

# Supporting Information

**A *tamB* homolog is involved in maintenance of cell envelope integrity and stress  
resistance of *Deinococcus radiodurans***

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**Table S1. Plasmids and strains used in the experiments.**

<b>Plasmids</b>	<b>Description</b>	<b>Source</b>
pEASY-Blunt	<i>E. coli</i> cloning plasmid	Transgen (China)
CBP-HAT-GST	GST containing plasmid	Lab stock
pRADK	<i>E. coli</i> – <i>D. radiodurans</i> shuttle vector	Lab stock
pMD18-T Vector	Streptomycin resistant gene containing plasmid	Lab stock
<b>Strains</b>	<b>Description</b>	<b>Source</b>
<i>E. coli</i> DH5 $\alpha$	<i>E. coli</i> cloning strain	Transgen (China)
<i>E. coli</i> Trans T1	<i>E. coli</i> cloning strain	Transgen (China)
<i>D. radiodurans</i> R1	<i>Deinococcus radiodurans</i> wild-type, ATCC 13939	Lab stock

**Table S2. Primers used in the experiments.**

<b>Re-sequencing primers</b>	<b>Sequence</b>
P1-F	GTTGTGACCTGCGGCGTG
P1-R	GCGCCGCGCTCGG
P2-F	CGCCCGGCTCAACCTG
P2-R	GAGCACGGTCACGCCG
P3-F	GGAGTTGCGGGCGACC
P3-R	CGTCGGAGGTCAGATGCAGG
P4-F	ACCTGCATCTGACCTCCGAC
P4-R	GCGGTGGCTCGCACG
P5-F	CGGGCGCCACGCTG
P5-R	CGCTCAGCTCGTGCCC
P6-F	GGCACCTGACCGGCG
P6-R	CGCTGAGGGTGCCCC
P7-F	GGCACTCAGAGCGGGGC
P7-R	GTCCCGCTCGCCCCG
P8-F	GGCCAGCCTGACCCGG
P8-R	TCACGTTTTTCGGCGCG
P9-F	GCTGCAACTCATTCCGTCG
P9-R	GTGGAATAGGTCGCGTCGA
P10-F	GGCACCGACGGCAACG
P10-R	ATGAACTCGTTCCAGGTGCC
<b>Gene knockout primers</b>	<b>Sequence</b>
$\Delta$ DR_146T-P1	GTTGTGACCTGCGGCGTG
$\Delta$ DR_146T-P2	TATGGATCCGAGTCCCGCCCCGGTG
$\Delta$ DR_146T-P3	TATAAGCTTGGCCAACCTCCCCGGC
$\Delta$ DR_146T-P4	GCACGATGAACTCGTTCCAGG
$\Delta$ DR_146T-P5	CGCTCTACCAGAACGAACCG
$\Delta$ DR_146T-P6	CTCTGAGCCGGAATGACCAC
$\Delta$ DUF490-P1	GCGCCGGGGCGAC
$\Delta$ DUF490-P2	TATGGATCCTCACCCGGATGCGCTC
$\Delta$ DUF490-P3	TATAAGCTTGGCCAACCTCCCCGGC
$\Delta$ DUF490-P4	GCACGATGAACTCGTTCCAGG
$\Delta$ DUF490-P5	CGCTGCCCCGAGGAATACA
$\Delta$ DUF490-P6	CAAGGTTGCTGCCGAGGTT
$\Delta$ TamB-DUF490-P1	GACCTGCGGGCGGTCAT
$\Delta$ TamB-DUF490-P2	TATGGATCCTCAGGCCGCCTGCCG
$\Delta$ TamB-DUF490-P3	TATAAGCTTGTGGTCACCGGGCCG
$\Delta$ TamB-DUF490-P4	GAGCGTCCAGCCTTGCTC
$\Delta$ TamB-DUF490-P5	GCCGGACATCACCTGGAAG
$\Delta$ TamB-DUF490-P6	CGGTTGCCGTGACTTTGG
$\Delta$ DR_2577-P1	AGTCTGATCGCTCTTACCACGG
$\Delta$ DR_2577-P2	CGGGATCCTTGACGGTTTCGACGCGA
$\Delta$ DR_2577-P3	CCAAGCTTCAACGCCCTGGTCAAGGA

