# Supplementary Tables and Figures

**The autophagy-related genes *BbATG1* and *BbATG8* have different functions in differentiation, stress resistance and virulence of mycopathogen *Beauveria bassiana***

**Sheng-Hua Ying*†, Jing Liu†, Xin-Ling Chu, Xue-Qin Xie and Ming-Guang Feng**

*Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, People's Republic of China*

†: These authors contributed equally to this work.

**Running title:** Autophagy-related genes in entomopathogen *B. bassiana*

**Keywords:** *Beauveria bassiana*, autophagy-related genes, conidiation, conidial protein, blastospore development, oxidative stress, pathogenicity

***Correspondence:** Sheng-Hua Ying, Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, People's Republic of China; E-mail: yingsh@zju.edu.cn; Tel.: 86-571-88206199 Fax: 86-571-8820 6178

# Supplementary Table S1 Domain analysis of ATG1p

| | Gene ID | Species | Alignment start | Alignment end | Envelope start | Envelope end | HMM acc | HMM name | Type | HMM start | HMM end | HMM length | Bit score | E-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **FUNGI** | A2QIL5 | *Aspergillus niger* | 30 | 334 | 30 | 336 | PF00069.20 | Pkinase | Domain | 1 | 258 | 260 | 201.7 | 1.10E-59 |
| | A2QIL5 | | 649 | 886 | 646 | 909 | PF12063.3 | DUF3543 | Family | 5 | 204 | 238 | 296.4 | 1.10E-88 |
| | EJP64020 | *Beauveria bassiana* | 25 | 324 | 23 | 326 | PF00069.20 | Pkinase | Domain | 3 | 258 | 260 | 203.4 | 3.40E-60 |
| | EJP64020 | | 621 | 896 | 619 | 897 | PF12063.3 | DUF3543 | Family | 3 | 237 | 238 | 293.4 | 9.10E-88 |
| | P53104 | *Saccharomyces* | 24 | 322 | 24 | 325 | PF00069.20 | Pkinase | Domain | 1 | 257 | 260 | 203.4 | 3.20E-60 |
| | P53104 | *cerevisiae* | 586 | 893 | 583 | 894 | PF12063.3 | DUF3543 | Family | 5 | 237 | 238 | 214 | 1.70E-63 |
| | Q4P0K0 | *Ustilago maydis* | 16 | 333 | 15 | 334 | PF00069.20 | Pkinase | Domain | 2 | 259 | 260 | 188.8 | 9.00E-56 |
| | Q4P0K0 | | 708 | 957 | 707 | 958 | PF12063.3 | DUF3543 | Family | 2 | 237 | 238 | 204.3 | 1.50E-60 |
| | Q52EB3 | *Magnaporthe oryzae* | 20 | 323 | 19 | 324 | PF00069.20 | Pkinase | Domain | 2 | 259 | 260 | 198.6 | 9.60E-59 |
| | Q52EB3 | | 658 | 946 | 657 | 947 | PF12063.3 | DUF3543 | Family | 2 | 237 | 238 | 320.3 | 5.50E-96 |
| | Q5A649 | *Candida albicans* | 58 | 352 | 58 | 354 | PF00069.20 | Pkinase | Domain | 1 | 258 | 260 | 214.4 | 1.40E-63 |
| | Q5A649 | | 619 | 830 | 615 | 834 | PF12063.3 | DUF3543 | Family | 6 | 232 | 238 | 154.7 | 2.30E-45 |
| | Q5BCU8 | *Aspergillus nidulans* | 24 | 329 | 24 | 332 | PF00069.20 | Pkinase | Domain | 1 | 257 | 260 | 202.6 | 5.80E-60 |
| | Q5BCU8 | | 630 | 900 | 628 | 900 | PF12063.3 | DUF3543 | Family | 3 | 238 | 238 | 330.9 | 3.30E-99 |
| | XP_006672480 | *Cordyceps militaris* | 269 | 588 | 268 | 590 | PF00069.20 | Pkinase | Domain | 2 | 258 | 260 | 185.3 | 1.10E-54 |
| | XP_006672480 | | 864 | 1140 | 863 | 1141 | PF12063.3 | DUF3543 | Family | 2 | 237 | 238 | 287.4 | 6.30E-86 |
| | XP_007819690 | *Metarhizium anisopliae* | 24 | 328 | 23 | 329 | PF00069.20 | Pkinase | Domain | 2 | 259 | 260 | 198.2 | 1.20E-58 |
| | XP_007819690 | | 634 | 915 | 631 | 916 | PF12063.3 | DUF3543 | Family | 4 | 237 | 238 | 281.3 | 4.60E-84 |
| **PLANT** | NP_001078281 | *Arabidopsis thaliana* | 20 | 277 | 20 | 277 | PF00069.20 | Pkinase | Domain | 1 | 260 | 260 | 240.8 | 1.30E-71 |
| | EEE58765 | *Oryza sativa* | 24 | 280 | 24 | 280 | PF00069.20 | Pkinase | Domain | 1 | 260 | 260 | 248.6 | 5.40E-74 |
| | EEE58765 | | 631 | 711 | 554 | 714 | PF12063.3 | DUF3543 | Family | 157 | 235 | 238 | 30.1 | 2.50E-07 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ANIMAL** | NP_648601 | *Drosophila* | 11 | 273 | 9 | 274 | PF00069.20 | Pkinase | Domain | 3 | 259 | 260 | 216.4 | 3.40E-64 |
| | NP_648601 | *melanogaster* | 614 | 826 | 614 | 827 | PF12063.3 | DUF3543 | Family | 1 | 237 | 238 | 183.8 | 2.90E-54 |
| | NP_082171 | | 16 | 270 | 14 | 270 | PF00069.20 | Pkinase | Domain | 3 | 260 | 260 | 234.9 | 7.70E-70 |
| | NP_082171 | *Mus musculus* | 281 | 347 | 280 | 348 | PF04212.13 | MIT | Domain | 2 | 68 | 69 | 71.1 | 5.10E-20 |
| | NP_082171 | | 376 | 444 | 375 | 444 | PF04212.13 | MIT | Domain | 2 | 69 | 69 | 51.3 | 7.50E-14 |
| | Q6PHR2 | | 16 | 270 | 14 | 270 | PF00069.20 | Pkinase | Domain | 3 | 260 | 260 | 235.6 | 4.70E-70 |
| | Q6PHR2 | *Homo sapiens* | 281 | 347 | 280 | 348 | PF04212.13 | MIT | Domain | 2 | 68 | 69 | 72.3 | 2.10E-20 |
| | Q6PHR2 | | 376 | 443 | 375 | 444 | PF04212.13 | MIT | Domain | 2 | 68 | 69 | 52 | 4.60E-14 |

