

Supplementary Material

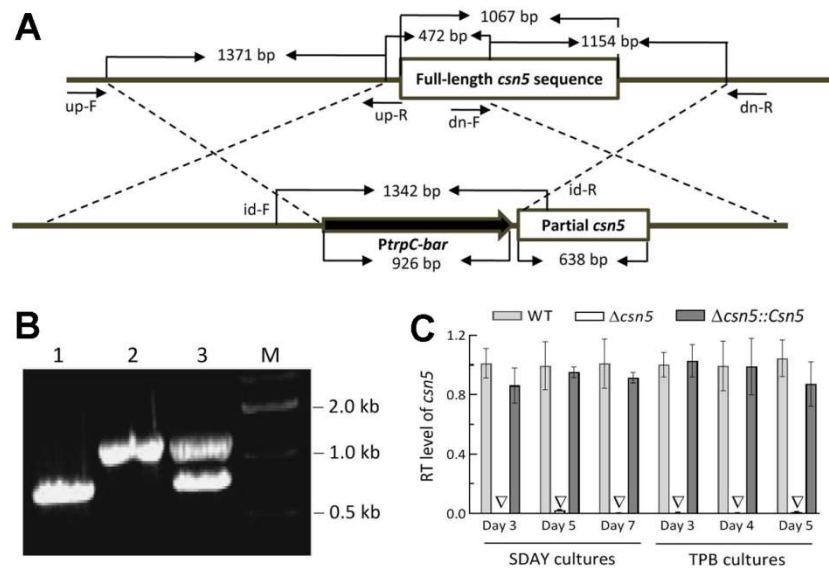


Figure S1. Generation and identification of *csn5* mutants in *B. bassiana*. **(A)** Schematic diagram for the disruption strategy of *csn5*. **(B)** The *csn5* mutants identified through PCR analysis (lanes 1–3: WT, $\Delta csn5$ and $\Delta csn5::Csn5$ respectively; M, DNA ladder) with paired primers in Table S1. The detected PCR bands denote the fragments of 888 bp in $\Delta csn5$, 1,342 bp in WT and both of them in $\Delta csn5::Csn5$ (888 + 926 – 1342 = 472 bp), indicating that *Csn5* was disrupted by the deletion of its promoter/coding fragment (43 + 429 = 472 bp) as expected. **(C)** Relative transcript (RT) levels of *Csn5* in the SDAY and insect hemolymph-mimicking TPB cultures of *csn5* mutants with respect to the WT standard during 7- and 5-day incubation at optimal 25°C respectively. Note that expression of *Csn5* was abolished in the $\Delta csn5$ cultures and restored in the $\Delta csn5::Csn5$ cultures. Error bars: standard deviations of the means from three cDNA samples derived from independent cultures of each strain.

Table S1. Paired primers designed for manipulation of *csn5* in *B. bassiana*.

Primers	Paired sequences (5'–3')*	Purpose
Csn5-F/R	CAATCACAACACCTTCAAAATGGAAACGGCTCTCAGGTCATG / CTCGCCCTTGCTC ACCATTGTACTGTTTTGATCCCCCAAGTCG	Cloning <i>csn5</i> cDNA (1014 bp) for fusion to <i>gfp</i>
upCsn5-F/R	ACGAGCTGTACAAGTAACCCGGGGAGCCTATGCGGTATCTATG / TGGCTGCAGGTC GACGGATCCGAGTTGGGAGTTAGTGTTC	Cloning <i>csn5</i> 5' fragment (1371 bp)
dnCsn5-F/R	GACCCATGGCTCGAGTCTAGAAAAATGTTGTCGGATGGTA / GGTGGTGGTGGCTAG CGTTAACTTCTGTCACTGGAGGATTG	Cloning <i>csn5</i> 3' fragment (1154 bp)
flCsn5-F/R	ATCCGTCGACCTGCAGCCAAGCTTAACCAAGCAGGTAAGGC / AACTAGTCAGAT CTTCTAGTGTACACCCACGACATTGAGG	Cloning full-length <i>csn5</i> for complementation (3991 bp)
pCsn5-F/R	CTCCAGCATTGCGCAACA / CTGAAACCCATCCGACCC	PCR detecting <i>csn5</i> (888 bp)
qCsn5-F/R	TCGTGACTTTGCAAAACAAGC / GACGCCAGTAGCCAGAAATC	qPCR detecting <i>csn5</i>