$\Delta$ DR_2577-P4	ACCTTGTAGCTGATCTTGAAGGTCT
$\Delta$ DR_2577-P5	CCGTTATCGTGACCTTGGG
$\Delta$ DR_2577-P6	CGTTATTGGCGTCGGTGTAG
<b>Tags labeled primers</b>	<b>Sequence</b>
DR_2577-His-P1	ACGACGGGCGATTTTCAG
DR_2577-His-P2	ATAGGATCCTTAGTGATGATGATGATGATGGAA GTTGACCTTGTAGCTGATCTTG
DR_2577-His-P3	TATAAGCTTAGCCCCTAGTTCCCGGC
DR_2577-His-P4	CGGGCTGCGGAAGTGC

**Table S3. Homologous proteins of DR\_146T in some sequenced *Deinococcus* bacteria**

<b>Species and gene number</b>	<b>Annotation</b>	<b>Length (aa)</b>
<i>D. gobiensis</i> : DGo_CA2115	Hypothetical protein	3354
<i>D. actinosclerus</i> : AUC44_09250	Hypothetical protein	3273
<i>D. soli</i> : SY84_03150	Hypothetical protein	3256
<i>D. puniceus</i> : SU48_10085	Hypothetical protein	3650
<i>D. geothermalis</i> : Dgeo_0508	Protein of unknown function DUF490	3243
<i>D. swuensis</i> : QR90_10050	Hypothetical protein	3272
<i>D. deserti</i> : Deide_16100	Conserved hypothetical protein, precursor	3511
<i>D. proteolyticus</i> : Deipr_1576	Protein of unknown function DUF490	3676
<i>D. maricopenensis</i> : Deima_1996	Protein of unknown function DUF490	3180
<i>D. peraridilitoris</i> : Deipe_3733	Hypothetical protein	3146

Sequence information of the homologs were obtained by BLAST searching in the NCBI database (<http://www.ncbi.nlm.nih.gov>) using DR\_146T as the query gene. The annotated homologs in the database that showed more than 25% identity (protein sequence) are listed.

**Table S4. Proteins identified in the cell envelope of *D. radiodurans* wild type by MS**

<b>Gene Number</b>	<b>Protein Description</b>	<b>Relative Abundance Index<sup>a</sup></b>
<b>DR_2577</b>	<b>S-layer protein</b>	++++
<b>DR_0383</b>	<b>S-layer-like array-like protein</b>	+++
<b>DR_1185</b>	<b>S-layer-like array-like protein</b>	+++
<b>DR_1115</b>	<b>S-layer-like array-like protein</b>	+++
<b>DR_2508</b>	<b>hexagonally packed intermediate-layer surface protein</b>	+++
<b>DR_1124</b>	<b>SLH family protein</b>	+
<b>DR_0379</b>	<b>outer membrane protein</b>	+++
DR_0989	cationic outer membrane protein OmpH	+
<b>DR_0630</b>	<b>cell division protein FtsA</b>	+
<b>DR_0631</b>	<b>cell division protein FtsZ</b>	+
<b>DR_0774</b>	<b>general secretion pathway protein D</b>	+++
<b>DR_1460</b>	<b>hypothetical protein</b>	+
<b>DR_1461</b>	<b>hypothetical protein</b>	+
<b>DR_1462</b>	<b>hypothetical protein</b>	+
DR_0986	extracellular solute-binding protein	+
DR_1290	extracellular solute-binding protein	+
DR_1712	extracellular solute-binding protein	+
DR_1955	extracellular solute-binding protein	+
DR_A0246	extracellular solute-binding protein	+
DR_0561	maltose ABC transporter periplasmic maltose-binding protein	++
DR_0564	amino acid ABC transporter periplasmic amino acid-binding protein	++
DR_0788	branched-chain amino acid ABC transporter periplasmic amino acid-binding protein	++
DR_1038	branched-chain amino acid ABC transporter periplasmic amino acid-binding protein	++
DR_B0014	hemin ABC transporter, periplasmic hemin-binding protein	++
DR_B0125	iron ABC transporter substrate-binding protein	++
DR_0363	peptide ABC transporter periplasmic peptide-binding protein	+
DR_0365	peptide ABC transporter permease	+
DR_1571	peptide ABC transporter periplasmic peptide-binding protein	+
DR_0959	peptide ABC transporter permease	+
DR_1027	amino acid ABC transporter, periplasmic amino acid-binding protein	+