## Supplementary Table S2 Domain analysis of ATG8p

| | Gene ID | Species | Alignment start | Alignment end | Envelope start | Envelope end | HMM acc | HMM name | Type | HMM start | HMM end | HMM length | Bit score | E-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **FUNGI** | A2QPN1 | *Aspergillus niger* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 171.7 | 3.00E-51 |
| | ACJ06588 | *Magnaporthe oryzae* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 167.6 | 5.50E-50 |
| | EJP69267 | *Beauveria bassiana* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 171.9 | 2.60E-51 |
| | P0C075 | *Candida albicans* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 160.3 | 1.10E-47 |
| | P38182 | *Saccharomyces cerevisiae* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 169.6 | 1.40E-50 |
| | Q5B2U9 | *Aspergillus nidulans* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 171.1 | 4.40E-51 |
| | XP_006672688 | *Cordyceps militaris* | 51 | 136 | 46 | 136 | PF02991.11 | Atg8 | Domain | 19 | 104 | 104 | 141.2 | 9.00E-42 |
| | XP_007818863 | *Metarhizium anisopliae* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 171.9 | 2.60E-51 |
| | XP_761714 | *Ustilago maydis* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 174.4 | 4.30E-52 |
| **PLANT** | NP_001061171 | *Oryza sativa* | 14 | 117 | 14 | 117 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 175.3 | 2.20E-52 |
| | NP_178631 | *Arabidopsis thaliana* | 14 | 117 | 14 | 117 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 173.5 | 8.20E-52 |
| **ANIMAL** | NP_727447 | *Drosophila melanogaster* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 165.7 | 2.20E-49 |
| | NP_009216 | *Homo sapiens* | 13 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 1 | 104 | 104 | 168.3 | 3.30E-50 |
| | NP_065615 | *Mus musculus* | 14 | 116 | 13 | 116 | PF02991.11 | Atg8 | Domain | 2 | 104 | 104 | 165.5 | 2.50E-49 |