* Underlined regions denote introduced cleavage sites of two pairs of restriction enzymes (*Xma*I/*Bam*HI and *Xba*I/*Hpa*I) for homogeneous recombination of the *bar*-separated 5' and 3' fragments for the disruption of *csn5* or the fragments for gateway exchange.

Table S2. Paired primers used for transcriptional profiling of *csn5*-related genes in *B. bassiana*.

Gene	Tag locus ^a	Annotation ^b	Sequences (5'–3') of paired primers
Used as internal standard			
<i>act</i>	BBA_04860	β-actin	GGCAACATTGTCATGTCTGG / TTTGCTGGAAGGTGGATAGG
Involved in asexual development and hydrophobicity			
<i>brlA</i>	BBA_07544	Developmental activator BrlA	GATGGATGACAAGTGCGATG / AAACCTGCACGAGAAACGAT
<i>abaA</i>	BBA_00300	Developmental activator AbaA	GCAAGTCTCCAGCCATAT / CTCCTCTTCGTCATAGTAGTC
<i>wetA</i>	BBA_06126	Developmental activator WetA	ATGCGGTACTACAGCCAAGG / GAGTTCCTGCTGGCTACTGG
<i>vosA</i>	BBA_01023	The velvet protein VosA	ACTCATGGGCTCATTGGTGG / CCGGCAAGAGAGATCCGAAA
<i>hyd1</i>	BBA_03015	Class I hydrophobin Hyd1	ATGGTGGAAAGGATCTGCAC / TGGGAAAAGAACCATCAGC
<i>hyd2</i>	BBA_06599	Class II hydrophobin Hyd2	TGTCAAGACTGGCGACATTT / ATTGGGACAAGCTGGTTGAG
Involved in response to heat shock			
<i>hsp30a</i>	BBA_08688	Heat shock protein 30	AGGCGTGAAAAAGGAAGACA / GGTTGACGCCTCTGTCGTAT
<i>hsp30b</i>	BBA_02057	Heat shock protein 30	ATTGCTCGCAACAACAACAA / CAAGATACCGTCTGGAAGC
<i>hsp40a</i>	BBA_06930	Chaperone DnaJ, Mdj1	AAACCCTGGCGATGATACTG / AGAAACGGCTGAAAAGGTCA
<i>hsp40b</i>	BBA_08440	Heat shock protein, Hsp40, DnaJ	CAAGAAGTGCAAGGGAAAGC / TACCGAGACGCGAAAAGACT
<i>hsp40c</i>	BBA_03736	Chaperone protein dnaJ 2, Mas5	CAAGAAGTGCAAGGGAAAGC / TACCGAGACGCGAAAAGACT
<i>hsp60</i>	BBA_05467	Chaperonin GroL	AGAAGAACAAGCGCGACATT / GGTGATGAAGTAGGGCGAAA
<i>hsp70a</i>	BBA_00941	Hsp70 protein	ACGCTTGAAAGTCTGCCCTA / GGATTTACACGAGCCTGGA
<i>hsp70b</i>	BBA_05586	Heat shock protein 70-2	CACGTAATCTTGGCTCGGTA / ACCTCGGGGTCTGAGAATT
<i>hsp90</i>	BBA_06516	Heat shock protein 90	GGTCAGTTCGGTGTGGTTT / CGTCCTTGAGGTGGAGGATA