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DR_1277	ABC transporter periplasmic substrate-binding protein	+
DR_2154	amino acid ABC transporter periplasmic amino acid-binding protein	+
DR_2278	amino acid ABC transporter periplasmic amino acid-binding protein	+
DR_2588	iron ABC transporter periplasmic substrate-binding protein	+
DR_A0210	peptide ABC transporter, periplasmic peptide-binding protein	+
DR_A0263	branched-chain amino acid ABC transporter periplasmic amino acid-binding protein	+
DR_0695	v-type ATP synthase subunit I	++
DR_0700	v-type ATP synthase subunit A	++
DR_0697	v-type ATP synthase subunit E	++
DR_0699	v-type ATP synthase subunit F	++
DR_0701	v-type ATP synthase subunit B	+
DR_0698	v-type ATP synthase subunit C	+
DR_1536	serine protease	++
DR_1459	serine protease	+
DR_2518	serine/threonine protein kinase	+
DR_0300	serine protease Do, putative	+
DR_0327	periplasmic serine protease	+
DR_1756	periplasmic serine protease Do	+
DR_1769	serine/threonine protein kinase-like protein	+
DR_1937	serine protease	+
DR_2325	serine protease	+
DR_A0064	serine protease	+
DR_0025	HSP20 family protein	+
DR_0116	hypothetical protein	++
DR_0505	5'-nucleotidase family protein	+++
DR_0560	hypothetical protein	++++
DR_0581	hypothetical protein	+++
DR_0769	hypothetical protein	+++
DR_0960	hypothetical protein	+++
DR_0969	hypothetical protein	+++
DR_0972	hypothetical protein	+++
DR_1021	hypothetical protein	+++
DR_1114	hypothetical protein	+++
DR_1140	hypothetical protein	+++
DR_1180	hypothetical protein	++
DR_1202	hypothetical protein	++
DR_1306	hypothetical protein	++
DR_1370	hypothetical protein	++
DR_1388	hypothetical protein	++

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DR_1407	hypothetical protein	++
DR_1484	hypothetical protein	++
DR_1714	hypothetical protein	++
DR_1737	signal peptidase I	+
DR_1768	hypothetical protein	++
DR_1923	hypothetical protein	++
DR_1964	general secretion pathway protein E	+
DR_2070	membrane lipoprotein	++
DR_2317	potassium channel subunit beta	+
DR_2620	cytochrome c oxidase subunit I	+
DR_A0069	cleavage and polyadenylation specificity factor-like protein	+
DR_A0241	beta lactamase-like protein	+
DR_A0302	hypothetical protein	++
DR_B0037	hypothetical protein	++
DR_B0041	hypothetical protein	++
DR_B0092	Dps family DNA-binding stress response protein	+

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a, Relative protein abundance was indicated by the index value ( $i$  = unweighted spectrum count of each protein/mass). +,  $i \leq 0.20$ ; ++,  $0.20 < i \leq 0.40$ ; +++,  $0.40 < i \leq 0.60$ ; +++++,  $0.60 < i \leq 0.80$ ; ++++++,  $i > 0.80$ .



**Table S5. Proteins identified in the SDS-PAGE gel containing SlpA with a molecular weight of 123 kDa by MS<sup>a</sup>**

<b>Gene Number</b>	<b>Protein Description</b>	<b>Relative Abundance Index<sup>b</sup></b>
<b>DR_2577</b>	<b>S-layer protein SlpA</b>	+++
DR_2508	hexagonally packed intermediate-layer surface protein	++
DR_B0037	hypothetical protein	+
DR_0363	peptide ABC transporter periplasmic peptide-binding protein	+
DR_1124	SLH family protein	+
DR_1185	S-layer-like array-like protein	+
DR_0116	hypothetical protein	+

a, The protein band containing SlpA with a molecular weight of 123 kDa in the wild type from the SDS-PAGE experiment (Fig. 7c) was subjected to MS analysis. b, Relative protein abundance was indicated by the index value ( $i$  = unweighted spectrum count of each protein/mass). +,  $i \leq 0.20$ ; ++,  $0.20 < i \leq 0.40$ ; +++,  $0.40 < i \leq 0.60$ ; +++++,  $0.60 < i \leq 0.80$ ; ++++++,  $i > 0.80$ .

