

**Supplementary Table S1.** Primers used in this study

Primer	Sequences (5'→3')*	RE
Mutant construction		
1555-UP-F	att <u>ctcgag</u> caggggtgccgaccatt	XhoI
1555-Up-R	gtatgata <u>tcggtggc</u> gaccacgccgg	EcoRV
1555-Dw-F	aaggat <u>cccgaagtcgtg</u> cagcacg	BamHI
1555-Dw-R	tat <u>ctgcag</u> cacgcccccacctg	PstI
1832-Up-F	att <u>ctcgagtggctg</u> accgccttaca	XhoI
1832-Up-R	gtatgata <u>tccttcagaa</u> actgggccacctcg	EcoRV
1832-Dw-F	aaggat <u>ccgacgcctacc</u> acggcg	BamHI
1832-Dw-R	tat <u>ctgcagcgg</u> caact gccggg	PstI
1832-Flag-F	cgggtaagctgtcggcatggactacaaagaccatgacgggtgat	
1832-Flag-R	tggctttgtagtccatgccgacagcttaccgaatgc	
Plasmid construction		
1832-F	taagg <u>gccc</u> atgacccaaggccaagaccaa	Apal
1832-R	gtatgata <u>tc</u> cagccgagctggctgagca	EcoRV
C36S-F	atggtcttgtggctggtgccggccttg	
C36S-R	caaggccggcaccagccacaagacat	
Protein purification		
1832-His-F	gatg <u>cgccg</u> caaccaaggccaagaccaagtgc	NotI
1832-His-R	gatt <u>ctcgaggccgagctgg</u> tcgagcacg	XhoI
2623-His-F	att <u>catatg</u> agtctttatgacgtggcaa tcgtcggagcc	NdeI
2623-His-R	att <u>ctcgagg</u> gccgggcgcagttcgccg	XhoI
0615-His-F	gatt <u>catatgga</u> actgcgacacctgcg	NdeI
0615-His-R	gatt <u>ctcgagcctgggcgg</u> ctcggtgggca	XhoI
1022-His-F	gatt <u>catatg</u> accacccaaccttctcg	NdeI
1022-His-R	tatg <u>tcgac</u> cttcgggcacttcggtgacga	SalI
1262-His-F	gatt <u>catatga</u> agaacttgctccgtgccatc	NdeI
1262-His-R	gatt <u>ctcgaga</u> acctcgccccgcgcaaaag	XhoI