<i>hsp104</i>	BBA_02283	Heat shock protein HSP98	ATGTTCCCGACAACCTGAAG / GCACCATGAGAAGGTGAAT
Involved in ubiquitin activation (E1 family)			
<i>ubi1/2</i>	BBA_06852	Ubiquitin family protein	GCCAAGCTGCTAGCATTACC / CGCCATGAAAAGGAAAACAT
<i>ubi3</i>	BBA_09309	Ubiquitin family protein	ATCCTCCCCACCATTACCTC / CTGGGTCTGCTCTGTCTAGC
<i>ubi4</i>	BBA_03881	Polyubiquitin precursor Ubi4	GCTCCTCTTTAGCAGCATGG / GCAAACTGCACGTAATCCA
Involved in ubiquitin conjugation (E2 family)			
<i>ubc1</i>	BBA_07096	E2 ubiquitin-conjugating protein UBC1	CGATAGCTACCCGTTCAAGC / GGGTTGCTCGTTCATCATCT
<i>ubc2</i>	BBA_01469	E2 ubiquitin-conjugating protein RAD6	GTGACTTCAAGCGAATGCAA / GCTCCTCAAAGTCATCACA
<i>ubc3</i>	BBA_06665	E2 ubiquitin-conjugating enzyme	GGACGAGCGAGAAAAGTACG / TCCATGTCACTGTCTGCTACC
<i>ubc4</i>	BBA_01937	E2 ubiquitin-conjugating protein UBC4	ATTGTGGGACCTCACGAGAC / ACCTCTCCAGTGCCTAGAA
<i>ubc5</i>	BBA_09007	E2 ubiquitin-conjugating protein UBC5	TCTACCACCAAAACGTCACA / TCTCCATGGCGTACTTTTC
<i>ubc6</i>	BBA_03114	E2 ubiquitin-conjugating protein UBC6	AATATTCCCGCCCACTACC / ACATTCTGTGAGACGGAACC
<i>ubc7</i>	BBA_02005	E2 ubiquitin-conjugating protein UBC7	GAGCTCAAGTTTCCCAAGGA / GAATCTTTTGACCGACTGG
<i>ubc8</i>	BBA_06237	E2 ubiquitin-conjugating protein UBC8	TCGCCAATGTTTGACATGAT / ATCGTCGTATCTTCGCTCT
<i>ubc9</i>	BBA_06098	E2 SUMO-conjugating protein UBC9	TACAGCAAGAGCGCAAGCTA / GGGTATTTCATCGGGGAAAGT
<i>ubc10</i>	BBA_00143	E2 ubiquitin-protein ligase peroxin 4	GTCGTCCAACGTCTCCAAAT / CACTGCATCAAGCTCTCGTC
<i>ubc11</i>	BBA_00216	E2 ubiquitin-protein ligase UBC11	AGCTTGCAGACGGAAGTAT / GGGTAGTTGGAGGGGAAGTC
<i>ubc12</i>	BBA_04666	NEDD8-conjugating protein UBC12	ACCTTTGCCATCAACCAGAG / GGCTCGAGGAACAAAAGT
<i>ubc13</i>	BBA_09651	E2 ubiquitin-conjugating protein UBC13	ACGAAGGTGGCATTTCCTAAG / GGGGACCAAGTTTCTTCA
<i>ubc14</i>	BBA_07061	Ubiquitin-conjugating protein DMA2	GCTGGGAGGAGTTGTGTCAT / TTGGGTTCTTGCCAGTTAC

Table S2 (continued)