**Figure S1. DNA sequence comparison of the re-sequenced DR\_146T loci with NCBI data.** Five gaps and four base errors in the NCBI gene loci were shown (red box). The gaps led to invalid start and/or invalid stop codons (black dashed box) in the predicted DR\_1462, DR\_1461 and DR\_1460 sequences. The start and stop codons of DR\_146T were marked with black solid line box.

		DR_146T start codon →	
Query	1	<span style="border: 1px solid black; padding: 0 2px;">GTG</span> AGGGCCAAAGCGgcgccccgcgccccccccggcgccgcccgcgcccggcTGGCCC	60
Sbjct	1	GTGAGGGCCAAAGCGGCGCCCGCGCCCGCCCGGCGCCGC-C-G-GCGCCGCTGGCCC	58
Query	61	TGGTGGGTGCTGGGCGGGCGCTGCTGCTCGGCGCGGTGGCTACGCCCCGCAACTGTTC	120
Sbjct	59	TGGTGGCTGTGGGCGGGCGCTGCTGCTCGGCGCGGTGGCTACGCCCCGCAACTGTTC	118
		DR_1462 start codon →	
Query	4201	GACCTCAGCGCCTGCGCCTGAACGTGAGCGGCCCTACCTCAGTGGCGGGCAGCGGC	4260
Sbjct	4199	GACCTCAGCGCCTGCGCCTGAACGTGAGCGGCCCTACCTCAGTGGCGGGCAGCGGC	4258
		DR_1461 start codon →	
Query	4321	<span style="border: 1px solid black; padding: 0 2px;">GCC</span> CGCGTGGTCATTCCGGCTCAGAGCTTCCCGCTGCGGGTGCGCCCGGCCACCGCGAGC	4380
Sbjct	4319	-CCCGCTGGTCATTCCGGCTCAGAGCTTCCCGCTGCGGGTGCGCCCGGCCACCGCGAGC	4377
Query	4381	CTCAGGCGAGGCGGCTGACTACGCGAGCGGGCGCTGGTCGGGCGGGTTGGACGCGGCC	4440
Sbjct	4378	CTCAGGCGAGGCGGCTGACTACGCGAGCGGGCGCTGGTCGGGCGGGTTGGACGCGGCC	4437
		DR_1462 stop codon	
Query	4681	CGCCTCGAACGGACCCTTTATCAGAACGAACCGCTCAACCTACCGCGGCCGTGGACTGG	4740
Sbjct	4678	CGCCTCGAACGGACCCTTTATCAGAACGAACCGCTCAACCTACCGCGG-CGTGGACTGG	4736
Query	5881	CAGCGGGCGAACGGACAGGCGCAGGTGGACCTACGGCGCAGGCCAGCGCGCGCAGGGC	5940
Sbjct	5877	CAGCGGGCGAACGGACAGGCGCAGGTGGACCTACGGCGCAKGGCCAGCGCGCGCAGGGC	5936
Query	7201	GCGGCGGTGCGAACTTTCCGGCGACGTGCAGGCGAGCTACGGTCCCGGGAACGCCGAGCTG	7260
Sbjct	7197	GCGGCGGTGCGAACTTTCCGGCGACGTGCAGGCGAGCTACGGTCCCGGGAACGCCGAGCTG	7256
Query	7981	GCGCTGACGGTGAAGACGGCTTCCCTTCCCGACCCCTCACCTGCGCGGCGACGGAGAg	8040
Sbjct	7977	GCGCTGACGGTGAAKACGGCTTCCCTTCCCGACCCCTCACCTGCGCGGCGACGGAGAG	8036
Query	8041	ggggggTATGCCCTCAGCGCGGGCAGCTCGGTACGGGGAACTCACCTCACGCCGGG	8100
Sbjct	8037	GGGGGTATGCCCTCAGCGCGGGCAKCTCGGTACGGGGAACTCACCTCACGCCGGG	8096
Query	9901	GTCTTCGGGCAGGGCACCITGCAGGCGTCCGCTCGGCGCGGTGCTCGCCGCCGTCGCG	9960
Sbjct	9897	GTCTTCGGGCAGGGCA-CITGCAGGCGTCCGCTCGGCGCGGTGCTCGCCGCCGTCGCG	9955
Query	9961	GGTCCACGCCGGGCGAGGGCGTGGTACCAGGCGGTTGTTTTCCGTTTTCCCCCTCGCC	10020
Sbjct	9956	GGTCCACGCCGGGCGAGGGCGTGGTACCAGGCGGTTGTTTTCCGTTTTCCCCCTCGCC	10015
		DR_1460 start codon →	
Query	10021	GACCCGCTGGCCGGGCGCGCCTCGGTGGTCGCCGAGCGCATCCGGGTGAGCGCGCGCCG	10080
Sbjct	10016	GACCCGCTGGCCGGGCGCGCCTCGGTGGTCGCCGAGCGCATCCGGGTGAGCGCGCGCCG	10075
		DR_1461 stop codon	
Query	12001	GGCCGC <span style="border: 1px solid black; padding: 0 2px;">TGA</span> 12009 DR_146T stop codon	
Sbjct	11996	GGCCGC <span style="border: 1px dashed black; padding: 0 2px;">TGA</span> 12004 DR_1460 stop codon	

