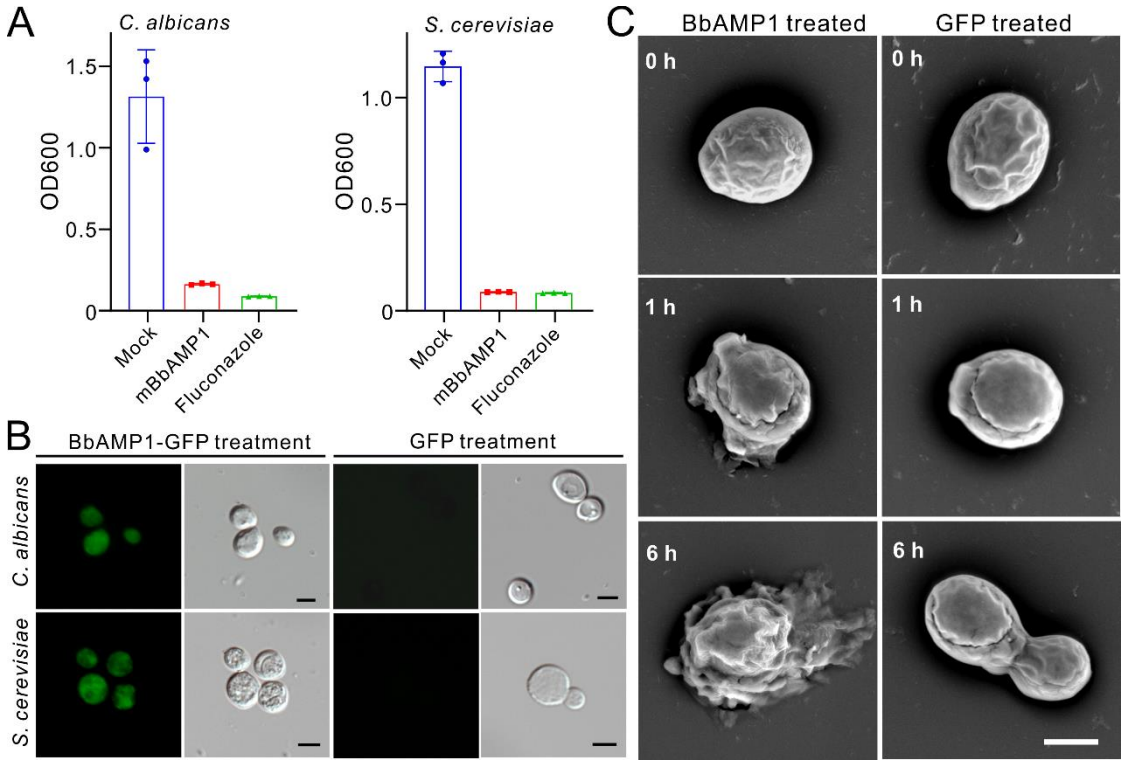

Fig. S4 BbAMP1 inhibition of yeast cells. (A) Antifungal assay of mBbAMP1 against two yeast species. The yeast cells were incubated in YPD broth with or without the addition of mBbAMP1 (at a final concentration of 20 μM) for overnight before the measurement of OD_{600} value. The antifungal drug fluconazole (at a final concentration of 100 μg mL^{-1}) was used as a control. (B) Binding of yeast cells by BbAMP1-GFP. The yeast cells were incubated with either BbAMP1-GFP or GFP protein for one hour before imaging. Bar, 3 μm. (C) Lysis of *S. cerevisiae* cells by BbAMP1. The treatment with a pure GFP protein was used as a control. Bar, 3 μm.