Gene	Tag locus ^a	Annotation ^b	Sequences (5'–3') of paired primers
Involved in ubiquitin-protein ligation (E3 family)			
<i>upl1</i>	BBA_00743	Ubiquitin-protein ligase TUL1	GTTTCGCCTTTGTCATCCTC / GCAAAGTCTCCCCATTGTGT
<i>upl2</i>	BBA_00804	RBR-type E3 ubiquitin transferase	ACGTCTTTGGCACCATTAGG / GTCCCCGTAAAAGTCGCATA
<i>upl3</i>	BBA_01028	E3 ubiquitin-protein ligase BRE1	AGGGGAAGTTAAACCCGAGA / TGCTGTTCCAACCTCTGCAC
<i>upl4</i>	BBA_01431	E3 ubiquitin-protein ligase HEL1	GCCTGCTTTGCTGATAGTCC / GTTCGCAACCAAGAGAAAGC
<i>upl5</i>	BBA_02443	E3 ubiquitin-protein ligase PRP19	TTGATGCCTCACTCACAACC / CTGCTAGCCAGAAACCGTTC
<i>upl6</i>	BBA_03508	E3 ubiquitin-protein ligase RAD18	CTGCGGAGTAATTGGTCCAT / CTACACTCGGCGTAGGTGGT
<i>upl7</i>	BBA_04432	E3 ubiquitin-protein ligase UBR1	GCGCTAAGTGGCATCAAG / ACCAAGGTGGATAAGCGATG
<i>upl8</i>	BBA_05204	E3 ubiquitin-protein ligase HEL1	CTCGCCGAGTCCAACACTC / CCAGCGTTTCGTAGAGTTCC
<i>upl9</i>	BBA_05776	E3 ubiquitin-protein ligase HEL2	GGAGCAGCATTTCAAAAAGG / GAGGTCAAAACAGCCATGT
<i>upl10</i>	BBA_06925	Ubiquitin-protein ligase peroxin 12	TTTCGAGTCTGTCGGTAGCC / TAAATGCTTGGCCAAAGAC
<i>upl11</i>	BBA_07173	E3 ubiquitin-protein ligase IRC20	CTTTTAGTGCGGTCGAGGAG / ATCGGCCGTTCTTTATTGTG
<i>upl12</i>	BBA_07231	E3 ubiquitin-protein ligase HRD1	GCAAGAAGTCAGGGAACAGC / TCCAGCCAAAGTACCCATTTC
<i>upl13</i>	BBA_07605	E3 ubiquitin-protein ligase RKR1	CGAGGCCAAGTACTTTCGAG / CATGGCCTGTCTGCACTCTA
<i>upl14</i>	BBA_09149	E3 ubiquitin-protein ligase SSM4	GCGCGAAGAATTTGAGGTAG / GCGCAATTCATGTACGTGTG
<i>upl15</i>	BBA_09504	Ubiquitin-protein ligase PSH1	ATTGCGGAACAACATCAACA / TGACGATCGCCTTCTCTCTT
<i>upl16</i>	BBA_01822	NEDD4 family E3 ubiquitin-protein ligase	TGGTGAGCAAAAGTCACGTC / GTATGTGCCTTGGGCAAGTT
<i>upl17</i>	BBA_03558	Putative ubiquitin-protein ligase UFD4	ATCAGCTCATAAGCCGAGT / AGACGCAAGATGGAAGCTGT
<i>upl18</i>	BBA_05494	Putative E3 ubiquitin-protein ligase HUL4	GTCCGGAAGAGATTGAGCTG / TCACTGCCGGTGATAAATGA
<i>upl19</i>	BBA_05518	E3 ubiquitin-protein ligase TOM1	GCGAACTATTACCGAGAGC / CCACTGTGTTGTCCCTCGTT
<i>upl20</i>	BBA_05644	Ubiquitin-ubiquitin ligase HUL5	CTCTGGCCTCAGAACACACA / CTCCAGGCACGGTTTGTAAAT