**Figure S2. Predicted protein sequence encoded by DR\_146T compared with the predicted protein sequences of DR\_1462, DR\_1461 and DR\_1460 from NCBI database. Point mutation sites were indicated by red rectangle in the sequences.**

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Re-seq 1 MRAKAAPRARPRRRRAPRWPWW 23 V 1440 PARVVIPAQSFPLR-----VRPATASLTA- 1463
DR_1462 ----- 1 M 1418 P-----PAWSFRLRASRCGCARPPRASRQAA 1443
      A A G G L G V P

Re-seq 1464 GGLTYASGRWSGGLDAAYALWTGELKRAGRVRIVGDGKVLAAARPSGPLNGRVGLLPTLSGEVSTDVAPLLPT 1535
DR_1461 1 MRRRRAICVPCAAPCACARKHWGAAPPRGHSGSELPAAAGAPGHREPHGRRPDLRERALVGRVGRGLRPVDRR 72
      A A R R

Re-seq 1536 LPGDLRAALRPGQLFAKVTATGATLRLERTLYQNEPLNLTA ----- 1577
DR_1461 73 AETGGAGPHCGRRQGARGAALWSPERAGRPPSYSQRRSFDGRGAAAADLAGRPTRGFASRSTVVRQGHSHGRH 145
      A A R R

