Supplementary Information for

Edge strand of *Escherichia coli* BepA interacts with immature LptD on the β-barrel assembly machine to direct it to on- and off-pathways

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This file includes:

Supplementary Materials and Methods

Media

E. coli cells were grown in L rich medium (10 g/L bacto-tryptone, 5 g/L bacto-yeast extract, 5 g/L NaCl; pH adjusted to 7.2 with NaOH) or M9 synthetic medium (without $CaCl_2$; (1)) supplemented with maltose (final 0.2%), glycerol (final 0.4%), all amino acids (except Met and Cys; final concentration of 20 μg/mL each). 50 μg/mL ampicillin (Amp), 20 μg/mL chloramphenicol (Cm), 25 μg/mL kanamycin (Km), 25 μg/mL tetracycline (Tet) and 50 μg/mL spectinomycin (Spc) were added as appropriate for growing plasmid-bearing cells and selection of transformants and transductants. Bacterial growth was monitored with Mini photo 518R (660 nm; TAITEC Co., Saitama, Japan).

Strain Construction

RM2091 (JE6631, *purC80*::Tn*10*) was constructed by transferring the *purC80*::Tn*10* marker, which is located near the *bepA* gene, from CAG18470 (2) into JE6631(3), respectively, by P1 transduction. RM2243 (AD16, *bepA(E137Q) purC80*::Tn*10*) was constructed as follows. pRM330 (a plasmid carrying *bepA(E137Q)*, see below) was introduced into RM2091 to yield cells with pRM330 integrated into the chromosome by homologous recombination in the *bepA* region. They were then grown on an L-agar plate containing 5% sucrose to select cells that had lost the integrated plasmid. The plasmid-cured cells were screened for those having the chromosomal *bepA(E137Q)* allele at the *bepA* locus. The *bepA(E137Q)* allele was finally transferred to AD16 (4) by joint P1 transduction with the *purC80*::Tn*10* marker. One of such strains was named RM2243. RM3654 (AD16, Δ*bepA*, *bamA*$^+$ *zae502*::Tn*10*) and RM3655 (AD16, *bamA(S439C) zae502*::Tn*10*) were constructed as follows, pRM845 (a plasmid carrying *bamA(S439C)*, see below) was introduced into YH188 (JE6631, *zae502*::Tn*10*) (5) to yield cells with pRM845 integrated into the chromosome by homologous recombination in the *bamA* region. They were then grown on an L-agar plate containing 5% sucrose to select cells that had lost the integrated plasmid. The plasmid-cured cells were screened for those having the chromosomal *bamA(S439C)* allele at the *bamA* locus. The *bamA(S439C)* allele was transferred to SN56 (6) by joint P1 transduction with the *zae502*::Tn*10* marker. Strains having the *bamA*$^+$ allele and the *bamA(S439C)* allele were picked up and named RM3654 and RM3655, respectively. RM2831 (HM1742, *kan araC*-P_{araBAD}-*lptD*) were constructed by essentially the same procedure as the construction of strains with a chromosomal C-terminal his_{10}-tagged gene (7). First, a *kan araC*-P_{araBAD}-*lptD* fragment having a sequence identical to the upstream or downstream region of the *lptD* start codon at the respective ends of the fragment, was PCR-amplified from pRM741 (a plasmid carrying a *kan* cassette at the upstream of an *araC*-P_{araBAD}) using a pair of primers, ara-lptD-f (5'-TTGTCACGCGCAACGTTACCGATGATGGAACAATAAAATCAACGTCATATGAATATCCTCCTTAG-3') and ara-lptD-r (5'-GGTGGCAATCATGGTGGCCAGGAGAGTGGGGATACGTTTTTTCATGGTGAATTCCTCCTGCTAG-3'). Then, the chromosomal *lptD* locus of the *E. coli* DY330 strain was replaced by this fragment using the λ-Red recombination system (8). The *kan araC*-P_{araBAD}-*lptD* was finally transferred to HM1742 by P1 transduction.