<i>upl21</i>	BBA_00267	Ubiquitin ligase complex subunit HRD3	AGCTTGTCGATTTGGCCTTG / CAGGTATAGTGCCTCCGAGT
<i>upl22</i>	BBA_03528	Putative ubiquitin-protein ligase ASI3	TTGCATGCACATCCGAACAA / AGAGCGGCCACATAGTAGAC
<i>upl23</i>	BBA_05538	Ub ligase molybdopterin-converting factor	GACGCCATTGACAACATCGA / CAGCCCATCGTCGGTACTAT
Involved in deubiquitination			
<i>usp1</i>	BBA_02705	Ubiquitin-specific protease UBP1	GAGCGTGCACTACGAGATGA / ACAGATCGCTGTCCGAGAGT
<i>usp2</i>	BBA_07124	Ubiquitin-specific protease UBP2	AGCGATCTTCAGGCACAGAT / ATCAAAGCTGACCTCGTGCT
<i>usp3</i>	BBA_08453	Ubiquitin-specific protease UBP3	ACTCTTTGCCAAGACGCCTA / GCCAACCTTGAGGATCGAA
<i>usp4</i>	BBA_04102	Putative ubiquitin-specific protease UBP12	CTCTTGACCGCAAGGTTCTC / GGGCAAGTCAATCAGCAAAT
<i>usp5</i>	BBA_00871	Ubiquitin-specific protease YUH1	CGGAGATTGCTTCAACCTG / TTCTCCTCTCCAGACTCCCA
<i>usp6</i>	BBA_01234	Ubiquitin-specific protease YUH1	CTTCACATCGCTTCTCCAGC / CGGCGGTCTGTGTGAAGTC
<i>usp7</i>	BBA_01266	Ubiquitin-specific protease YUH1	GCAATAAGAGGCGCAATCCA / GCACCTTGACGTCCTTGATC
<i>usp8</i>	BBA_01860	Ubiquitin-specific protease UBP15	GCGACAACTACGGCTACTG / GTAATCAGCCGCGACACAAT
<i>usp9</i>	BBA_02115	Ubiquitin-specific protease UBP13	CAACGCCAGGCTATCAACTC / TCCTTGAGCCCGGTAAATGT
<i>usp10</i>	BBA_02323	Ubiquitin-specific protease YUH1	GTCCAACGTGAATCCTGACC / GGGATAAAGCGAGCAGGTC
<i>usp11</i>	BBA_03889	Ubiquitin-specific protease UBP13	CCAAGCTCAACAAGGCCATT / CGTAAATGGAGCGCGATAGG
<i>usp12</i>	BBA_04237	Ubiquitin-specific protease UBP11	CCAAGCTCAACAAGGCCATT / CGTAAATGGAGCGCGATAGG
<i>usp13</i>	BBA_04750	Ubiquitin-specific protease UBP15	CTCGCAAGAAGGAACATGG / AACGAGGGCAAACTGAACAC
<i>usp14</i>	BBA_04857	Ubiquitin-specific protease UBP14	TCCCCAGCCATTCATCAGTT / CCACCATAGAGCCTTTTCGC
<i>usp15</i>	BBA_06326	Ubiquitin-specific protease UBP14	TTCTGACGCCAACTCTGCTA / TGAACAACCACTCAACAGCG
<i>usp16</i>	BBA_07695	Ubiquitin-specific protease UBP6	CTTTACACGAGGATGAGCGC / CGCCGCTCATGTACTTTTGA
<i>usp17</i>	BBA_10345	Ubiquitin-specific protease UBP8	CCAGCGTCACCTATGTTTGG / TTTGCGGCCAGAGAAAGAAC

