

Supporting Information

The Spt10 GNAT superfamily protein modulates development, cell cycle progression and virulence in the fungal insect pathogen, *Beauveria bassiana*

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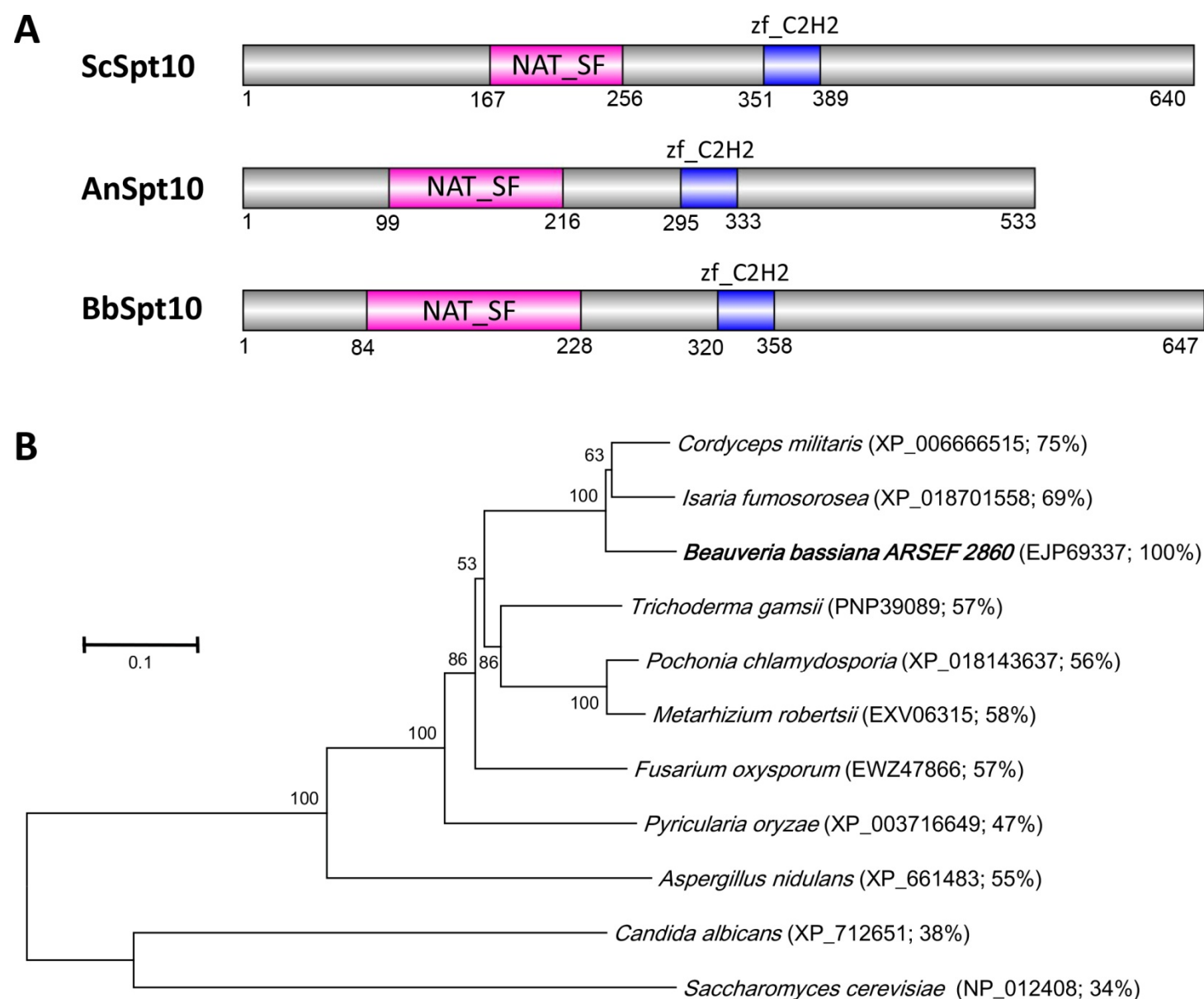


Figure S1. Bioinformatic properties of fungal Spt10 homologues. **(A)** Sequence features of Spt10 homologs found in *Beauveria bassiana* (Bb), *Saccharomyces cerevisiae* (Sc), and *Aspergillus nidulans* (An). The domain of each protein was predicted at <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. **(B)** Phylogenetic relationship of *B. bassiana* Spt10 with the homologs found in other representative fungi. A neighbor-joining method in MEGA7 at <http://www.megasoftware.net> was used in the phylogenetic analysis. Each fungal name is followed by the NCBI accession code of each protein and its sequence identity (%) to *B. bassiana* Spt10 in parentheses. Poisson model was used with 1000 bootstrap replications in uniform rates. Scale bar: branch length proportional to genetic distance assessed with the neighbor-joining method.

