

Supplementary Material

**Photoreactivation Activities of Rad5, Rad16A and Rad16B Help
Beauveria bassiana to recover from Solar Ultraviolet Damage**

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Table S1. Paired primers used for manipulation and detection of target genes in *B. bassiana*.

Primers	Paired sequences (5'–3') ^a	Purpose ^b
cRad5-F/R	<u>CAATCACAACACCTTCAAAATGGACTTTGCCAACTCACA</u> / <u>TCCTCGCCCTTGCT</u> <u>CACCATGCTAAGCAGTTCTTTCATGT</u>	Cloning <i>rad5</i> cDNA (3357 bp) for fusion to <i>GFP</i>
cRad16B-F/R	<u>CAATCACAACACCTTCAAAATGCAGTCTGGAACCCAGC</u> / <u>TCCTCGCCCTTGCT</u> <u>TCACCATCTTGAACAGGCTGCGGATAT</u>	Cloning <i>rad16B</i> cDNA (3264 bp) for fusion to <i>GFP</i>
cRad16A-F/R	<u>C AATCACAACACCTTCAAAATGCCTGCTGGACGGTCTTC</u> / <u>TCCTCGCCCTTGCT</u> <u>TCACCATTTTGCAGAGCTTGGCAGCG</u>	Cloning <i>rad16A</i> cDNA (3018 bp) for fusion to <i>GFP</i>
adRad5-F/R	<u>GCCATGGAGGCCAGTGAATTCATGGACTTTGCCAACTCACA</u> / <u>CAGCTCGAGCTC</u> <u>GATGGATCCGCTAAGCAGTTCTTTCATGT</u>	Cloning <i>rad5</i> cDNA (3357 bp) for ligation to AD
bdRad5-F/R	<u>ATGGCCATGGAGGCCGAATTCATGGACTTTGCCAACTCACA</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCGCTAAGCAGTTCTTTCATGT</u>	Cloning <i>rad5</i> cDNA (3357 bp) for ligation to BD
adRad16A-F/R	<u>GCCATGGAGGCCAGTGAATTCATGCTGCTGGACGGTCTTC</u> / <u>CAGCTCGAGCTC</u> <u>GATGGATCCATTACACGGAACAAAACT</u>	Cloning <i>rad16A</i> cDNA (3018 bp) for ligation to AD
bdRad16A-F/R	<u>ATGGCCATGGAGGCCGAATTCATGCTGCTGGACGGTCTTC</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCATTACACGGAACAAAACT</u>	Cloning <i>rad16A</i> cDNA (3018 bp) for ligation to BD
adRad16B-F/R	<u>GCCATGGAGGCCAGTGAATTCATGCAGTCTGGAACCCAGC</u> / <u>CAGCTCGAGCTC</u> <u>GATGGATCCCTTGAACAGGCTGCGGATAT</u>	Cloning <i>rad16B</i> cDNA (3264 bp) for ligation to AD
bdRad16B-F/R	<u>ATGGCCATGGAGGCCGAATTCATGCAGTCTGGAACCCAGC</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCCTTGAACAGGCTGCGGATAT</u>	Cloning <i>rad16B</i> cDNA (3264 bp) for ligation to BD
adELC1-F/R	<u>GCCATGGAGGCCAGTGAATTCATGGCGACGACTCAGACTCC</u> / <u>CAGCTCGAGCTC</u> <u>GATGGATCCGTTTGTCTGATAGCTTGGTCCAA</u>	Cloning <i>ELC1</i> cDNA (333 bp) for ligation to AD
bdELC1-F/R	<u>ATGGCCATGGAGGCCGAATTCATGGCGACGACTCAGACTCC</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCGTTTGTCTGATAGCTTGGTCCAA</u>	Cloning <i>ELC1</i> cDNA (333 bp) for ligation to BD
adCul1-F/R	<u>GCCATGGAGGCCAGTGAATTCATGATCTCTGGGAGAGGAAG</u> / <u>CAGCTCGAGCT</u> <u>CGATGGATCCCGCCACATAGCGGTACAC</u>	Cloning <i>cul3A</i> cDNA (2520 bp) for ligation to AD
bdCul1-F/R	<u>ATGGCCATGGAGGCCGAATTCATGATCTCTGGGAGAGGAAG</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCCGCCACATAGCGGTACAC</u>	Cloning <i>cul3A</i> cDNA (2520 bp) for ligation to BD
adCul2-F/R	<u>GCCATGGAGGCCAGTGAATTCATGCACTCCCTCGCGGATCA</u> / <u>CAGCTCGAGCTC</u> <u>GATGGATCCCGCCAAAGTACGTGTACGCGT</u>	Cloning <i>cul3B</i> cDNA (2493 bp) for ligation to AD
bdCul2-F/R	<u>ATGGCCATGGAGGCCGAATTCATGCACTCCCTCGCGGATCA</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCCGCCAAAGTACGTGTACGCGT</u>	Cloning <i>cul3B</i> cDNA (2493 bp) for ligation to BD