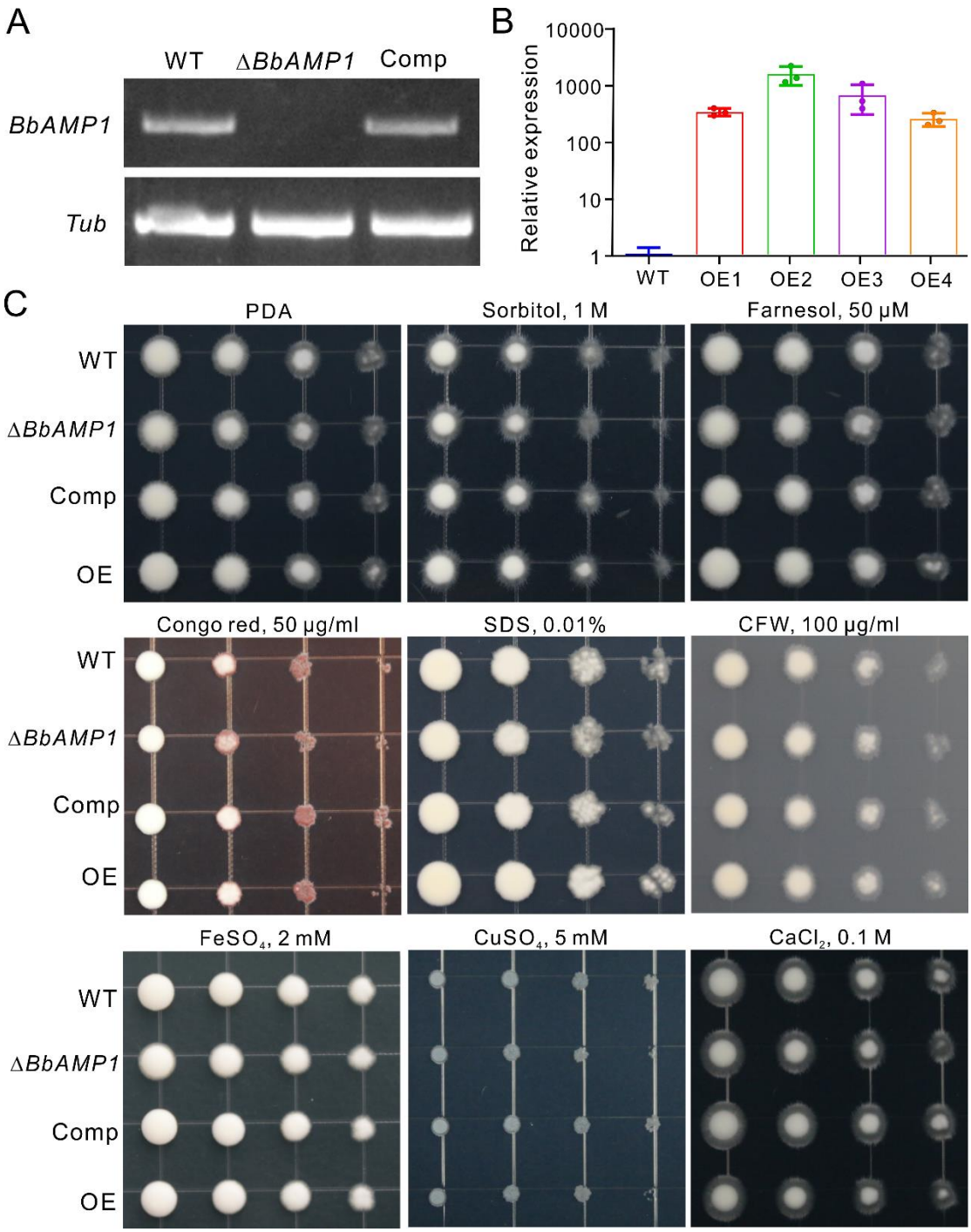

Fig. S5 Generation and phenotyping of *BbAMP1* mutants. (A) PCR verification of the gene complementation (Comp) strain. (B) qPCR examination of the independently acquired overexpression (OE) mutants. The highest expression strain OE2 was then simplified as OE and used for further experiments. (C) No obvious difference of fungal growth between strains after inoculation on PDA or PDA amended with stress factors at the indicated concentrations for two weeks. CFW, Calcofluor white.