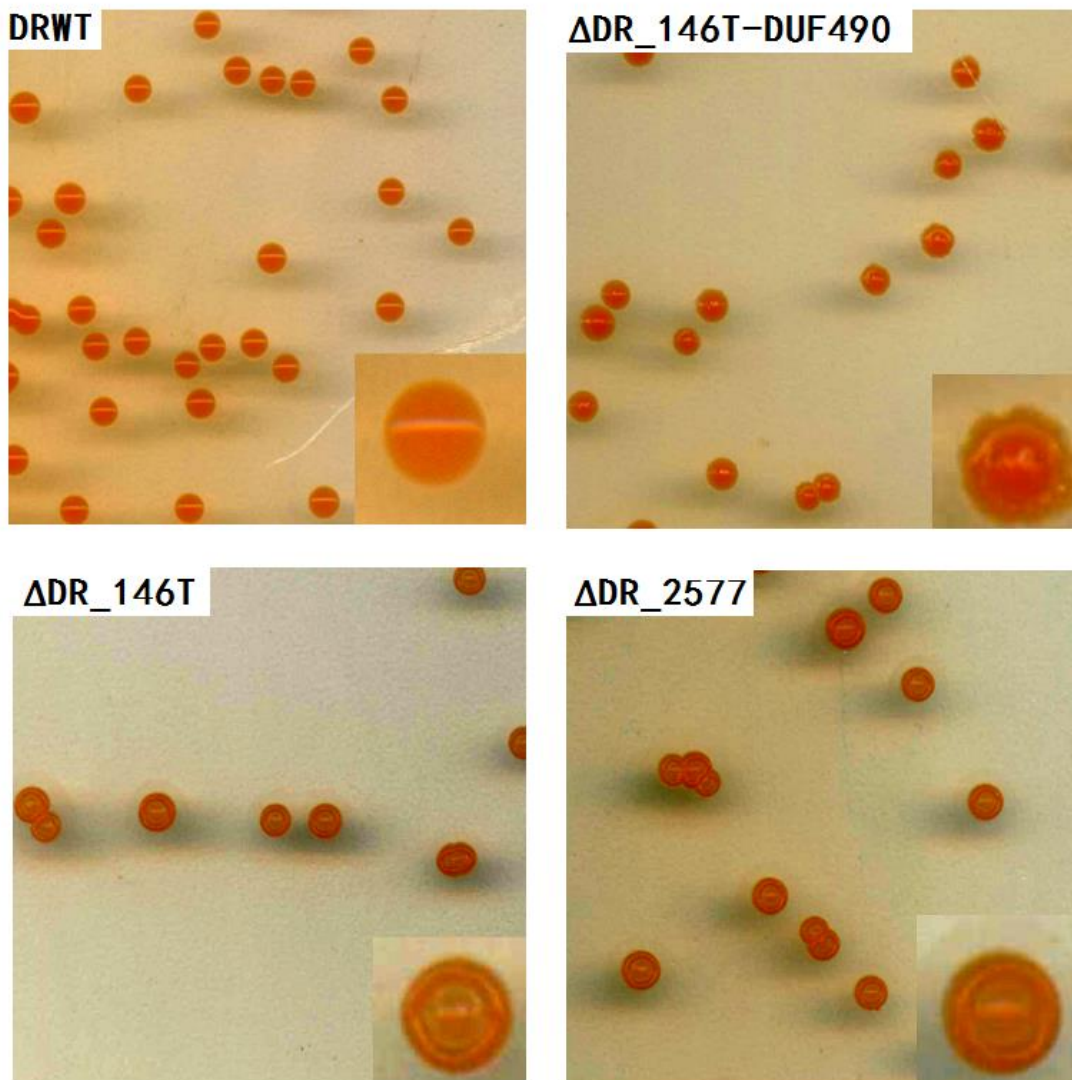
Re-seq ----- 1578 VD 1974 2414 2665 2689
DR_1461 146 APRTDPLSERTAQPHER 163 VD LTAQGG SYGAG TVKTA AGQLG TLQALPL 3313
      VD *** LTA GQ *** SY AG *** TV TA *** AG LG *** T +
      LTAGG SYAAG TVXTA AGXLG TCRRCRS 1888
      559 999 1250 1274