**Supplementary Table S3 PCR primers used in this study**

| Primer name | Sequence (5′–3′) [a] | Restriction sites | Purpose of primer design |
|---|---|---|---|
| $P_{ATG1}1$ | CCGGAATTCCGCTCCCGTCCGTCTATCTGA | *Eco*RI | Generating 5′-fragment for disruption vector |
| $P_{ATG1}2$ | CGCGGATCCACTAATCCAAGAATATCGCCAG | *Bam*HI | |
| $P_{ATG1}3$ | AAGCTCTAGACGCCGATTCAGTCACATACCG | *Xba*I | Generating 3′-fragment for disruption vector |
| $P_{ATG1}4$ | GGACTAGTGCTTCGCCTGGTTATTTGCTT | *Spe*I | |
| $P_{ATG1}5$ | GTTGAACTGCTCCGCAAGATAG | / | Screening mutants |
| $P_{ATG1}6$ | GCTGCTCTGGGATGATTAGGC | / | |
| $P_{ATG1}7$ | ggggACAAGTTTGTACAAAAAAGCAGGCTTAACTAAGCTTGACGGTGGCGGC | / | Obtaining the full gene for complementation |
| $P_{ATG1}8$ | ggggACCACTTTGTACAAGAAAGCTGGGTCTGACATCGAGGCCTGTCATGCTAC | / | |
| $P_{ATG1}9$ | CGCCGATTCAGTCACATACCG | / | Probe for Southern blotting |
| $P_{ATG1}10$ | TGCCGAAGAGTTGTTTTGTCC | / | |
| $P_{ATG8}1$ | CCGGAATTCGTTCTCGGTCCCATTTGTCTTG | *Eco*RI | Generating 5′-fragment for disruption vector |
| $P_{ATG8}2$ | CGCGGATCCCATGTTGGGCAAATATGTGGTA | *Bam*HI | |
| $P_{ATG8}3$ | AAGCTCTAGAGAGCAGCATCTACGAGGAACA | *Xba*I | Generating 3′-fragment for disruption vector |
| $P_{ATG8}4$ | GGACTAGTCGAGACCCTGTACGCTGGAGA | *Spe*I | |
| $P_{ATG8}5$ | GTCTTGCACCTTTCTGTACCAC | / | Screening mutants |
| $P_{ATG8}6$ | GTGTTCTCGCCAGAGTAGGTG | / | |
| $P_{ATG8}7$ | ggggACCACTTTGTACAAGAAAGCTGGGTGGGAGCGTGTCCAGCATTCTTC | / | Obtaining the full gene for complementation |
| $P_{ATG8}8$ | ggggACAAGTTTGTACAAAAAAGCAGGCTTCCAGCCACCTTTTTGCCCTC | / | |
| | | / | |