adMMS2-F/R	<u>GCCATGGAGGCCAGTGAATTCATGGCCAAGTTCTCTGTAA</u> / <u>CAGCTCGAGCTC</u> <u>GATGGATCCCTGAGTAGGTAGAACCCT</u>	Cloning <i>mms2</i> cDNA (420 bp) for ligation to AD
bdMMS2-F/R	<u>ATGGCCATGGAGGCCGAATTCATGGCCAAGTTCTCTGTAA</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCCTGAGTAGGTAGAACCCT</u>	Cloning <i>mms2</i> cDNA (420 bp) for ligation to BD
adRad1-F/R	<u>GCCATGGAGGCCAGTGAATTCATGTCGACAAACAATGCGCC</u> / <u>CAGCTCGAGCTC</u> <u>GATGGATCCGTAATCTTCTCGTCCATGA</u>	Cloning <i>rad1</i> cDNA (2820 bp) for ligation to AD
bdRad1-F/R	<u>ATGGCCATGGAGGCCGAATTCATGTCGACAAACAATGCGCC</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCGTAATCTTCTCGTCCATGA</u>	Cloning <i>rad1</i> cDNA (2820 bp) for ligation to BD
adRad2 -F/R	<u>GCCATGGAGGCCAGTGAATTCATGGGCGTGAACGGTCTTTG</u> / <u>CAGCTCGAGCT</u> <u>CGATGGATCCCTGCTTCCGCCGTTTATTGC</u>	Cloning <i>rad2</i> cDNA (3462 bp) for ligation to AD
bdRad2 -F/R	<u>ATGGCCATGGAGGCCGAATTCATGGGCGTGAACGGTCTTTG</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCCTGCTTCCGCCGTTTATTGC</u>	Cloning <i>rad2</i> cDNA (3462 bp) for ligation to BD
adRad6 -F/R	<u>GCCATGGAGGCCAGTGAATTCATGTCAACGCGCCAGACG</u> / <u>CAGCTCGAGCT</u> <u>CGATGGATCCGCTCTCCAGCTCTTCTCTA</u>	Cloning <i>rad6</i> cDNA (456 bp) for ligation to AD
bdRad6 -F/R	<u>ATGGCCATGGAGGCCGAATTCATGTCAACGCGCCAGACG</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCGCTCTCCAGCTCTTCTCTA</u>	Cloning <i>rad6</i> cDNA (456 bp) for ligation to BD
adRad18 -F/R	<u>GCCATGGAGGCCAGTGAATTCATGCCTGTTGACGATGTTGCGGATT</u> / <u>CAGCTCG</u> <u>AGCTCGATGGATCCCTAAATACTGCTTTCCTGATTCCG</u>	Cloning <i>rad18</i> cDNA (1275bp) for ligation to AD
bdRad18 -F/R	<u>ATGGCCATGGAGGCCGAATTCATGCCTGTTGACGATGTTGCGGATT</u> / <u>CGCTGCA</u> <u>GGTCGACGGATCCCTAAATACTGCTTTCCTGATTCCG</u>	Cloning <i>rad18</i> cDNA (1275 bp) for ligation to BD
adRad10-F/R	<u>GCCATGGAGGCCAGTGAATTCATGGCTGACGAATACGGCGC</u> / <u>CAGCTCGAGCT</u> <u>CGATGGATCCCTTTCGAAGCTTTGCCAGCG</u>	Cloning <i>rad10</i> cDNA (1176 bp) for ligation to AD
bdRad10-F/R	<u>ATGGCCATGGAGGCCGAATTCATGGCTGACGAATACGGCGC</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCCTTTCGAAGCTTTGCCAGCG</u>	Cloning <i>rad10</i> cDNA (1176 bp) for ligation to BD
adRad14 -F/R	<u>GCCATGGAGGCCAGTGAATTCATGGAGCGCCCAAGACGCC</u> / <u>CAGCTCGAGCT</u> <u>CGATGGATCCAGCTCCAGCTCCTCACTT</u>	Cloning <i>rad14</i> cDNA (1149 bp) for ligation to AD
bdRad14 -F/R	<u>ATGGCCATGGAGGCCGAATTCATGGAGCGCCCAAGACGCC</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCAGCTCCAGCTCCTCACTT</u>	Cloning <i>rad14</i> cDNA (1149 bp) for ligation to BD
adRad23 -F/R	<u>GCCATGGAGGCCAGTGAATTCATGAAGGTACCTTCAGAGA</u> / <u>CAGCTCGAGCT</u> <u>CGATGGATCCCTTGCTCGCAGGCGGCTGCT</u>	Cloning <i>rad23</i> cDNA (1191 bp) for ligation to AD
bdRad23 -F/R	<u>ATGGCCATGGAGGCCGAATTCATGAAGGTACCTTCAGAGA</u> / <u>CGCTGCAGGTC</u> <u>GACGGATCCCTTGCTCGCAGGCGGCTGCT</u>	Cloning <i>rad23</i> cDNA (1191 bp) for ligation to BD