^a Gene accession codes of *B. bassiana* genome under the NCBI accession NL_ADAH00000000.

^b Putative E2, E3 and UBP genes are annotated with respect to their corresponding homologs in budding yeast.

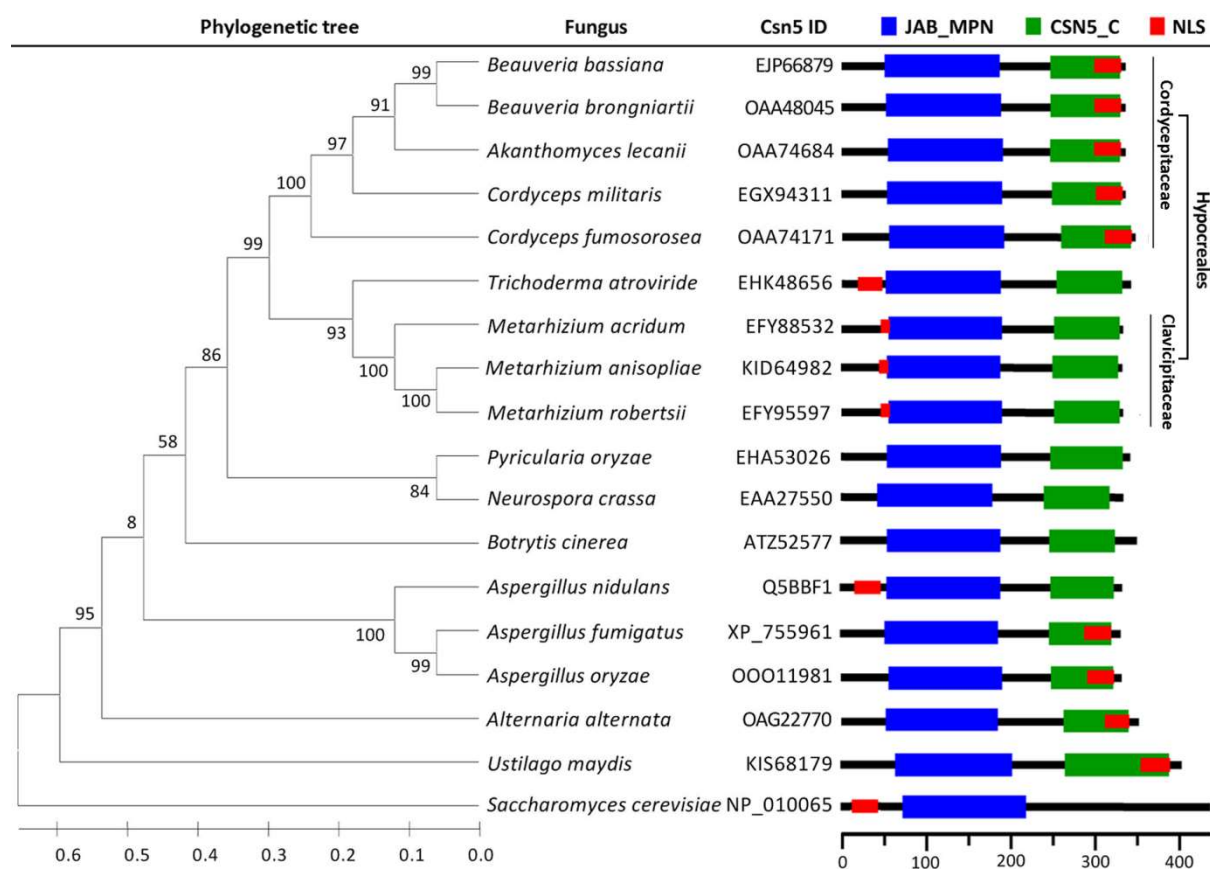


Figure S2. Phylogenetic relationships and sequence features of Csn5 orthologues found in entomopathogenic (Hypocreales) and nonentomopathogenic fungi. Scale for phylogenetic tree: branch length proportional to genetic distance assessed with the maximum likelihood method in MEGA7 at <http://www.megasoftware.net/>. Bootstrap values of 1000 replications are shown at nodes. Main domains and NLS motif are predicted at <https://www.ncbi.nlm.nih.gov/Structure/> and <http://nls-mapper.iab.keio.ac.jp/>, respectively.