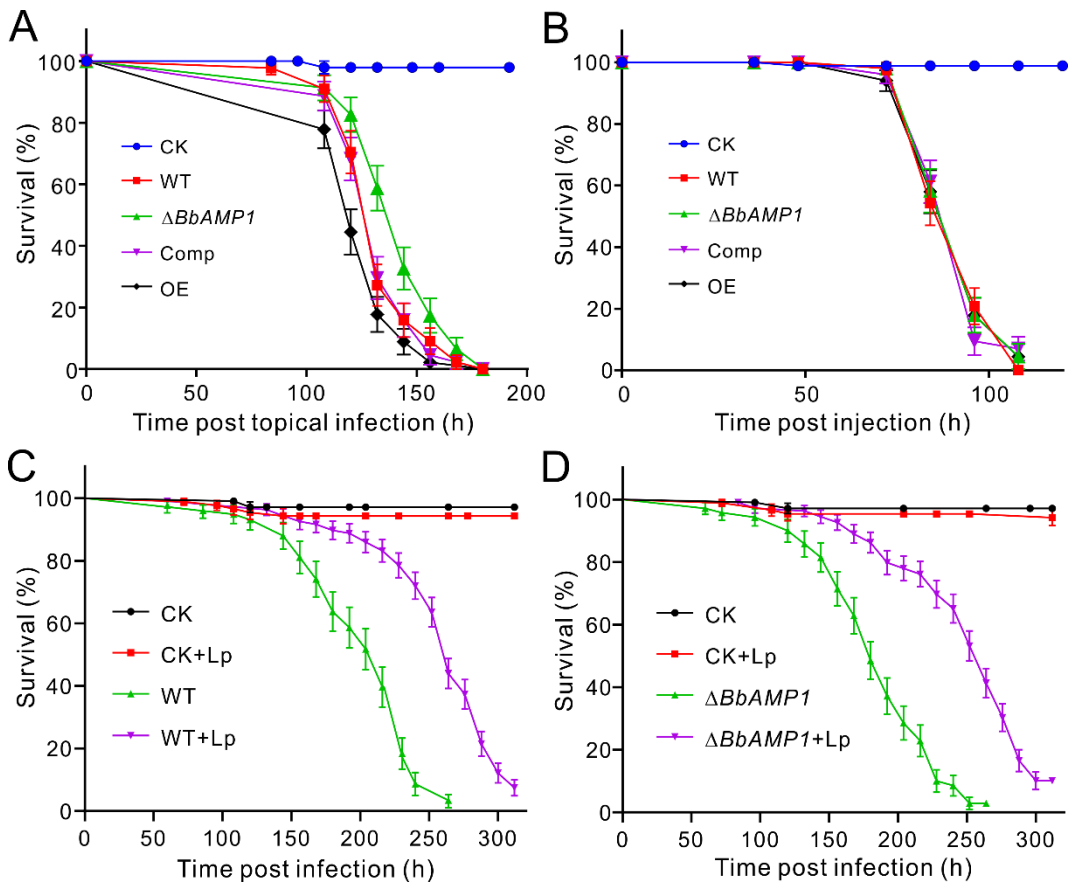

Fig. S6 Insect survival assays. (A, B) Survivals of the wax-moth larvae after topical infection (A) and injection (B) with different strains of *B. bassiana*. Significant difference of insect survivals was observed between WT and Δ*BbAMP1* ($\chi^2 = 5.26$; $p = 0.022$) and between WT and OE ($\chi^2 = 4.11$; $p = 0.043$). The mock control (CK) was treated with 0.05% Tween 20. (C, D) Survivals of the axenic flies after topical infection with the WT (C) and Δ*BbAMP1* (D) spores with and without the addition of the *L. plantarum* (Lp) cells. Relative to the treatments without bacterial cells, fly survivals were significantly extended after treating with the WT ($\chi^2 = 63.94$; $p < 0.0001$) and Δ*BbAMP1* ($\chi^2 = 76.33$; $p < 0.0001$) strains plus the addition of *L. plantarum*. The flies treated with 0.05% Tween 20 with or without the addition of Lp were included as the controls (CK and CK+Lp).