Table S1 (continued)

Primers	Paired sequences (5'–3') ^a	Purpose ^b
adWC1-F/R	<u>GCCATGGAGGCCAGTGAATTC</u> ATGGAAGGATACTACCTCC / <u>CAGCTCGAGCTCGATGGATCC</u> AGGTAAGCTCGTTTCACGCT	Cloning <i>wc1</i> cDNA (2889 bp) for ligation to AD
adWC2-F/R	<u>GCCATGGAGGCCAGTGAATTC</u> ATGTCCCAGGGACACGCGCC / <u>CAGCTCGAGCTCGATGGATCC</u> GCTTCCGGCACCAACTCTG	Cloning <i>wc2</i> cDNA (1497 bp) for ligation to AD
adPhr1-F/R	<u>GCCATGGAGGCCAGTGAATTC</u> ATGGCGCCTCGTGCAACGAA / <u>CAGCTCGAGCTCGATGGATCC</u> CGCCACAGCCGCTTGACG	Cloning <i>phr1</i> cDNA (1761 bp) for ligation to AD
bdPhr1-F/R	<u>ATGGCCATGGAGGCCGAATTC</u> ATGGCGCCTCGTGCAACGAA / <u>CGCTGCAGGTGACGGATCC</u> CGCCACAGCCGCTTGACG	Cloning <i>phr1</i> cDNA (1761 bp) for ligation to BD
adPhr2-F/R	<u>GCCATGGAGGCCAGTGAATTC</u> ATGACAAAGCCAGAGTCAT / <u>CAGCTCGAGCTCGATGGATCC</u> GTTTTCTGCTTCTTCGCTG	Cloning <i>phr2</i> cDNA (1869 bp) for ligation to AD
bdPhr2-F/R	<u>ATGGCCATGGAGGCCGAATTC</u> ATGACAAAGCCAGAGTCAT / <u>CGCTGCAGGTGACGGATCC</u> GTTTTCTGCTTCTTCGCTG	Cloning <i>phr2</i> cDNA (1869 bp) for ligation to BD
bdWC1-F/R	<u>ATGGCCATGGAGGCCGAATTC</u> ATGGAAGGATACTACCTCC / <u>CGCTGCAGGTGACGGATCC</u> AGGTAAGCTCGTTTCACGCT	Cloning <i>wc1</i> cDNA (2889 bp) for ligation to BD
bdWC2-F/R	<u>ATGGCCATGGAGGCCGAATTC</u> ATGTCCCAGGGACACGCGCC / <u>CGCTGCAGGTGACGGATCC</u> GCTTCCGGCACCAACTCTG	Cloning <i>wc2</i> cDNA (1497 bp) for ligation to BD
upRad5-F/R	<u>ACGAGCTGTACAAGTAA</u> CCCGGGT GAGGCGTGAAATCCAGAA / <u>TGGCTGCAGTTCGACGGATCC</u> CGAGCCGACCAATCTCCATAC	Cloning <i>rad5</i> 5'-end (1471bp) for disruption
dnRad5-F/R	<u>GACCCATGGCTCGAGTCTAGA</u> ACCAAGTTTACCAGACAAGCCA / <u>GGTGGTGGTGCTAGCGTTA</u> ACGGACAAGAAAACCTCCATCAC	Cloning <i>rad5</i> 3'-end (1402 bp) for disruption
upRad16A-F/R	<u>ACGAGCTGTACAAGTAA</u> CCCGGG ACAAGTGAGAAAAGGGAACGTC / <u>CGGTACCAAGCTTGGCTG</u> CAGAGTAGCAGAACCCTGAGAAAG	Cloning <i>rad16A</i> 5'-end (1443 bp) for disruption
dnRad16A-F/R	<u>GACCCATGGCTCGAGTCTAGA</u> TGGAGTGTTTCTCGTTTCA / <u>GGTGGTGGTGCTAGCGTTA</u> ACCAACACGGCAAGACAAGAATC	Cloning <i>rad16A</i> 3'-end (1409 bp) for disruption
upRad16B-F/R	<u>AGCTGTACAAGTAA</u> CCCGGG GCTGGCTGTGATAGGTGGAGTA / <u>TGGCTGCAGTTCGACGGATCC</u> GTTTGTAAACGGTGTGTGAGATTG	Cloning <i>rad16B</i> 5'-end (1509 bp) for disruption