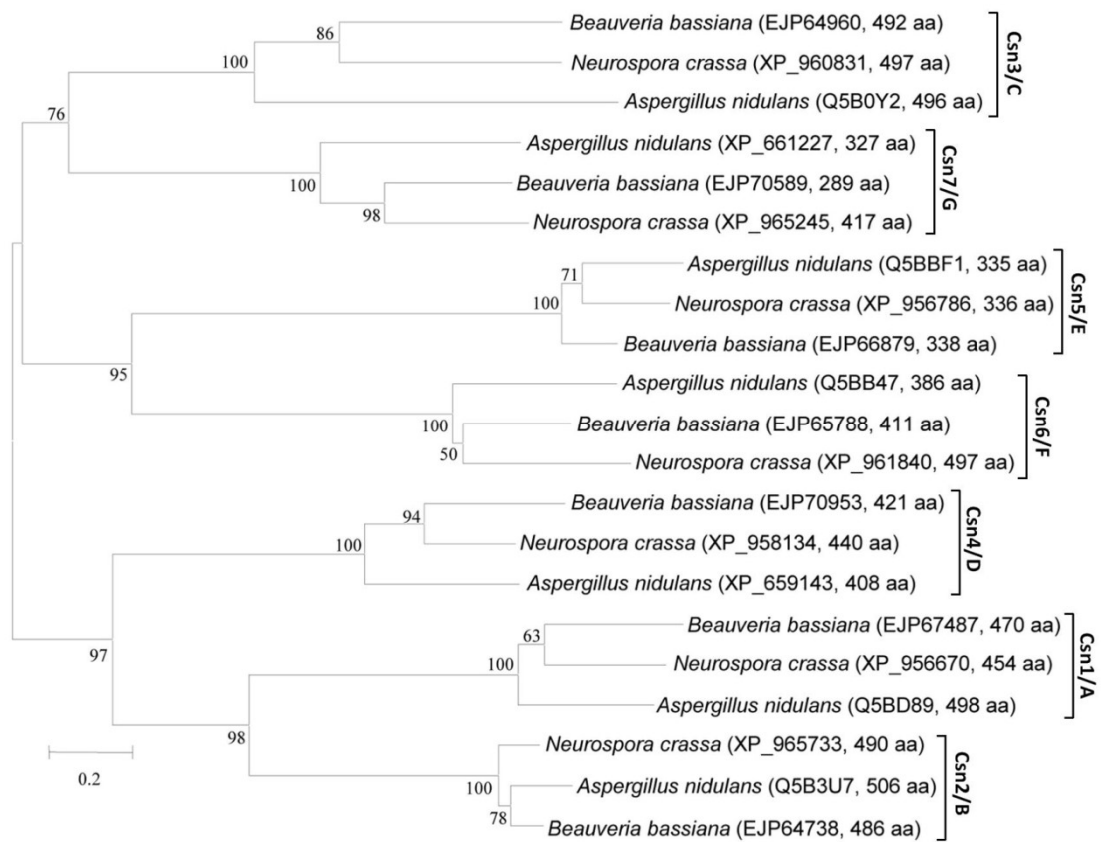


Figure S3. Phylogenetic relationships of CSN subunits between *B. bassiana* and two model fungi. Scale: branch length proportional to the genetic distance assessed with the maximum likelihood method in MEGA7 at <http://www.megasoftware.net/>. Bootstrap values of 1000 replications are shown at nodes. The NCBI accession code of each subunit and the length of its amino acid sequence are listed in the parentheses following the fungal name.