Plasmids Construction

pSTD689-derived plasmids carrying a *bepA* mutants were constructed from pRM290 (pSTD689-*bepA*) (9) by site-directed mutagenesis. Derivatives of pRM291 (pSTD689-*bepA(E137Q)*) (9) carrying an additional Cys mutation and derivatives of pUC-*bepA(E137Q)*-his_{10} (pUC18-*bepA(E137Q)*-his_{10}) (6) carrying an *amber* mutation were constructed by site-directed mutagenesis. pUC18-*bepA(Pro)*-his_{10} and pUC18-*bepA(amb)*-his_{10} plasmids were constructed from pUC-*bepA*-his_{10} (pUC18-*bepA*-his_{10}) (6) by site-directed mutagenesis. To construct pUC18-*bepA(Pro, amb, E137Q)*-his_{10} plasmids, a mutation for the individual Pro substitutions was introduced into each of the pUC18-*bepA(amb, E137Q)*-his_{10} plasmids by site-directed mutagenesis. pNB91 (pMW118-*bepA(E137Q)*) was constructed by subcloning an EcoRI-HindIII *bepA(E137Q)* fragment prepared from pUC-*bepA(E137Q)* (pUC18-*bepA(E137Q)*) (6) into the same sites of pMW118. pRM807 (pMW118-*bepA(A106P, E137Q)*) and pRM808 (pMW118-*bepA(F107P, E137Q)*) were also

constructed by subcloning an EcoRI-HindIII *bepA* fragment from each of pSTD639-*bepA* plasmids into the same sites of pMW118.

Plasmids carrying *lptD(amb)-his$_{10}$* were constructed from pRM309 (pRM294-*lptD-his$_{10}$*) (10) by site-directed mutagenesis. pRM821 (pRM294-*lptD(Y331amb, D749amb)-his$_{10}$*) and pRM822 (pRM294-*lptD(Y331amb, Y726amb)-his$_{10}$*) were constructed from pRM626 (pRM294-*lptD(Y331amb)-his$_{10}$*) by site-directed mutagenesis. pRM829 (pRM294-*lptD(E733C)-his$_{10}$*) and pRM831 (pRM294-*lptD(Y331, E733C)-his$_{10}$*) were constructed from pRM309 and pRM626, respectively, by site-directed mutagenesis. Plasmid carrying *lptD(Cys)-his$_{10}$* were constructed as follows. Each mutation for the Cys substitutions was introduced to pRM309 by site-directed mutagenesis. The BamHI-HindIII *lptD(Cys)-his$_{10}$* fragment of the resulting plasmids was subcloned into the same site of pTWV228 (Takara Bio inc., Shiga, Japan) to generate pTWV228-*lptD(Cys)-his$_{10}$*.

pRM320 (pUC118-*bepA-yfgD*) was constructed by PCR amplification of the *bepA-yfgD* fragment from the genome of MC4100 using a pair of primers, bepA-f (5'-GCGCGCGGATCCATTTGAGTGGGCTAATCTTCG-3') and yfgD-r (5'-GCGCGCGTCGACCGAACTACGCGAAGTTAATCC-3'), and, cloning it into the BamHI-SalI site of pUC118 (Takara Bio) after digestion with these enzymes. For the construction of pRM324, the *bepA(E137Q)* mutation was introduced into pRM320 by site-directed mutagenesis. pRM330 (pK18mobsacB-*bepA(E137Q)-yfgD*) was constructed by subcloning the BamHI-SalI *bepA(E137Q)-yfgD* fragment from pRM324 into the same sites of pK18mobsacB (11).

pRM823 (pUC118-*bamA*) was constructed by *in vitro* recombination using In-Fusion HD cloning kit (Takara Bio) of a EcoRI-BamHI fragment from pUC118 and a *bamA* fragment prepared by PCR amplification from the genome of MC4100 using a pair of primers, bamA-f (5'-GCGCGAATTCAGGAAGAACGCATAATAACG-3') and bamA-r (5'-GCGCGGATCCTTACCAGGTTTTACCGATG-3'). For the construction of pRM836, the *bamA(S439C)* mutation was introduced into pRM823 by site-directed mutagenesis. pRM845 (pK18mobsacB-*bamA(S439C)*) was constructed by subcloning the EcoRI-BamHI *bamA(S439C)* fragment from pRM823 into the same sites of pK18mobsacB.