dnRad16B-F/R	<u>GACCCATGGCTCGAGTCTAGA</u> CATAACACGAAGCCCAAAAC / <u>GGTGGTGGTGCTAGCGTTA</u> ACTGCTGGCAGGCAAGTATCT	Cloning <i>rad16B</i> 3'-end (1551 bp) for disruption
flRad5-F/R	<u>ATCCGTCGACCTGCAGCCAAGCTTT</u> GAGGCGTGAAATCCAGAAT / <u>ACACTAGTCAGATCTTCTAG</u> AAAGCCCTCGTCAAATACTGC	Cloning full-length <i>rad5</i> (4853 bp) for complementation
flRad16B-F/R	<u>ATCCGTCGACCTGCAGCCAAGCTT</u> TGTGACTGTTTCGCTCTTC / <u>ACACTAGTCAGATCTTCTAG</u> AGTTTGGGCTTCGTGTTATG	Cloning full-length <i>rad16B</i> (4515 bp) for complementation
upRad16A2-F/R	<u>ACAGTACACGAGGACTCTAGA</u> AACAAGTGAGAAAAGGGAACGTC / <u>CTGCCCTGAGAGGAATTC</u> AGTAGCAGAACCCTGAGAAAG	Cloning <i>rad16A</i> 5'-end (1443 bp) for double disruption
dnRad16A2-F/R	<u>CCGTCTCTCCGATGCACTAGT</u> GTTGAGTGTTTCTCTGTTTCA / <u>GGTGGTGGTGCTAGCGTTA</u> ACCAACACGGCAAGACAAGAATC	Cloning <i>rad16A</i> 3'-end (1409 bp) for double disruption
pRad5-F/R	GTTGGTGAGAAGGTGACGAAG / TCCACCAAGGTCCATCAT	PCR detecting <i>rad5</i>
pRad16A-F/R	GTGCCTACACCGTATCAACA / CTTTGTATCGCCATTACCC	PCR detecting <i>rad16A</i>
pRad16B-F/R	TGTGACTGTTTCGCTCTTC / GTTTTGGGCTTCGTGTTATG	PCR detecting <i>rad16B</i>
qRad5-F/R	CCCCGAAAACCAAGGCAAAG / AACGCTTCCGTCAAACCTTGC	qPCR detecting <i>rad5</i>
qRad16A-F/R	TCAACTGACGAACCCGACTG / TCGTGACCAAGAGCTCAAT	qPCR detecting <i>rad16A</i>
qRad16B-F/R	CAAGCGCCATCATTACCACG / GGTGGTGGCGACTTTTGTTT	qPCR detecting <i>rad16B</i>
qActin-F/R	GGCAACATTGTCATGTCTGG / TTTGCTGGAAGGTGGATAGG	qPCR detecting β -actin gene

^a Underlined regions denote DNA fragments to exchange for the corresponding fragments of constructed vectors at the sites (in bold) of restriction enzymes for ligation to AD or BD (*EcoRI/BamHI*), homologous recombination of 5' and 3' DNA fragments of *rad5*, *rad16A* or *rad16B* (*XmaII/BamHI* and *XbaI/HpaI*) in the WT background or 5' and 3' DNA fragments of *rad16A* in the null mutant of *rad16B* (*EcoRI/XbaI* and *SpeI/HpaI*), or complementation of *rad5* or *rad16B* (*HindIII/XbaI*).

^b Tag loci for cloned genes: *rad5* (BBA_03842), *rad16A* (BBA_07794), *rad16B* (BBA_01511), *phr1* (BBA_01664), *phr2* (BBA_01034), *wc1* (BBA_10271), *wc2* (BBA_01403), *rad1* (BBA_07749), *rad10* (BBA_03417), *rad2* (BBA_00274), *rad6* (BBA_01469), *rad18* (BBA_03508), *rad14* (BBA_03454), *rad4A* (BBA_02814), *rad23* (BBA_01030), *mms2* (BBA_08836), *elc1* (BBA_03154), *cul3A* (BBA01191), *cul3B* (BBA_02238).