| | | | |
|---|---|---|---|
| $P_{ATG8}9$ | CGAGAACACCTTTGGCAGCAT | / | Probe for Southern blotting |
| $P_{ATG8}10$ | CGTATCAAACCTGCCTACAC | / | |
| $P_{CP}1$ | AAAAAGAATTCGTGTTGGACTTCCTGACGAT | *Eco*RI | Generating 5′-fragment for disruption vector |
| $P_{CP}2$ | AAAAAGGATCCTGTCTTACACGGGGAGTTGC | *Bam*HI | |
| $P_{CP}3$ | AAAAATCTAGAACCTCACCTTCAAAAACCTCC | *Xba*I | Generating 3′-fragment for disruption vector |
| $P_{CP}4$ | AAAAAAGATCTTGCTTGCTTATCTAGCCTGC | *Bgl*II | |
| $P_{CP}5$ | ACCTCGTAGTTTTAGGCAGAT | / | Screening mutants |
| $P_{CP}6$ | ATGATTGACAAGGCTGATGG | / | |
| $P_{CP}7$ | ggggACAAGTTTGTACAAAAAAGCAGGCTGTTGGACTTCCTGACGAT | / | Obtaining the full gene for complementation |
| $P_{CP}8$ | ggggACCACTTTGTACAAGAAAGCTGGGTTAGCCGCCACTACCAT | / | |
| $P_{CP}9$ | CCAACCCGAGTCTACCGCCA | | Probe for Southern blotting |
| $P_{CP}10$ | GCTCTCCGTTTTCTCATCAT | | |
| GA8-F1 | CATGCCATGGTGAGCAAGGGCGAGGAGC | *Nco*I | Fusion of GFP gene to *BbATG8* |
| GA8-R1 | CATCCTTGAACTTGCTACGCATCTTGTACAGCTCGTCCATGCCG | / | |
| GA8-F2 | ATGCGTAGCAAGTTCAAGGATG | / | |
| GA8-R2 | CGCGGATCCTCAAATGCTGCCAAAGGTGTTCTC | *Bam*HI | |
| Sur-F | AAGAAGCCTGTCGACGTGCCAACGCCACAGTG | *Hin*dIII | Amplification of *Sur* gene |
| Sur-R | CATGCTCGAGGGTACCGTCGACGTGAGAGCATGCAATTC | *Xho*I-*Kpn*I | |

*a*: The solid lines indicate the incorporated restriction sites. The sequences of *attB1* and *attB2*, required for Gateway recombination, are indicated with the dotted lines.

**Supplementary figures:**

**Figure S1. Phylogenetic analysis of *Beauveria bassiana ATG1 and ATG8* genes.** Relationships among the *ATG1* (**A**) and *ATG8* (**B**) genes were analyzed with Neighbor joining method and the bootstrap values > 50% from 1000 replicates are shown as numbers at each branch of the phylogenetic tree. GenBank accession numbers of autophagic genes are list after the abbreviations of their respective organism, which are shown as following groups, **fungi** (And: *Aspergillus nidulans*, Ang: *A. niger*, Bb: *Beauveria bassiana*, Ca: *Candida albicans*, Cm: *Cordyceps militaris*, Ma: *Metarhizium anisopliae*, Mg: *Magnaporthe grise*a, Sc: *Saccharomyces cerevisiae* and Um, *Ustilago maydis*); **plant** (At: *Arabidopsis thaliana* and Os: *Oryza sativa*); **animal** (Dm: *Drosophila melanogaster*, Hm: *Homo sapiens* and Mm: *Mus musculus*).