\* Restriction enzyme (RE) recognition sites are underlined in the sequences.

```

      *      20      *      40      *      60      *      80      *      100      *      120      *
DR_2623 : ---MSLYDVAIVGAGPVGIAAAGCKRAGLSYVVLKKGCVVNAI-----FEYPTYMGFFTTAPELEIGNHPFV-TGHDKEIDRRDALMYRLVTQRENINVRQYTTVNRVHAAPAGFTLEIEAQDGTTPG : 119
7A76    : ---MQKETVIIIGGGPCGAAATSLQKVGINPLVIEKGNIVNAI-----YNYPTHQTFFSSSEKLEIGDVAFI-TENRKEVRNQALAYYREVVRKRSVRVNAFERVEKVQKDGEAFQVETTKRDGSKE : 119
7A7B    : ---MQKVESTIIIGGGPCGSAATEQMRKGIDTLIEKGNVVEST-----YNYPTHQTFFSSSDKLSIGDVFFI-VEESKERRNQALVYYREVVKHHQLKVNAFEVLTVKKMNNKFTITTT-----TKD : 114
4ZN0    : GSHMASMYDLIIIGGGFAGLTAGIYAVRYGLDTLILERNISGQISMADIVENYEGFPSI-----SGLEIM-----ERFETHAQEVGVK-TTITEVLSVRSEGTKKIITTT-----DSG : 102
4C5O    : ---MDSVDVVVIGGGQSGISAGYFLRRSGLSYVILDAAEASPGGAWQH---AWH-SLHLFSPAGWSSIPGWMPASQGPYIARAENVIAVLAQYEQKYALPVLRLPIRVQRVSHFGERLRVV--AR--DGR : 117

      140      *      160      *      180      *      200      *      220      *      240      *      260
DR_2623 : VVEARRVVVATGYNDNPLSMGITBGEDSE---NV-SHYYTEAHPEM-GLNVTVIGAGNSAADAALDLWRSGVNVIMVVAPELK-----STIKYWVR-----PDLENRIKEGSIHAHNSRVVE : 227
7A76    : IYIAKYIVVATGYNDPNYMNVPGEELK---KV-AHYEKEGHPYE-DRDVVIGGKNSSDAALDLVKSGARVTVLYRGIEYS-----PSIKPWIL-----PEFEALVRNGTIQMHFGAHVKE : 227
7A7B    : VYECRSLTIATGYMGQHNTEVEGADLP---KV-FHYEKEAHPYE-DQDVVIGGKNSAIDAALDLKAGANVTVLYRGGDYS-----PSIKPWIL-----PNFTALVNHEKIDMEFNANVTQ : 222
4ZN0    : DLEAKAVIIATGA--NPKHIGVPEKELISKGV-SYCAICDGPFEERNKIIVAVGGNSAVTDAFLSKVAQKYLVHVRDHLK-----AARVLQDR-----VDGTPNIEILILNSHVLE : 207
4C5O    : QWLRARVISATGTIGEAYTPEYQGLESFAGIQLHSAHYSTPAPFA-GMRVAIIGGNSGAQILAEVSTVAETTWLITRTEPALADDVDGRVLFERATERWKAQQEGREPDLPF-GGFGDIDVM--VPPVLD : 243

      *      280      *      300      *      320      *      340      *      360      *      380
DR_2623 : HPEH-----VVVQGEDGRTFELPTDFTFALTGMRPFLSFLDGLNLTATQPDE---CLVLTENYESVPGLEFVVGSAGFAGKTNQV---FIENGREHADHAVAETIERQLRSGELRPA-- : 332
7A76    : ITE-H-----TLTFTVDGEALTIKNDVFTAMTGXHPDHSFLTKMGVQIDEE--TGRPFYTEDRMETNAENIFLAGVIAAGNNANEI---FIENGREHGDALIACTIASRE---K----- : 326
7A7B    : ITE-D-----TVTYEVNGESKTIHNDYVFAMIGYHPDYEFLLKSVGQINTNEFGTAPMYNKETYETNIENCYIAGVIAAGNDANTII---FIENGKEHGGIIAQSMLAKK---QTPLES : 328
4ZN0    : VVGTRE-EGIKKVEKIIEDVNSRETRELSTNGVEIYVGIHPNTEFVDVEK---DE---GGFIKTDRWMETSEKGYAAGDCRDTPIWQLV---TAVRDCAIAATAAYEYI-EKIR----- : 311
4C5O    : ARARGVLAAPPPPARFSPTGMQWADGTERAFLAVIWCTGREFALSHLKGDLVTPTQGVQEV---DGSGLRALAVPSVWLLGYG---DWNMGMASATLIGVTRVAREAVRQVTAYCA-DHQDR--- : 357