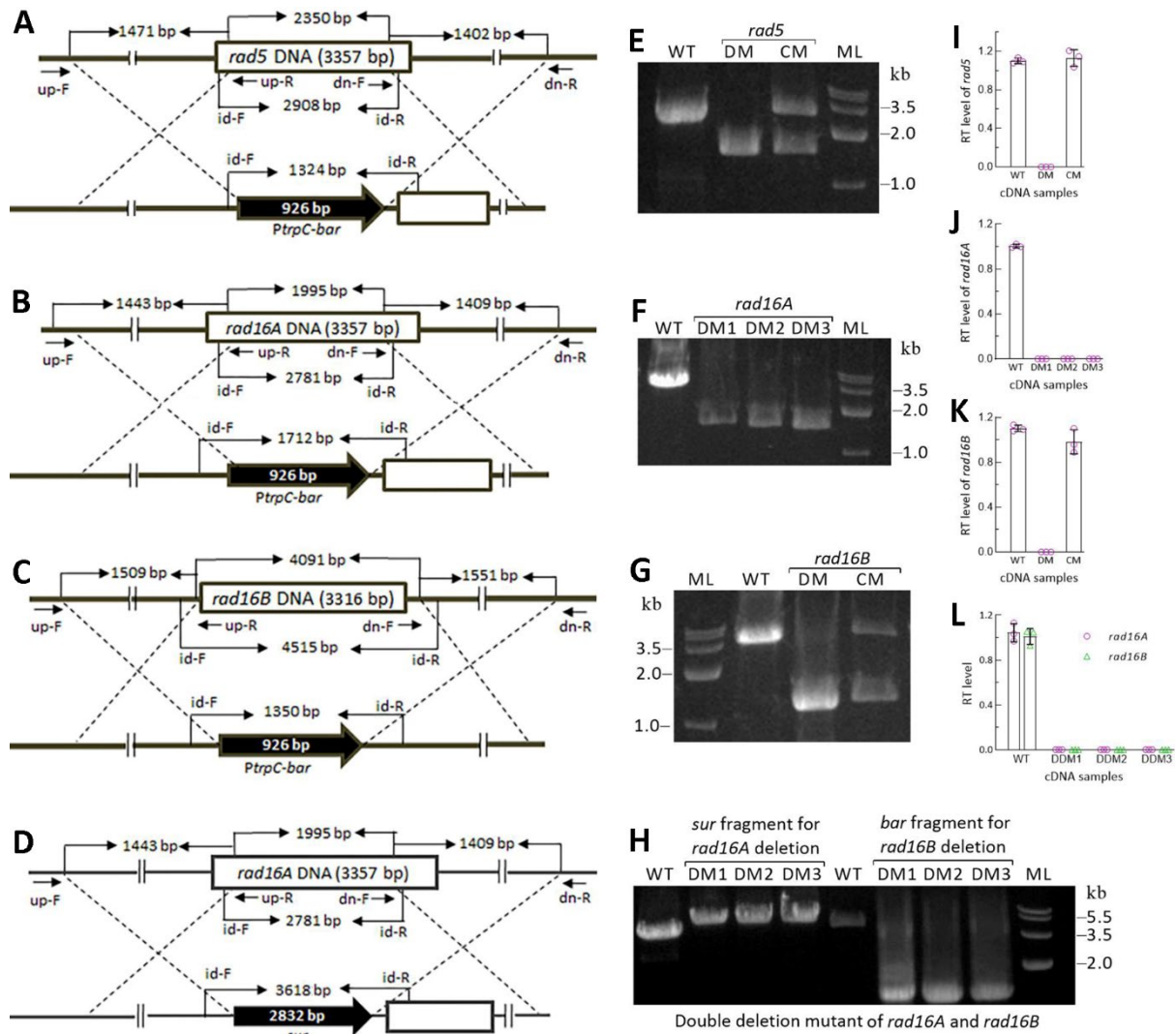


Figure S1. Generation and identification of *rad5*, *rad16A* and *rad16B* mutants in *B. bassiana*. (A–C) Schematic diagrams for the strategies of singular *rad5*, *rad16A* and *rad16B* deletions in the WT background. (D) Schematic diagram for the strategy of *rad16A* deletion in the null mutant of *rad16B*. (E–H) The *rad5*, *rad16A* and *rad16B* mutants identified via PCR analysis with paired primers (Table S1), respectively. The detected DNA fragments indicate a success in deleting the partial or full-length coding and partial flanking regions of each target gene from the WT strain as expected (2908 + 926 – 1324 = 2350 bp for *rad5*, 2781 + 926 – 1712 = 1995 bp for *rad16A* in WT, 4515 + 926 – 1350 = 4091 bp for *rad16B* in WT, and 2781 + 2832 – 3618 = 1995 bp for *rad16A* in the null mutant of *rad16B*). DM, deletion mutant. CM, complementation mutant. ML, molecular ladder of genomic DNA. (I–K) Relative