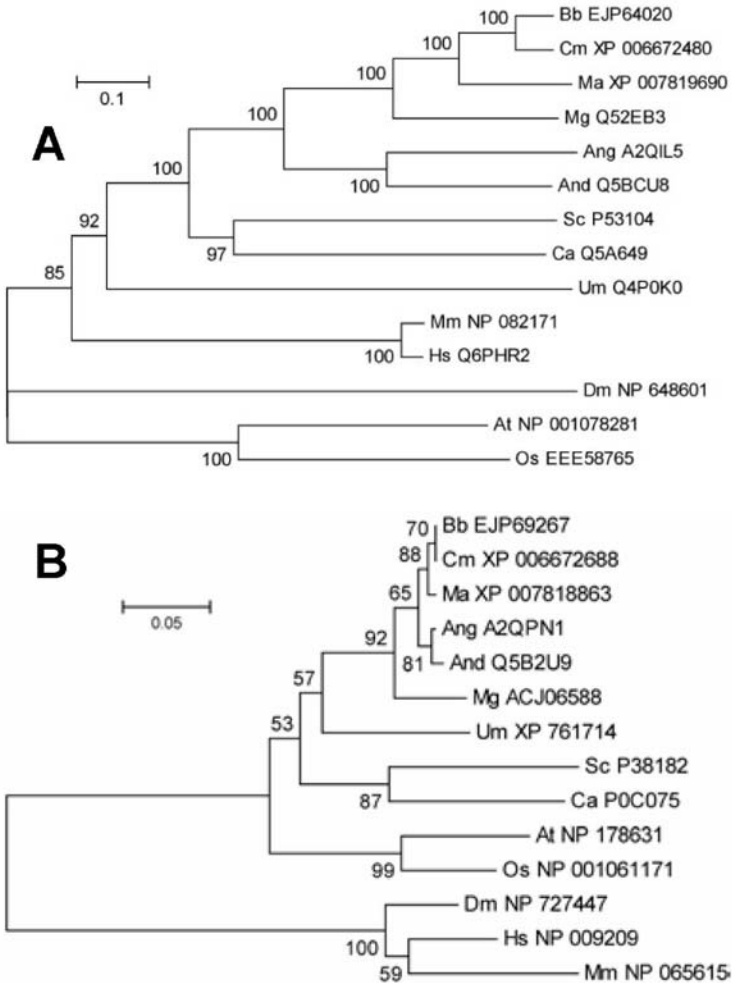

**Figure S2. Molecular manipulation of *B. bassiana ATG1* and *ATG8* genes.** (**A**) Schematic diagram showing gene locus, gene disruption vector, and expected homologous recombination events. ATGx refers to ATG1 or ATG8 gene, and RE represents the required restriction enzymes. (**B**) PCR screen of correct recombinant strains. Lane 1 and 4: wild type; lane 2: Δ*BbATG1* mutant; lane 3: Δ*BbATG1::BbATG1* strain; lane 5: Δ*BbATG8* mutant; lane 6: Δ*BbATG8::BbATG8* strain; lane 7: Δ*BbATG1*Δ*BbATG8* mutant and lane M: DNA molecular size standards. (**C**) Southern blotting analysis of digested genomic DNA from indicated strains. Lane 1 and 5: wild type; lane 2: Δ*BbATG1* mutant; lane 3: Δ*BbATG1::BbATG1* strain; lane 6: Δ*BbATG8* mutant; lane 7: Δ*BbATG8::BbATG8* strain; lane 4 and 8: Δ*BbATG1*Δ*BbATG8* mutant. The electrophoretic positions and sizes of the DNA fragments are shown.