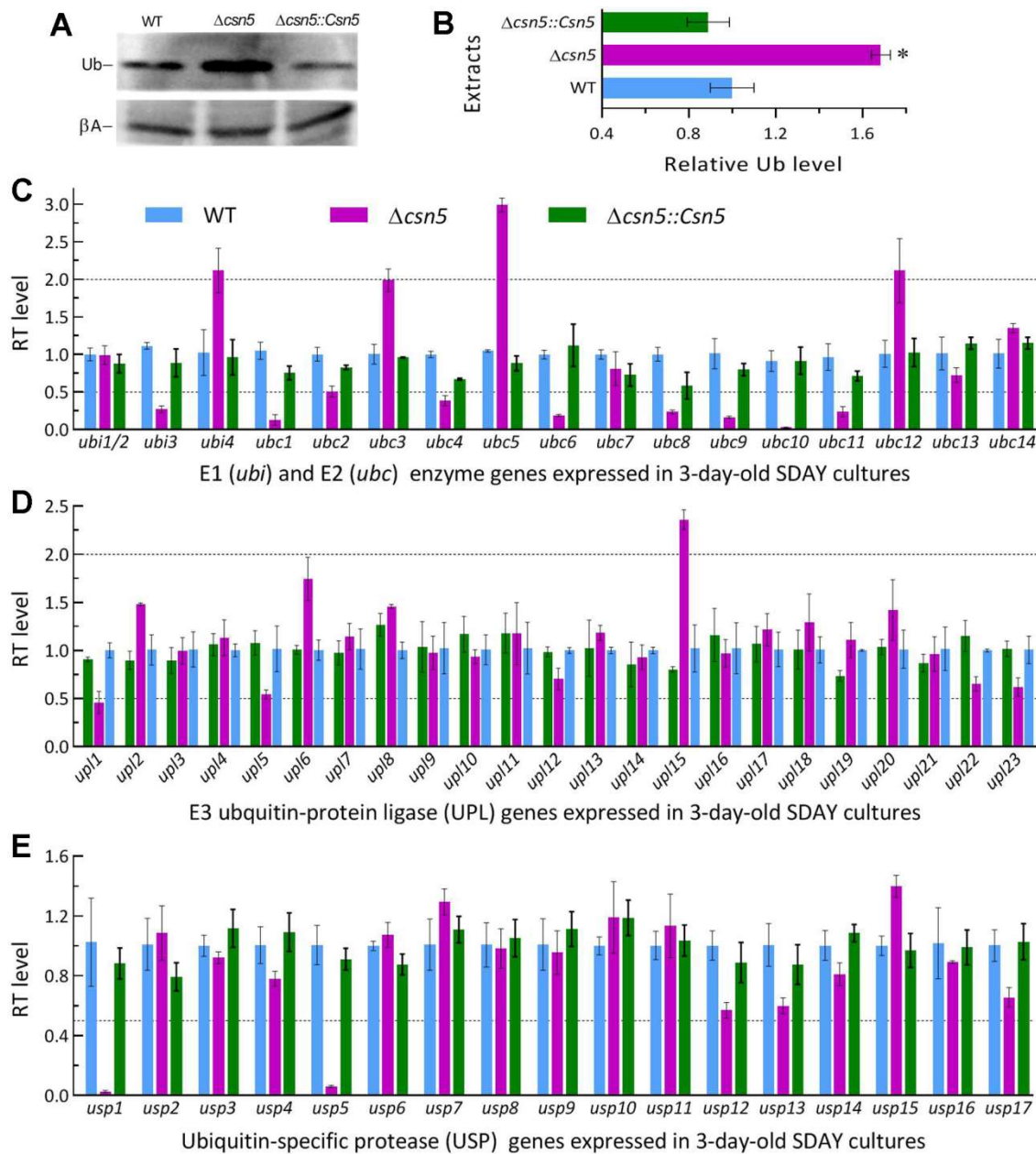


Figure S4. Impact of *csn5* deletion on accumulation level of intracellular free ubiquitin and expression levels of putative UPS genes in *B. bassiana*. (**A**, **B**) Western blot for free ubiquitin (Ub) accumulation probed with monoclonal anti-Ub antibody in the protein extracts of 3-day-old SDBY cultures and relative Ub accumulation levels computed as ratios of Ub blot intensities over those of β -actin (β A) as a reference probed with anti- β A antibodies, respectively. Each lane was uploaded with 20 μ g of protein sample. * $P < 0.001$ in Tukey's HSD test. (**C**–**E**) Relative transcript (RT) levels of E1, E2, E3 and ubiquitin-specific protease (USP) genes in the 3-day-old SDAY cultures of *csn5* mutants with respect to the WT standard. The dashed lines indicate a significant level of one-fold change (50% downregulation or 100% upregulation relative to WT). Error bars: standard deviations of the means from three independent replicates.