transcript (RT) levels of *rad5*, *rad16A* and *rad16B* in the 3-day-old SDAY cultures of their mutants versus WT, respectively. (L) RT levels of *rad16A* and *rad16B* in the 3-day-old cultures of three double-deletion mutants (DDM1–3) versus WT. Note that the expression of each target gene was not detectable in its DM but well restored in its CM. Error bars: standard deviations of the means from three cDNA samples derived from independent cultures of each strain.

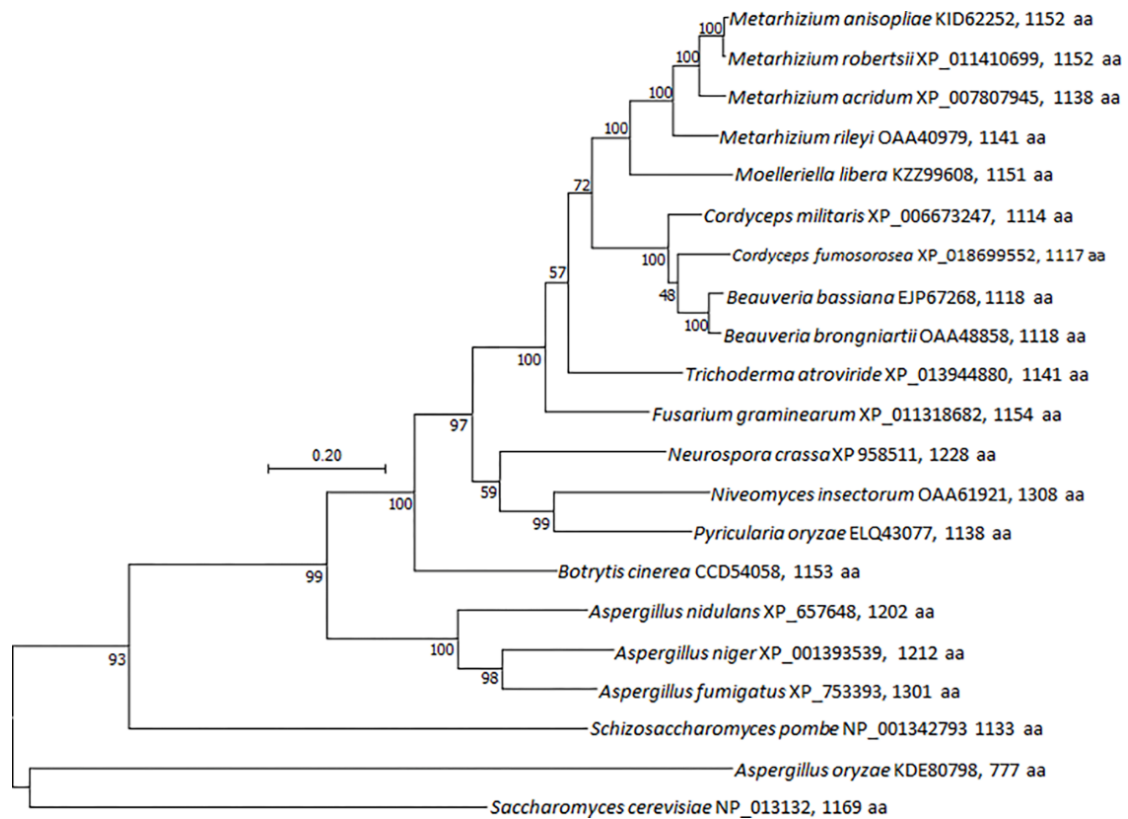


Figure S2. Phylogenetic relationships of Rad5 orthologs found in selected ascomycetes. The maximum likelihood method in the online program MEGA11 at <http://www.megasoftware.net/> was used in the analysis. Bootstrap values of 1000 replications are shown at nodes. Scale bar: branch length proportional to genetic distance. Each fungal name is followed by the NCBI accession code of each protein and the length of its amino acid sequence, respectively.