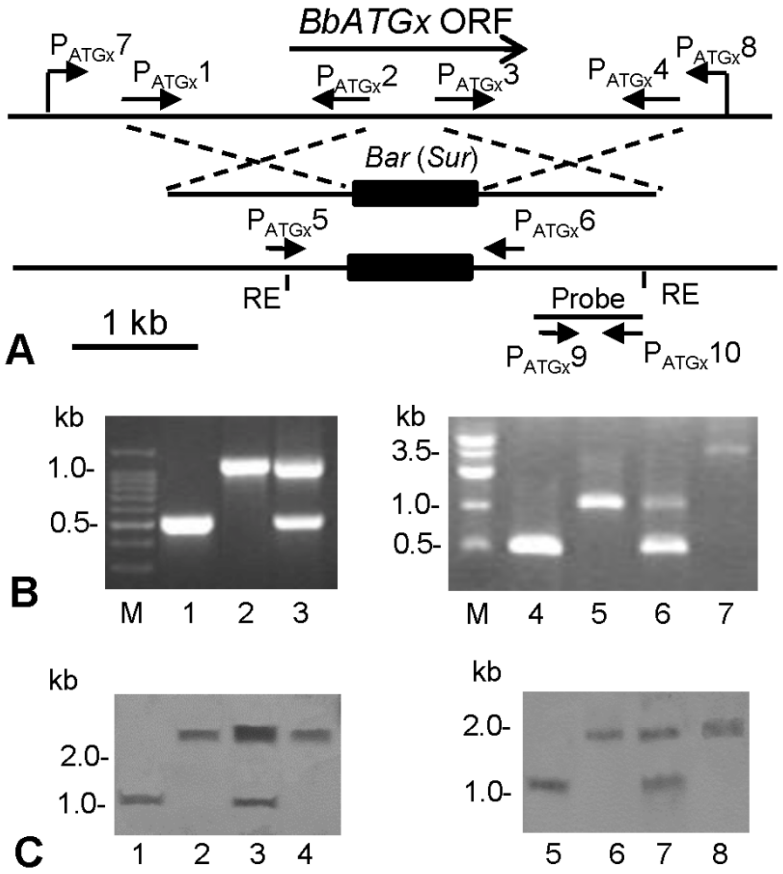

**Figure S3. Gene disruption of *BbATG1* and *BbATG8* abolishes the autophagic process in *B. bassiana*.** Fungal strains were incubated in SDB media for 2 days, and exposed to starvation stress in CPZ media without carbon and nitrogen sources. Transmission electron images show that the autophagic bodies were accumulated in vacuoles of wild type and complemented strains, and no obvious accumulation was found in gene disruption mutants. Scale bar represents 0.2 μm.

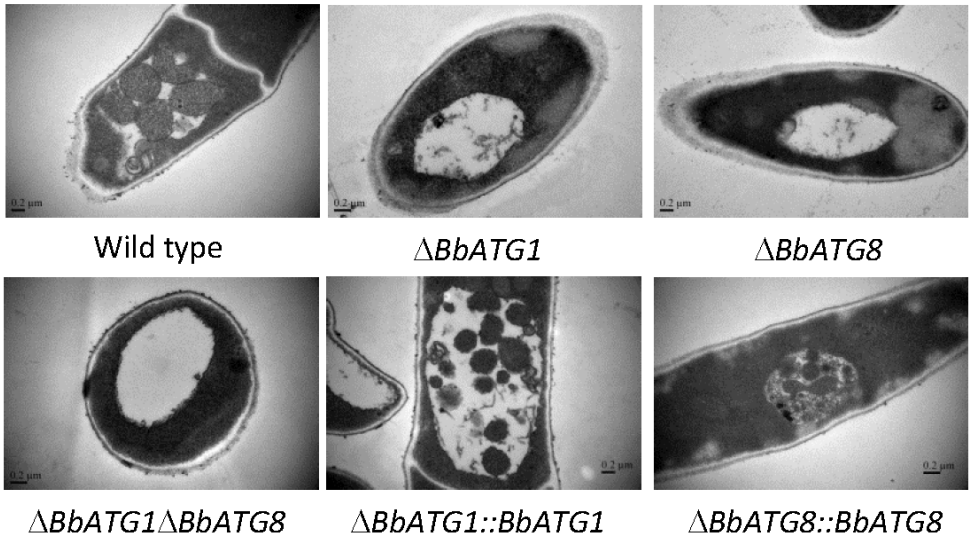

**Figure S4. The patterns of conidial germination on oligotrohpic surface.** Disruption of *BbATG1* and *BbATG8* genes impaired the germination ability of conidia on water agar plates (A) and host cuticles (B). Scale bars, 10 μm.