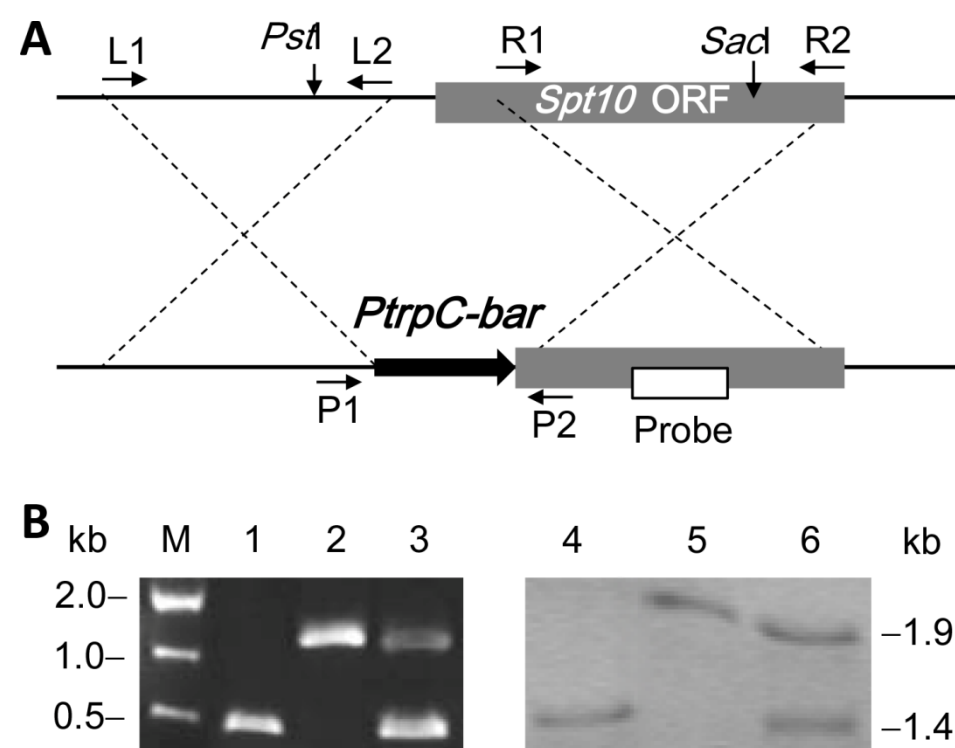


Figure S2. Generation and identification of *B. bassiana* *spt10* mutants. **(A)** Schematic diagram for the strategy of *spt10* deletion. **(B)** The *spt10* mutants identified via PCR (lanes 1–3) and Southern blotting (lanes 4–6) analyses with paired primers and amplified probe (Table S1). Lanes 1 and 4: wild-type. Lanes 2 and 5: $\Delta spt10$ mutant. Lanes 3 and 6: $\Delta spt10::spt10$ mutant. Genomic DNAs were digested with *Pst*I/*Sac*I at the marked sites for the Southern blotting hybridization of *spt10*.