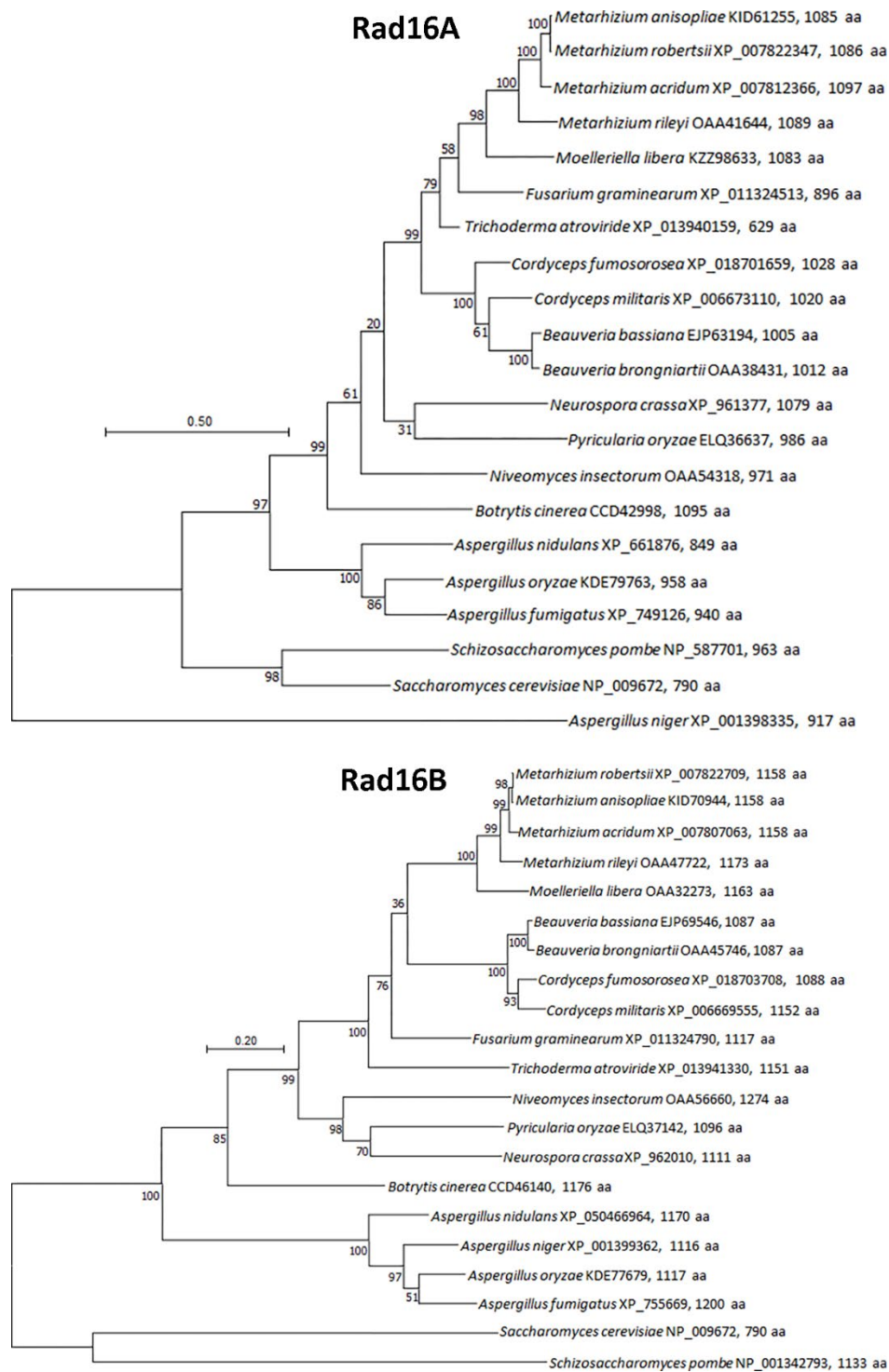


Figure S3. Phylogenetic relationships of Rad16 homologs found in selected ascomycetes. The maximum likelihood method in the online program MEGA11 at <http://www.megasoftware.net/> was used in the analysis. Bootstrap values of 1000 replications are shown at nodes. Scale bar: branch length proportional to genetic distance. Each fungal name is followed by the NCBI accession code of each protein and the length of its amino acid sequence, respectively. Note the existence of two Rad16 paralogs (Rad16A and Rad16B) in all the examined fungi except *S. cerevisiae*.