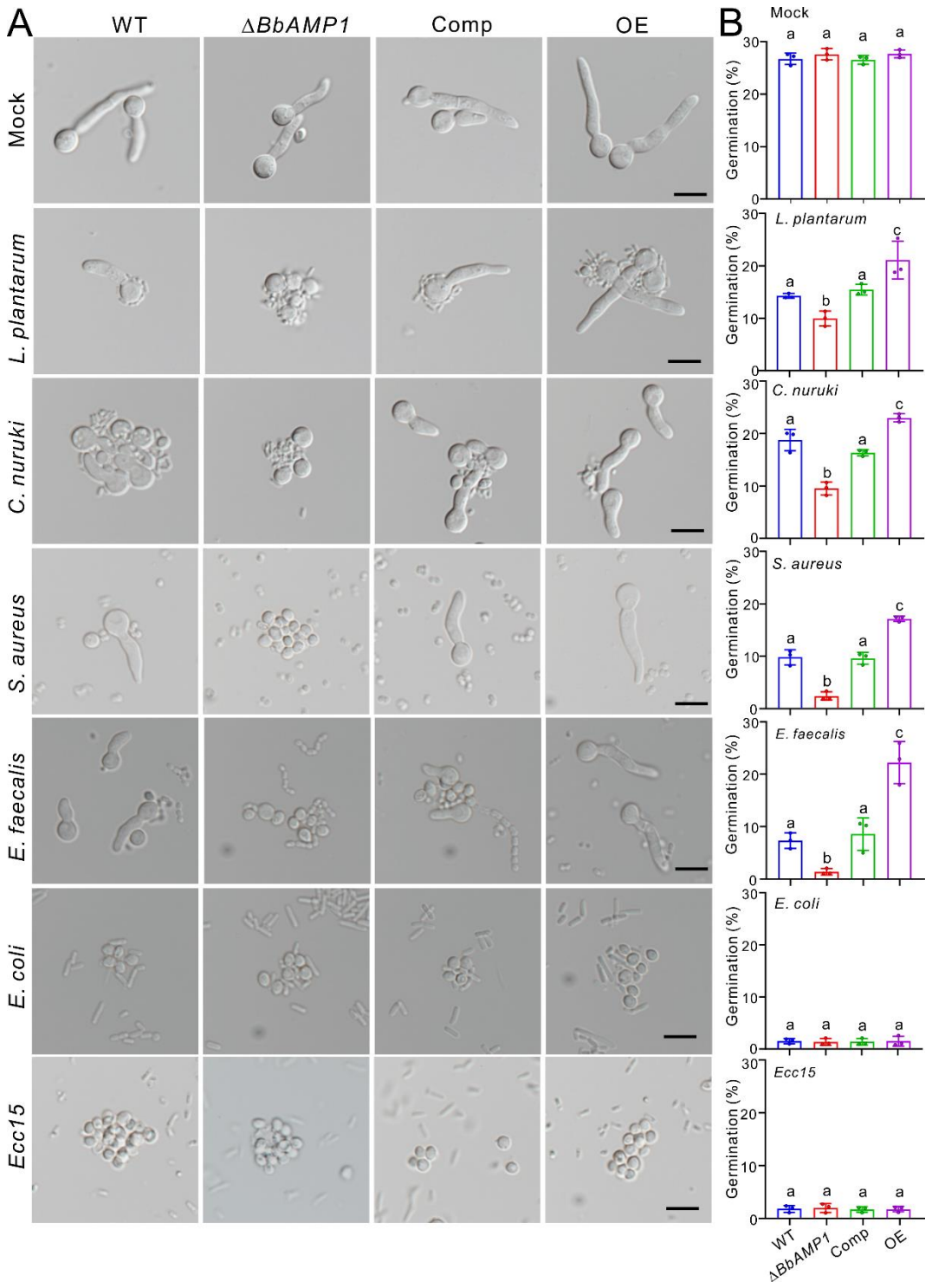

Fig. S7 Inhibition of spore germinations by different bacteria. (A) Microscopic imaging of bacterial inhibition of fungal spore germinations. Bar, 5 μm. (B) Statistical comparison of the spore germination rates of different *Beauveria* strains after challenge with different bacteria each at a final OD_{600} of 0.01. Values are mean ± SD. The letters shown above each column represent the significance of difference as tested by one-way ANOVA analysis. Different letters within each panel indicate $p < 0.05$.