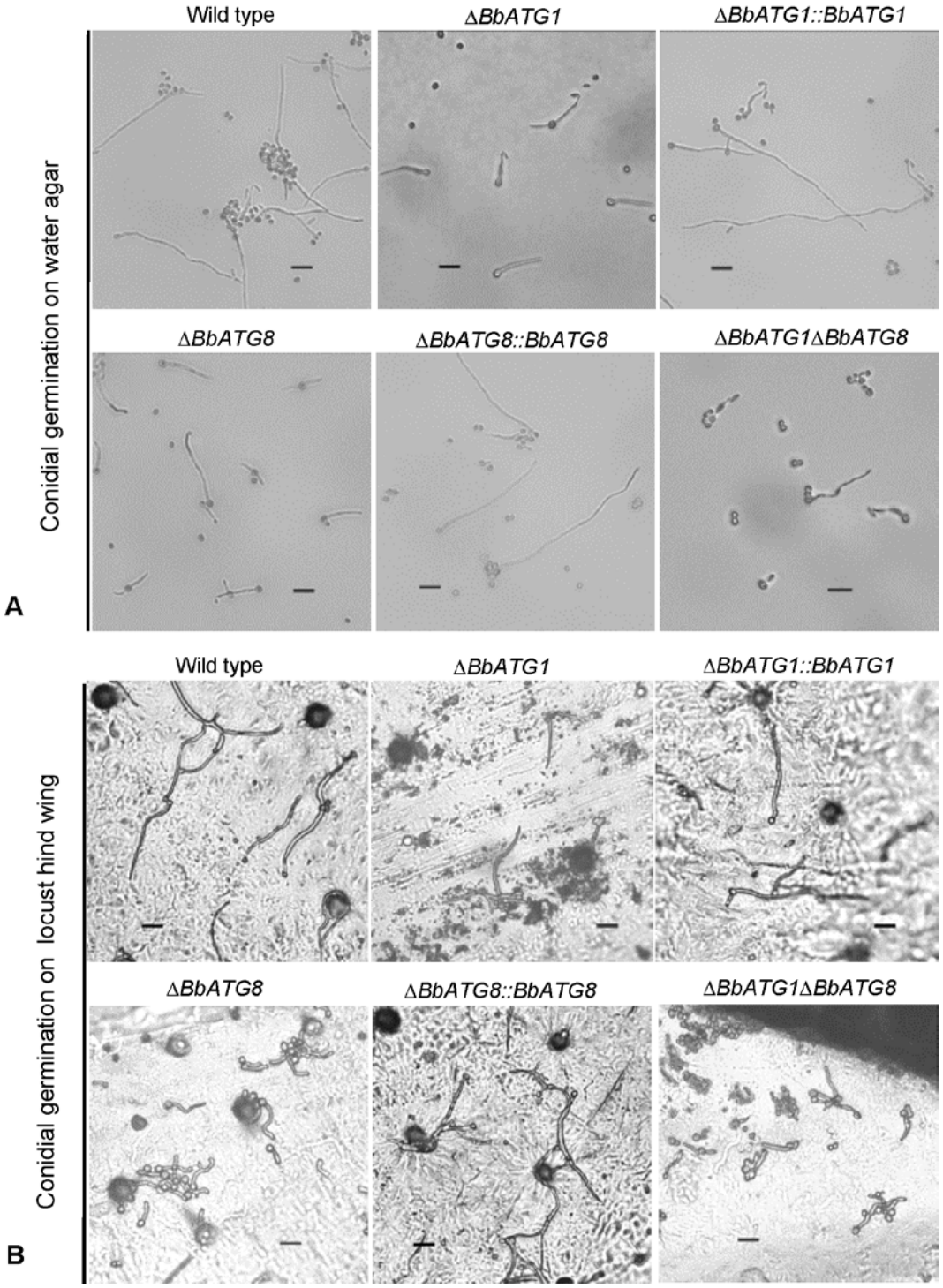

**Figure S5. Zymograms for superoxide dismutases (SOD) of wild type, Δ*BbATG8* mutant and complementation strains.** The 2-day-old mycelia from SDB media were exposed to an 8-h stress under 2 mM menadione, and the mycelia were sampled every 2 hours. Mycelial soluble proteins were extracted, and aliquots of 30 μg protein were resolved by native polyacrylamide gel electrophoresis. The SOD enzymes were visualized on the gel stained with nitroblue tetrazolium solution.

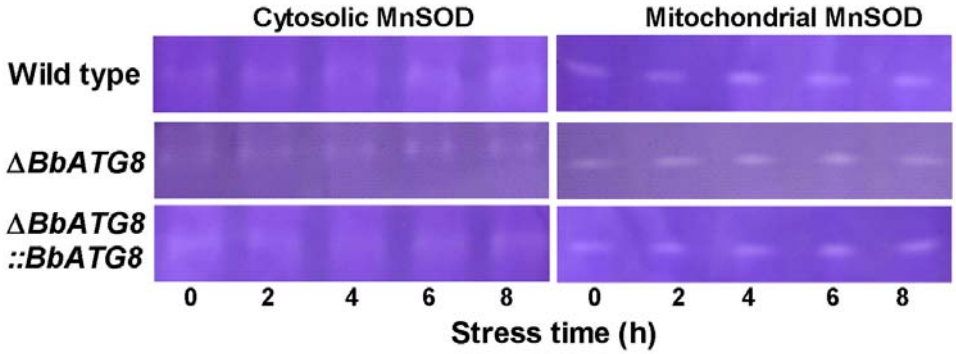

**Figure S6. Representative images showing formazan precipitation in fungal cells.** Hyphal bodies were harvested from the haemoceol of *Galleria mellonella* infected via injection of conidia from each indicated strain and stained with nitroblue tetrazolium. Scale bar: 5 μm.