```

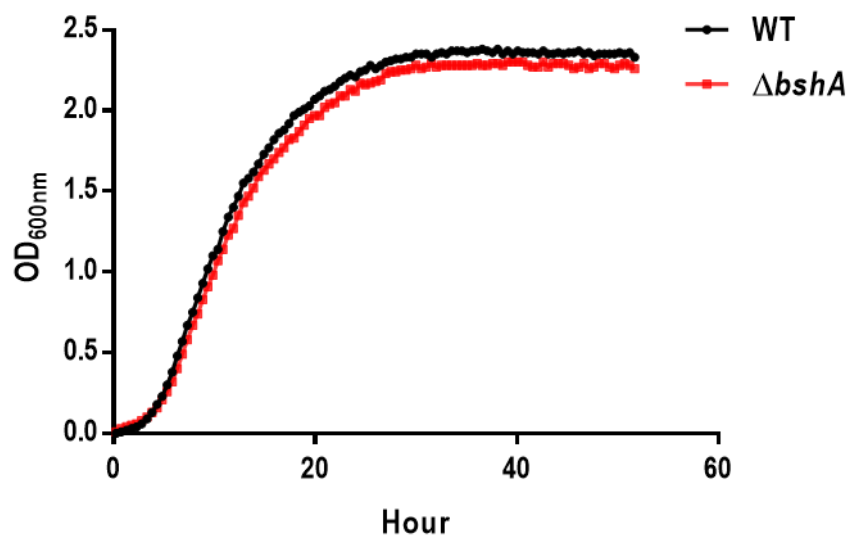
**Supplementary Figure S1.** Multiple alignment of amino acid sequences of *D. radiodurans* Bdr (DR\_2623) and its homologs. The conserved GXGXXG motifs are indicated by red line. The program Genedoc ([www.psc.edu/biomed/genedoc](http://www.psc.edu/biomed/genedoc)) was used to visualize the alignment in quantify mode, which highlights residues most-frequently found in each column of the alignment. Black letters on light gray shading represent 60% identity. White letters on gray and black shading represent 80% and 100% identities, respectively. Protein sequences obtained from the Protein Data Bank (PDB) are distinguished by the PDBid, 7A76; *Bacillus cereus*, 7A7B; *Staphylococcus aureus*, 4ZN0; *Metanosarcina maezi*, 4C5O; *Stenotrophomonas maltophilia*.

```

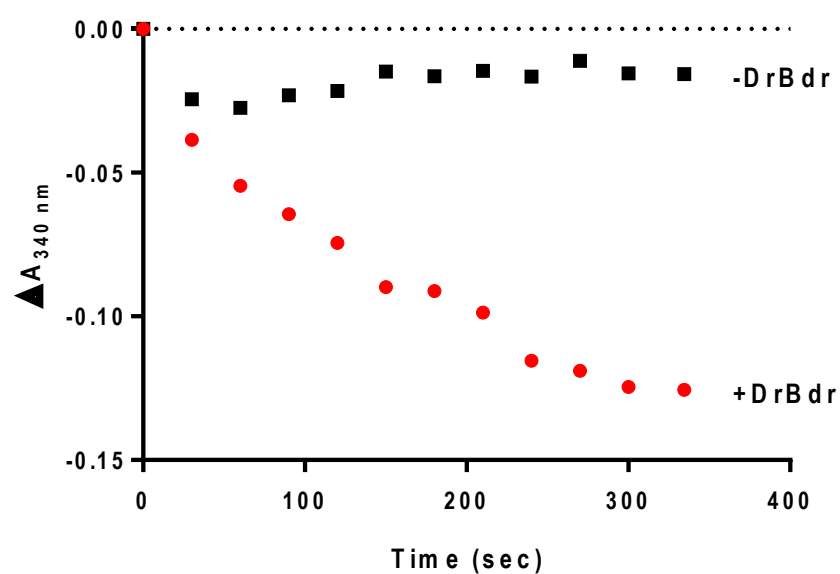
*          20          *          40          *          60          *
WP_010888467.1 : -----MTQGQDQVLSELTTPEDVFCFLKDYPLAAVFKAGTCHKTMQGGFGVLETFLORYELE
WP_011530593.1 : -----MTQNATQQEQLVPLTTPEDVFRFLQAYPLAAIFKAGTCHKTMQGGFGVVFSLQRIELE
WP_013556600.1 : -----MSEQLVPLTTPPEEVTFLAEHPLAAVFKAGTCHKTMQGGFGVLETFIKGHELE
WP_013614326.1 : -----MTQATQAEQLVPLTTPPEEVQTFLAEPPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_014684431.1 : -----MTQT-ASQDPQLVPLTTPPEEVQFLQDYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_015236909.1 : -----MTQLVPLTTPPEEVTFLAQFPPLAAIFKAGTCHKTMQGGFGVLETFYLAHELE
WP_019009878.1 : MTTFNA-----QTEAQQLVPLTTPEDVFCFLKEHPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_019588304.1 : -----MTQSVTDHKQLVPLTTPPEEVSFLAEYPLAAVFKAGTCHKTMQGGFGVLETFLOKHELE
WP_025567523.1 : -----MTQGQDQVLQLTTPPEEVBQFLKDHPLAAVFKAGTCHKTMQGGFGVLETFLORYELE
WP_027460378.1 : -----MTANATEQTVLPLTTPPEEVRFLQDYPLAAIFKAGTCHKTMQGGFGVVFSLQRIELE
WP_029478327.1 : MTQT---AKADTQASTQPEKQLVPLTTPPEEVQFLTDHPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_029483394.1 : -----MTQTADAERQLVPLTTPPEEVQFLQDYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_034338428.1 : -----MQLVPLTTPPEEVAFLTENPLSAVFKAGTCHKTMQGGFVVVEQFLKHHLELE
WP_034355856.1 : -----M-TQTSGPSPTAAEQQLVPLTTPPEEVTFLVEYPLAAVFKAGTCHKTMQGGFGVLETFLOKHELE
WP_039684670.1 : MT-----QSAPSESQAERQLVPLTTPPEEVBQFLTDYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_041227187.1 : -----MTQATQAENQQLVPLTTPPEEVBQFLTEYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_058976175.1 : -----MTQA-AQNEAQLVPLTTPPEEVBQFLTEYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_062157723.1 : -----MTQA-AQNEAQLVPLTTPPEEVBQFLSEYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_064014827.1 : MTTDPQQA-QQADAQAPEQLVPLTTPEDVFCFLKEHPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_075835341.1 : -----MTQTAEERQLVPLTTPPEEVBQFLTDYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_084048689.1 : MTQNAS-TSQTSAQAQAEQLVPLTTPPEEVAFLKEYPPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_088247843.1 : -----MTQT-AQNEFQLVPLTTPPEEVBQFLTEYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_092263697.1 : -----MTQNQASEQQLVPLTTPPEEVBQFLRDYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_102126180.1 : -----MTQTTTQPDQQLVPLTTPPEEVTFLREYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_103127591.1 : -----MTQSVTDHKQLVPLTTPPEEVAFLQEPPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_103309877.1 : MTQTAQTAAQADA-ASAEAQLVPLTTPEDVFAFLKEYPPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_109827323.1 : -----MTQTSQAQQLVPLTTPPEEVTFLAQYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_110885278.1 : -----MAQLPLTTPPEEVSFLTEHPTSAIFKAGTCHKTMQGGFVSLETFIKRYELE
WP_126351343.1 : -----MTQANANQTGEQLVPLTTPPEEVQTFLAQYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_129118708.1 : -----MTQNATEQQLVPLTTPEDVFSFLKEYPPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_139401242.1 : MT-----QSAPSESQAERQLVPLTTPPEEVBQFLTDYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_143720486.1 : -----MTQTDQTAAQQLVPLTTPPEEVTFLAQYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_160976666.1 : -----MTQA-AQNEAQLVPLTTPPEEVBQFLTEYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_161882651.1 : -----MTQTDSTAQQLVPLTTPPEEVTFLAQYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_183985038.1 : -----MQLVPLTTPPEEVTFLQFPPLGAVFKAGTCHKTMQGGFVSLETFLORIELE
WP_184025978.1 : -MTHST-PSQPTPPQAEPEQLVPLTTPEDVFSFLTAYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_184108763.1 : MT-----QTHDAQNDASTPAQLVPLTTPEDVFTFLKEYPPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_184127908.1 : MT-----QSAPSESQAERQLVPLTTPPEEVBQFLTDYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_188902460.1 : -----MTQTAQTETQAERQLVPLTTPPEEVBQFLTEYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_188963977.1 : -----MTFVLVPLTTPEDVETFLAENPLSGVFKAGTCHKTMQGGFGVLETFLOKHELE
WP_188967871.1 : -----MTQTAQAERQLVPLTTPPEEVBQFLKDYPLAAVFKAGTCHKTMQGGFGVLETFLOHELE
WP_188998928.1 : -----MQLVPLTTPPEEVAFLSEHPVSAIFKAGTCHKTMQGGFVVVEQFLKQRDLE
WP_189004503.1 : -----MTQATPAENQQLVPLTTPPEEVBQFLTEYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_189055013.1 : -----MTQA-AQNEAQLVPLTTPPEEVBQFLTEYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_189067741.1 : -----MTQA-AQNEAQLVPLTTPPEEVBQFLTEYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_189071423.1 : -----MTQA-AQTEAQLVPLTTPPEEVBQFLTEYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_189089339.1 : -----MTHVLVPLTTPEDVETFLAEHPTAGVFKAGTCHKTMQGGFGVLETFLOHELE
WP_189643621.1 : -----MTQATQAEQLVPLTTPPEEVQTFLAEPPLAAVFKAGTCHKTMQGGFGVLETFLORIELE
WP_191241542.1 : -----MT-QTAQNEQLVPLTTPEDVFTFLREYPLAAVFKAGTCHKTMQGGFGVLETFLORIELE