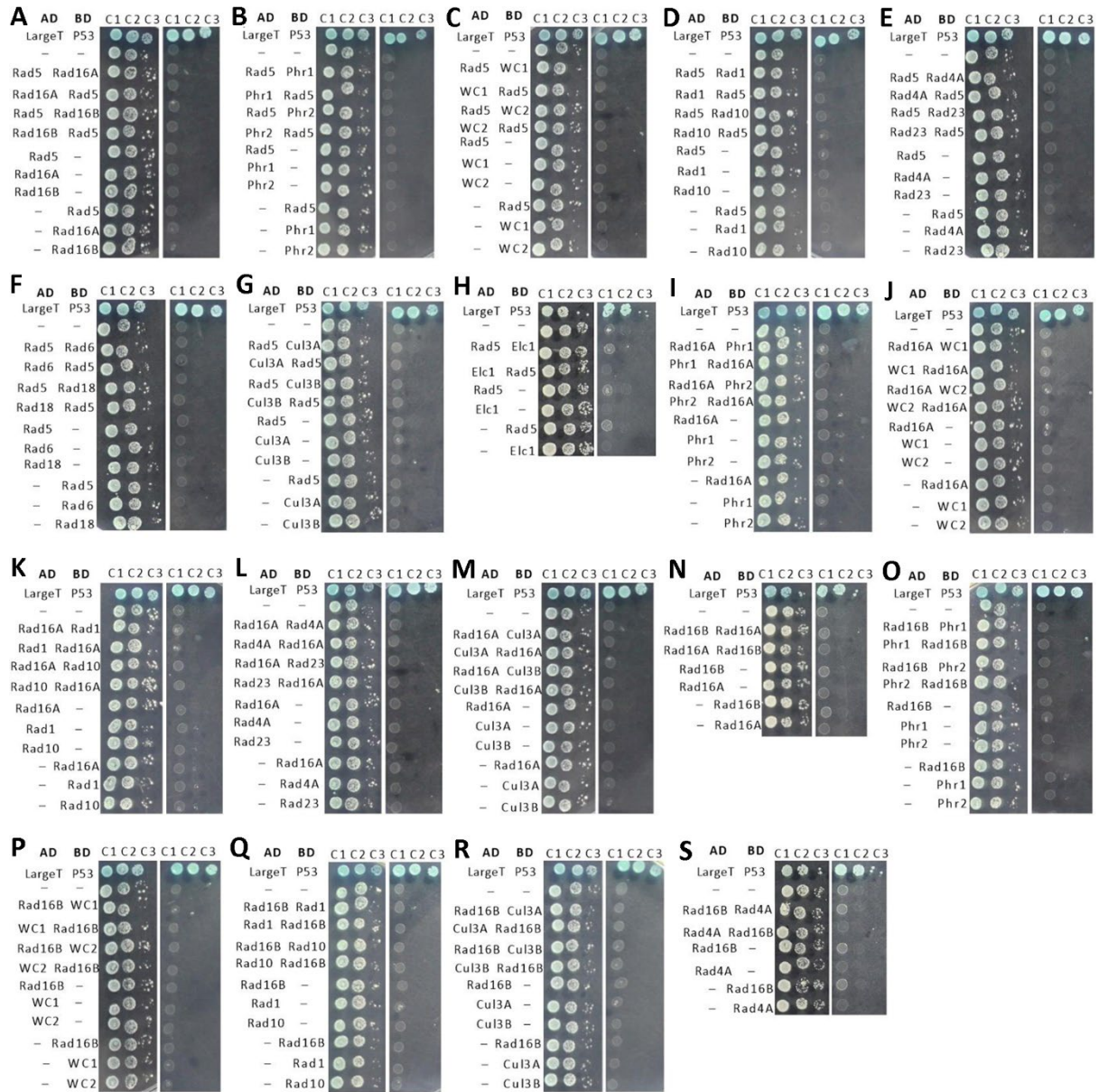


Figure S4. Y2H assays for negative protein-protein interactions in *B. bassiana*. (A–S) No signals for interactions of Rad5, Rad16A or Rad16B with photolyases (Phr1 and Phr2), photolyase regulators (WC1 and WC2), Cul3 paralogs (Cul3A/B) and other anti-UV RAD proteins compared to positive control (AD-LargeT-BD-P53) on quadruple-dropout plates (right image in each panel). All yeast colonies initiated with 5×10^4 (C1), 5×10^3 (C2) and 5×10^2 (C3) cells were incubated at 30°C for 3 days.