Supporting Tables:

Table S1. PCR primers used in this study.

Gene	Primers	Primer sequences	RE	Notes
BBA_01785 (*BbAMP1*)	KO UF	ATCGAATTCCTGCAGACGAAACGGAAGGATTGA	Pst I	Gene deletion
	KO UR	TCCCCCGGGCTGCAGCAGCCAGTGGATGGTGAA		
	KO DF	ATCTGATGAACTAGTGCTGCGAGGCTCATTGCT	Spe I	
	KO DR	CGCTCTAGAACTAGTCAAACGCTGGCTCGTCAA		
	TF	AACGGAAGGATTGATTAGAC		Deletion verification
	TR	GACGCAAGTTGCTTTAGG		
	OE F	AACAACTAGTTCTAGAATGAAGTTCTCCCTCG	Xba I	Gene overexpression
	OE R	TGGCGGCCGCTCTAGACTAGTTGCAGTAGCA		
	RT F	TGCTGGGACTCCGTTACTT		RT-PCR
	RT R	AGTAGCACACTTCGCCTTTG		
	Comp F	ATCGAATTCCTGCAGTGGCGTCTACATTGACA	Pst I	Gene complementation
	Comp R	CCTGTCGAGCTGCAGCTAGTTGCAGTAGCAC		
	qRT F	AAGCGCAGCAACGATGA		RT-qPCR
	qRT R	AGCAACGTACGGAGCAATAC		
	GST F	GGGCCCCTGGGATCCGACGACGACGACAAGAGCG CCTGTTGCAGC	BamH I	Protein expression
	GST R	GGAATTCGGGGATCCCTAATGATGATGATGATGAT GGTTGCAGTAGCACAC		
	pYes F	ACCGAGCTCGGATCCAACATGTCTAGCGCCTGTTG CAGC	BamH I	Yeast expression
	pYes R	GTTACTAGTGGATCCCTAGTTGCAGTAGCA		
	EX F1	AACAACTAGTTCTAGAATGAAGTTCTCCCTCG		Gene fusion
	EX R1	CTCGCCCTTGCTCACGTTGCAGTAGCACAC	Xba I	
	EX F2	GTGTGCTACTGCAACGTGAGCAAGGGCGAG		
	EX R2	TGGCGGCCGCTCTAGATTACTTGTACAGCTC		
BBA_09303 (*BbAMP2*)	KO UF	ATCGAATTCCTGCAGGATGGGAGAAGAAGAGGG	Pst I	Gene deletion
	KO UR	TCCCCCGGGCTGCAGCAATAGCTGTGGGAAGGA		
	KO DF	ATCTGATGAACTAGTCTGGGCGTAAGTGCAACA	Spe I	
	KO DR	CGCTCTAGAACTAGTCTCTGAGGGAAGCGGTGA		
	TF	GGTTGGGCGAGAAAGGTC		Deletion verification
	TR	TGTTGAATAGCAGCGAAG		
	RT F	TGAAGGCCTTTACTAGTCTCTTTG		RT-PCR
	RT R	CTATAGACACAGACGCAGGTTG		
	qRT F	TGCCCGCAATGAAATCAAC		RT-qPCR
	qRT R	CCGGTAGGCTTGATAGTTCTG		
BBA_08528 *(BbAMP3*)	KO UF	ATCGAATTCCTGCAG TCTGTTTCGGGTGGTCAA	Pst I	Gene deletion
	KO UR	TCCCCCGGGCTGCAG CCAGGTGCGTCATCATTT		
	KO DF	ATCTGATGAACTAGTGTTCGGAGCCGTCCCTAT	Spe I	
	KO DR	CGCTCTAGAACTAGTCCCGCTAATCAGCAAGCA		
	TF	GTCTCCTATCCCTCTTCCG		Deletion verification
	TR	CTTTCATTCACTGCCACA		
	RT F	CAAGTGGTTTTGGCGTTCT		RT-PCR
	RT R	CAGCCGCCATCGTTACTC		