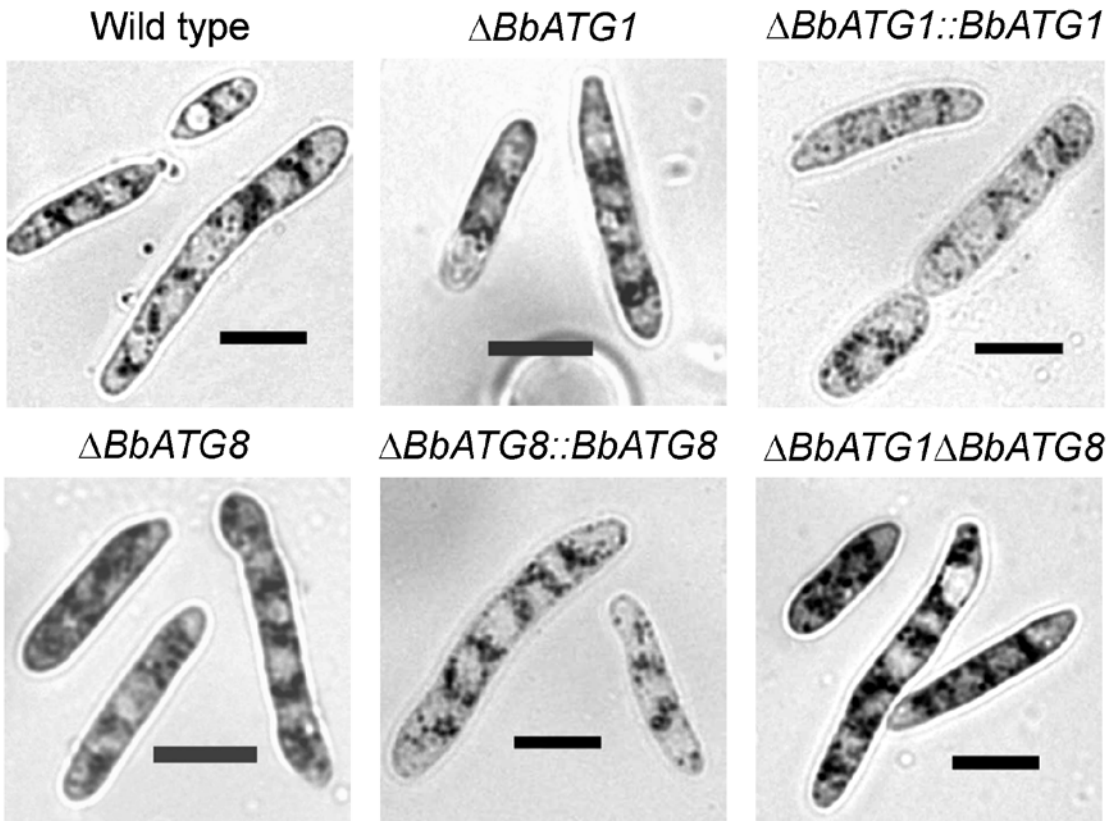

**Figure S7. Gene disruption and complementation of *B. bassiana BbCP15* gene.** (**A**) Schematic diagram describing the strategy of gene disruption by homologous recombination of disruption vector on the target gene locus. RE represents restriction sites for generation of DNA fragments in Southern blot. (**B**) PCR confirmation of correct recombinant strains. Lane 1: wild type; lane 2: complementation strain; lane 3: gene disruption strain and lane M: DNA molecular size standards. (**C**) Southern blotting analysis for determining correct recombination. Lane 1: wild type; lane 2: complementation strain and lane 3: gene disruption strain. The sizes of the resolved DNA fragments are shown.