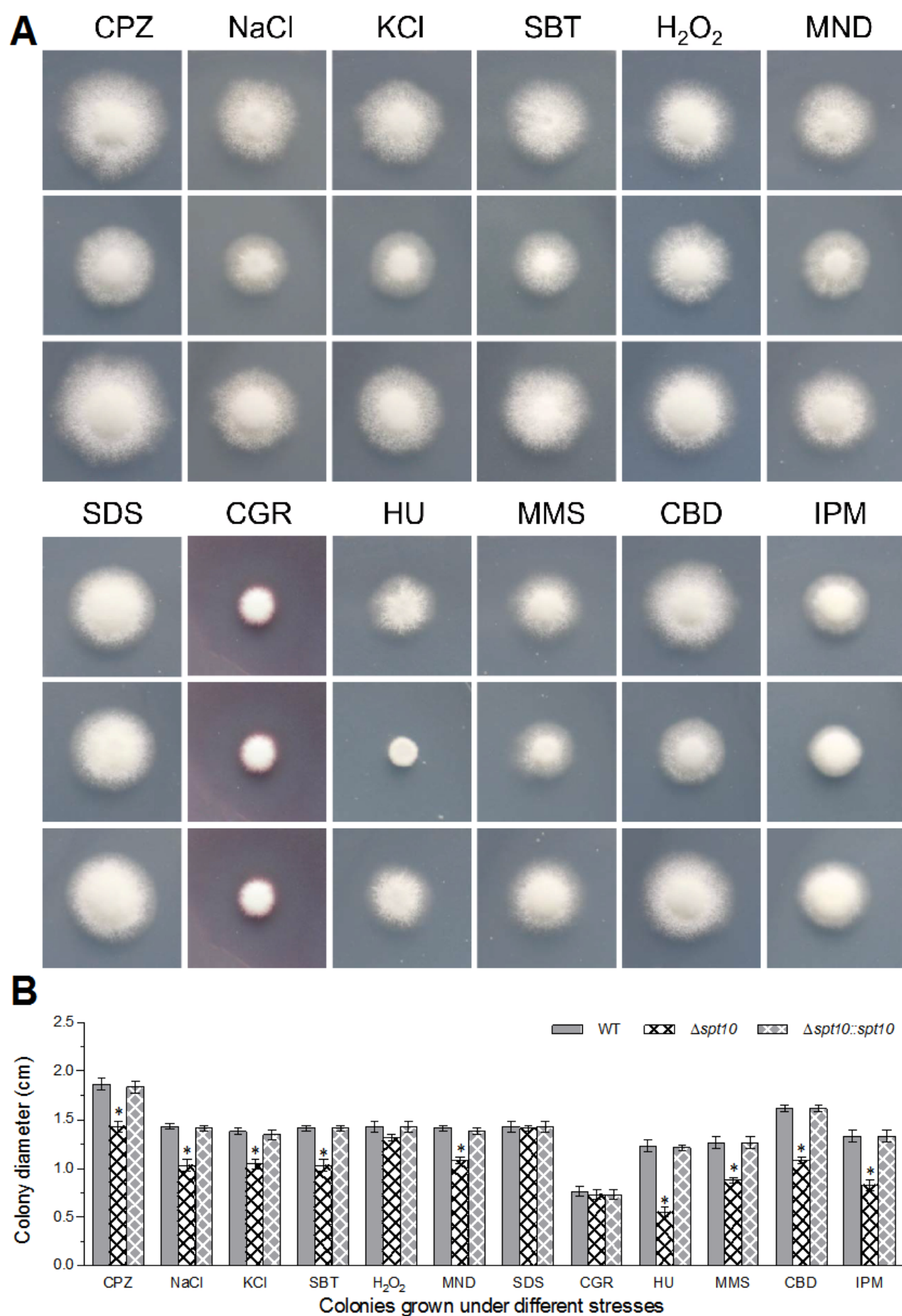


Figure S3. Multiple stress responses of *B. bassiana* in absence of *Spt10*. (**A**, **B**) Images and colony diameters of fungal colonies grown at 25°C for 8 d on CZA supplemented with NaCl (0.4 M), KCl (0.4 M), sorbitol (SBT; 0.6 M), H₂O₂ (2 mM), menadione (MND; 0.02 mM), SDS (0.1 mg/ml), congo red (CGR; 10 μg/ml), hydroxyurea (HU; 10mM), methyl methanesulfonate (MMS, 0.05%), carbendazim (CBD, 5 μg ml⁻¹) and ipodione metabolite (IPM, 10 μg ml⁻¹). The asteristed bar in each three-bar group differ significantly from those unmarked (Tukey's HSD, $p < 0.05$). Error bars: SD of the mean from three replicates.

Table S1. Paired primers designed for manipulation of *spt10* and identification of its mutants in *B. bassiana*.

Primers	Paired sequences (5'-3')*	Purpose
Spt10-F/R	ACCTTCAAACCCGGGGATCCATGCTGCCGCGTCGA/ATCTGCAGGTGCAGCGGATCCAGTCCACCCCTTGTAT	Cloning <i>spt10</i> cDNA(1941 bp)
Spt10up-F/R	AAAAAGAATTCCAAGACAGGCAAAGAGGATTCA / AAAAAAAGCTTCCAGCCAGTACAACACTTGCTT	Cloning <i>spt10</i> 5'-end (1545 bp)
Spt10dn-F/R	AAAAAACTAGTATCACCTATCTCTGCGGGCA / AAAAAAGTTAACCATCCAATCGTCCATGTCC	Cloning <i>spt10</i> 3'-end (1720 bp)
Spt10fl-F/R	<u>gggg</u> ACCACTTTGTACAAGAAAGCTTCCAAGACAGGCAAAGAGG / <u>gggg</u> ACAAGTTTGTACAAAAAAGCAAAACATTGAGCACAAAGGAGCAA	Cloning full-length <i>spt10</i> (4219 bp)
pSpt10-F/R	CGGTGTAAGTCAATACGGCAAG / CCAAAGTTTGGAAACAGTACG	PCR detecting <i>spt10</i>
sbSpt10-F/R	GCGTCTTCAACTTGGTGT / CCGTAGTCTTGTATTATGCC	Southern blotting <i>spt10</i> (462 bp)
qSpt10-F/R	GCTCATCACCTATCTCTG / GTAAAGGGCTCCATCATC	qPCR detecting <i>spt10</i>
q18S-F/R	TGGTTTCTAGGACCGCCGTAA / CCTTGGCAAATGCTTTCGC	qPCR detecting 18S RNA