	qRT F	TGCAACTTCAAGCGCAAC		RT-qPCR
	qRT R	TTAGCTGCAGACGGTAATTCT		
BBA_08974 (BbAMP4)	KO UF	ATCGAATTCCTGCAGTTTGAGGAAGAGCGAGAA	Pst I	Gene deletion
	KO UR	TCCCCCGGGCTGCAGTTTGCCAACAAGAGGGTA		
	KO DF	ATCTGATGAACTAGTTGGGATAGTCGGGCTGTT	Spe I	
	KO DR	CGCTCTAGAACTAGTCGATTTCAAGTTTGCCTCC		
	TF	TTGTCATGCTTGGGTAATC		Deletion verification
	TR	CAATCGCAAACCATCAGT		
	RT F	TCGTTCTTGGCCTTGGTG		RT-PCR
	RT R	GCAGATTATTTCCATCGCACT		
	qRT F	TGCAACTTCAAGCGCAAC		RT-qPCR
	qRT R	TTAGCTGCAGACGGTAATTCT		
β-tubulin gene	TubRT F	AACATGGTTCCTTTCCCTCGTCTTC		RT-PCR analysis
	TubRT R	TTCCTCATCATCAATGCCAGCGT		
	Tubq F	GTATGGACGAGATGGAGTTCAC		RT-qPCR analysis
	Tubq R	CTCGTATTCCTCTTCCTCATCATC		
BbAMP1 secretion	pSUC2-F	GGTGTGAAGTGGACCAAAGGTCTA		Yeast signal-sequence trap analysis
	pSUC2-R	CCTCGTCATTGTTCTCGTTCCCTT		
	pSUC2-BLys2F	TTTAATTAAGAATTCATGACTCGATTTACTACC	EcoR I	
	pSUC2-BLys2R	AGGGAGAACCTCGAGCTTGTAGCTGCACTTGGC	Xho I	
	pSUC2-BbAMP1F	TTTAATTAAGAATTCATGAAGTTCTCCCTCGTC	EcoR I	
	pSUC2-BbAMP1R	AGGGAGAACCTCGAGGGAGTCCCAGCAGAAGGC	Xho I	
Fly *Rpl32*	Rpl32F	GACGCTTCAAGGGACAGTATCTG		Fungal load assay
	Rpl32R	AAACGCGGTTCTGCATGAG		
Bb 18S rRNA	Bb18S-F	TGGTTTCTAGGACCGCCGTAA		
	Bb18S-R	CCTTGGCAAATGCTTTCGC		
Bacterial 16S RNA genes	27F	AGAGTTTGATCMTGGCTCAG		16S rRNA amplification Microbiome sequencing
	1492R	TACGGYTACCTTGTTACGACTT		
	515F	GTGCCAGCMGCCGCGG		
	806R	GGACTACNNGGGTATCTAAT		

Table S2. Statistical comparison of the median lethal time (LT_{50}) between WT and mutant strains against the females of *Drosophila melanogaster*.

Experiment*	**Strains**	**LT_{50} (h)**	**Significance**
Experiment 1	WT	168±3.920	-
	Δ*BbAMP1*	192±6.007	χ^2=8.872; P = 0.003
	Δ*BbAMP2*	168±4.676	χ^2=0.751; P = 0.386
Experiment 2	WT	176±6.461	-
	Δ*BbAMP3*	184±4.009	χ^2=0.064; P = 0.801
	Δ*BbAMP4*	176±5.545	χ^2=0.000; P = 0.997

*, Two batches of experiments were conducted with the WT and respective mutant strains.