Re-seq 3314 GAVLAAVAGSTPGE ----- 3327
DR_1461 1889 ARCSPPSRAPRRARAWSPGRFVSVSPSTRWPGAPRWSPSASG 1940

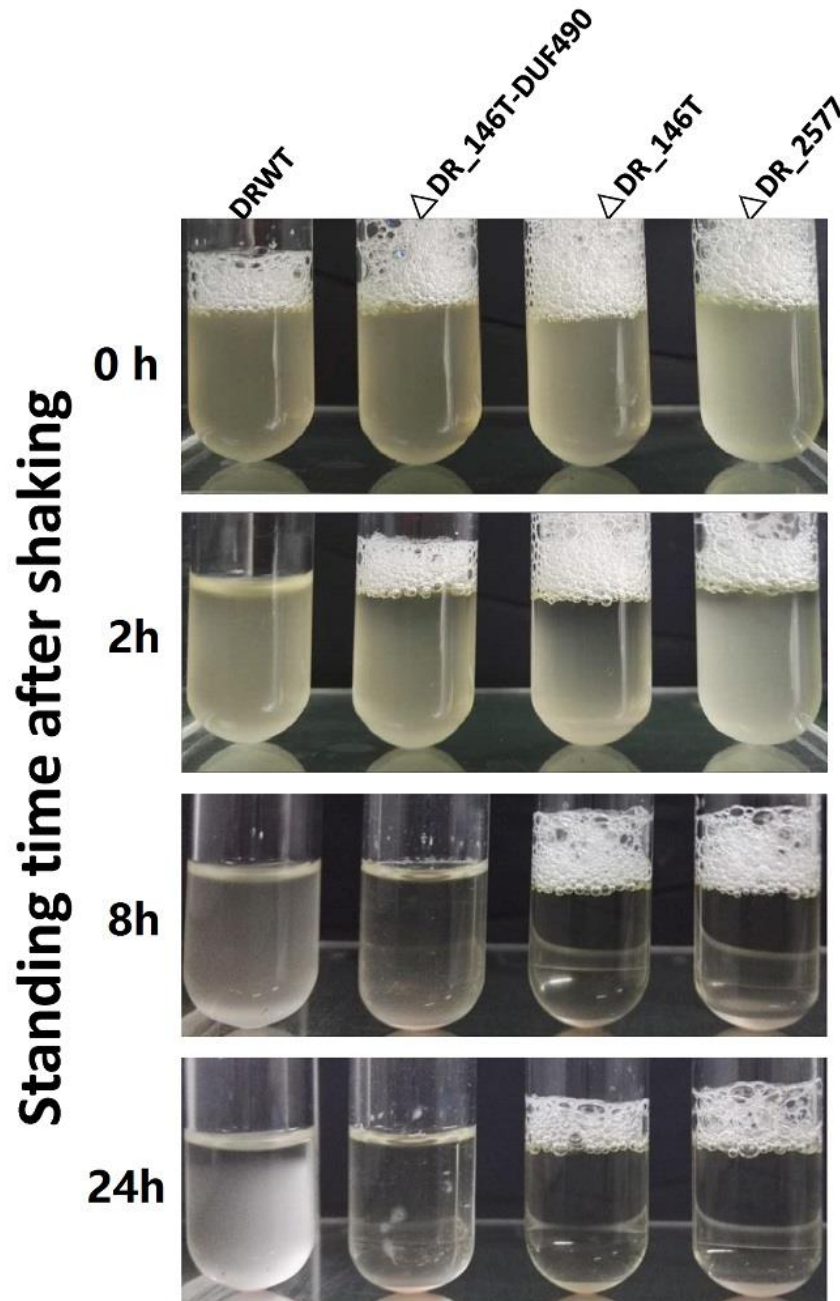
Re-seq 3328 VVT GR 4002
      +VT *** GR
DR_1460 1 MVT GR 675

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**Figure S3. Comparison of colony phenotype of the wild type with those of the mutants.** *D. radiodurans* wild type colonies were circular and smooth, while the colonies of DR\_146T mutant were ring-shaped and rugose. DRWT, *D. radiodurans* wild type;  $\Delta$ DR\_146T-DUF490, mutant deficient in DUF490 of DR\_146T;  $\Delta$ DR\_146T, mutant deficient in DR\_146T;  $\Delta$ DR\_2577, mutant deficient in DR\_2577. The inset diagram shows an amplified colony.



**Figure S4. Settlement of bacterial cultures.** *D. radiodurans* wild type and mutant cultures were placed on the table following vibration at room temperature. DRWT, *D. radiodurans* wild type;  $\Delta$ DR\_146T-DUF490, mutant deficient in DUF490 of DR\_146T;  $\Delta$ DR\_146T, mutant deficient in DR\_146T;  $\Delta$ DR\_2577, mutant deficient in DR\_2577.



**Figure S5. Peptides of DR\_146T detected in the cell envelope of *D. radiodurans* wild type cells by MS analysis.** The MS patterns for each peptide matched to DR\_146T are shown (a-i) and the peptides are listed (j). The peptide identification was performed using the MS data obtained to search against the updated *D. radiodurans* protein sequence database supplemented with the correct DR\_146T sequence.