```

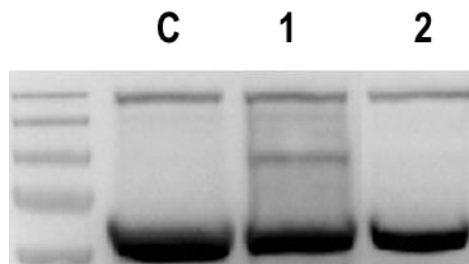
**Supplementary Figure S2.** Multiple alignment of amino acid sequences of deinococcal AbxC homologs. The TCHKT and its neighboring residues of 49 AbxC homologs are aligned, and the conserved TCHKT motifs are indicated by red line. Protein sequences obtained from the NCBI database are distinguished by the WP protein accession number. Black letters on light gray shading represent 60% identity. White letters on gray and black shading represent 80% and 100% identities, respectively.



**Supplementary Figure S3.** Growth curve of *bshA* mutant. The wild-type (WT) and *bshA* mutant ( $\Delta bshA$ ) cells grown to the stationary phase in the TGY medium were inoculated at 1:100 into a fresh TGY medium, samples were taken regularly during the incubation from cultures of cells. The optical densities were monitored at 600 nm. The results are representative of two independent experiments.



**Supplementary Figure S4.** NADPH consumption by the AbxC/BSH/DrBdr pathway in response to  $\text{H}_2\text{O}_2$ . The initial reaction mixtures contained 500  $\mu\text{M}$  BSH, 500  $\mu\text{M}$  NADPH, and 10  $\mu\text{M}$  of AbxC. The NADPH consumption was monitored at 340 nm in the absence of DrBdr (– DrBdr) or in the presence of 0.5  $\mu\text{M}$  of DrBdr (+ DrBdr) immediately after addition of 10 mM  $\text{H}_2\text{O}_2$ . The experiments were repeated twice and representative data are shown.



**Supplementary Figure S5.** Western blotting of *D. radiodurans* cells with FLAG-tagged AbxC. Soluble proteins were extracted from  $\Delta abxC$  (C, control),  $\Delta abxC$  expressing FLAG-tagged AbxC (lane 1), and  $\Delta abxC$  expressing FLAG-tagged AbxC following 10 mM DTT treatment (lane 2). Five microgram protein samples were separated on non-reducing SDS-PAGE and blotted onto nitrocellulose membrane. AbxC-interacting proteins were detected using the anti-FLAG antibodies.