* Underlined regions denote the restriction enzyme sites used for cloning *spt10* cDNA (*Bam*HI/*Bam*HI) or deleting *spt10* (*Eco*RI/*Hind*III and *Spe*I/*Hpa*I) and the fragments of gateway exchange for *Spt10* complementation.

Table S2. Paired primers used for transcriptional profiling of potential Spt10-targeted genes in *B. bassiana* via qPCR.

Gene	Tag loci*	Annotation	Sequences (5'-3') of paired primers
Involved in asexual development			
<i>fluG</i>	BBA_04942	Developmental protein FluG	CCTCCCTAGTTTGGTCGCTTTCTC / CGCTGTCGGTAATCTGCTCCTC
<i>flbA</i>	BBA_02968	Developmental regulator FlbA	CCAATCCACTCGCCGCTCTC / CGGAGGAAAGAGAATCGGTAGAGG
<i>flbB</i>	BBA_06988	bZIP transcription factor FlbB	GCACTGACACGCCGACAAGAGC / CCGCCGCCGAAGCCTGTTG
<i>flbC</i>	BBA_03181	C ₂ H ₂ conidiation transcription factor FlbC	TCCATCTCCAACCTTGCTGGGTCTC / GCGGGCGTAGGCGGAAGG
<i>flbD</i>	BBA_07259	MYB conidiophore development protein FlbD	CGGCAAGCGATGGGCAGAGATTG/ CGAGCAAGGTGACGGTAGAGGTG
<i>flbE</i>	BBA_01716	Conidiophore development protein FlbE	CAGACGATGAGACAGAGA / GGGCTTTATATGCGAGTAG
<i>brlA</i>	BBA_07544	C ₂ H ₂ conidiation transcription factor BrlA	GACCAGTTCAACAGACAAG / CAGTAATCTTCGTGCTTCTC
<i>abaA</i>	BBA_00300	Conidiation factor AbaA	GCAAGTCTCCAGCCATAT / CTCCTCTTCGTCATAGTAGTC
<i>wetA</i>	BBA_06126	Conidial maturation factor WetA	CGCAGACGAATTTGACTT / GCTGGTGGTTGAATACAT
<i>vosA</i>	BBA_01023	Velvet protein VosA	GGACAGACGAGTGATTGA / GGCATATACGACGCATCT
Involved in hyphal septation			
<i>apsA</i>	BBA_05547	Cell division/GTP binding protein ApsA	CTCTGCTCTGCTTCATAG / TTCTCGGTACGGTAGTTC
<i>apsB</i>	BBA_06894	Cell division/GTP binding protein ApsB	TTACCGACAACATTGAAC / GGATGAAGAAGACACAAG
<i>apsC</i>	BBA_04748	Cell division control protein 12 ApsC	GGAGAAGTTCTTCAAGGT / AGGAGTCACGATTGTTAA
<i>chsA</i>	BBA_03236	Class 2 chitin synthetase ChsA	GTGTCTTACTGATTGCTA / ATAATGATGGCGTAGATG
<i>chsB</i>	BBA_04667	Chitin synthetase 3a ChsB	CTCTGCCAATGTCACCAAG / TCAATGTCTTCCTGCTCCTT
<i>chsC</i>	BBA_03590	Chitin synthetase 1 ChsC	CGATCTGAACCAGCACAA / ATGACGGAGACGATGAGT
<i>bud3</i>	BBA_03175	RhoGEF domain-containing protein Bud3	GATAATCCTATAATACGACTC / GTAGACATCATTGACAAG
<i>bud4</i>	BBA_01075	GTP binding protein Bud4	GCGTCATCATCACCAGAA / TAATCTCATCATCCTCATCATACA
<i>rho4</i>	BBA_03155	Ras family protein Rho4	CACATACTCCAAGAAGATT / AAGACGGTAGGAACATAT
<i>sepH</i>	BBA_03315	Cell division control protein SepH	AACTGCCTCAACATCATC / TCATATACACGCCAACAAG

* Gene accession codes in *B. bassiana* genome under the NCBI accession NL_ADAH00000000 (Xiao *et al.*, 2012).