Immunoblotting analysis

Acid-denatured proteins were solubilized in SDS-sample buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 5 mg/mL bromophenol blue) with or without 10% β-ME, separated by SDS-PAGE and electro-blotted onto a PVDF membrane (Merck Millipore; Billerica, MA). The membrane was first blocked with 5% skim milk in PBST (Phosphate Buffered Saline with Tween 20), and then incubated with Penta-His HRP conjugate (1/2,000 or 1/3,000 dilution)), anti-BepA (1/10,000), anti-LptD (1/50,000) or anti-BamA (1/20,000). After washing with PBST, the membrane was incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (1/5,000) (Goat Anti-Rabbit IgG (H + L)-HRP Conjugate; Bio-Rad Laboratories, Inc., Hercules, CA) in PBST (This step was omitted for the detection using Penta-His HRP conjugate). Proteins were visualized with ECL™ Western Blotting Detection Reagents (GE Healthcare UK Ltd, *Amersham Place* Little Chalfont, England) or ECL™ Prime Western Blotting Detection Reagents (GE Healthcare) and LAS4000 mini lumino-image analyzer (GE Healthcare).

Pulse-chase analysis for assay of the LptD disulfide-isomerization

Cells were first grown at 30°C in M9-medium supplemented with 2 μg/mL thiamine, 0.4% glycerol, 0.2% maltose, all amino acids (except Met and Cys) with or without 0.05% arabinose until early log phase. After induction with 1 mM IPTG for 15 min, cells were pulse-labeled with 370 kBq/mL [^{35}S]Methionine for 1 min. At appropriate time points after addition of excess nonradioactive Met (final conc. 250 μg/mL), total cellular proteins were precipitated with 5% TCA, washed with acetone, solubilized in SDS-buffer (50 mM Tris-HCl (pH 8.1), 1% SDS, 1 mM EDTA) and diluted 33-fold with Triton-buffer (50 mM Tris-HCl (pH 8.1), 150 mM NaCl, 2% Triton X-100, 0.1 mM EDTA). After clarification, samples were incubated with anti-LptD antibodies and nProtein A Sepharose™ 4 Fast Flow (GE healthcare) at 4ºC over-night with slow rotation. Proteins bound to the antibody/ProteinA-Sepharose were recovered by centrifugation, washed with Triton buffer and then with 10 mM Tris-HCl (pH 8.1) and eluted by incubation at 98°C for more than 5 min in SDS-sample buffer. The samples were divided into two portions and one was treated with 10% β-ME. The proteins were

separated by SDS-PAGE, and visualized with BAS1800 phosphoimager. Relative $LptD^{NC}$ were calculated by the following equation: Relative $LptD^{NC} = [LptD^{NC}_{(X\ min)}]/[LptD^{C}_{(X\ min)} + LptD^{NC}_{(X\ min)}]$, where $LptD^{NC}$ and $LptD^{C}$ are the intensities of the respective bands.

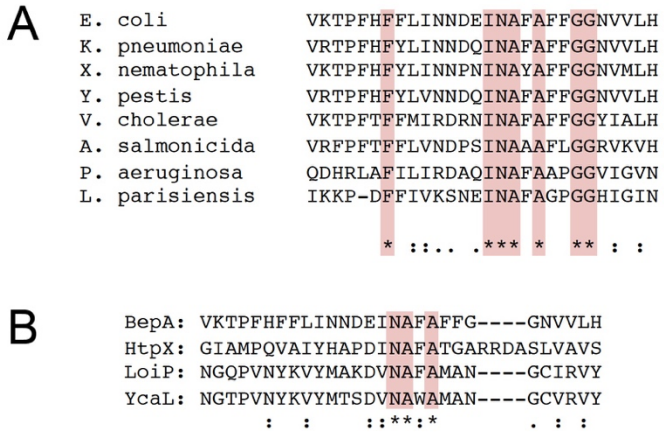

Fig. S1. Sequence alignment of BepA homologs and *E. coli* M48 family peptidases.

(*A*) Alignment of the edge strand amino acid sequences of BepA homologs of *E. coli*, *Klebsiella pneumoniae*, *Xenorhabdus nematophila*, *Yersinia pestis*, *Vibrio cholerae*, *Aeromonas salmonicida*, *Pseudomonas aeruginosa* and *Legionella parisiensis*. (*B*) Alignment of the edge strand amino acid sequences of BepA and other *E. coli* M48 family peptidase (HtpX, LoiP, and YcaL). The alignments were conducted by using the Clustal Omega program. Conserved residues are marked in red.

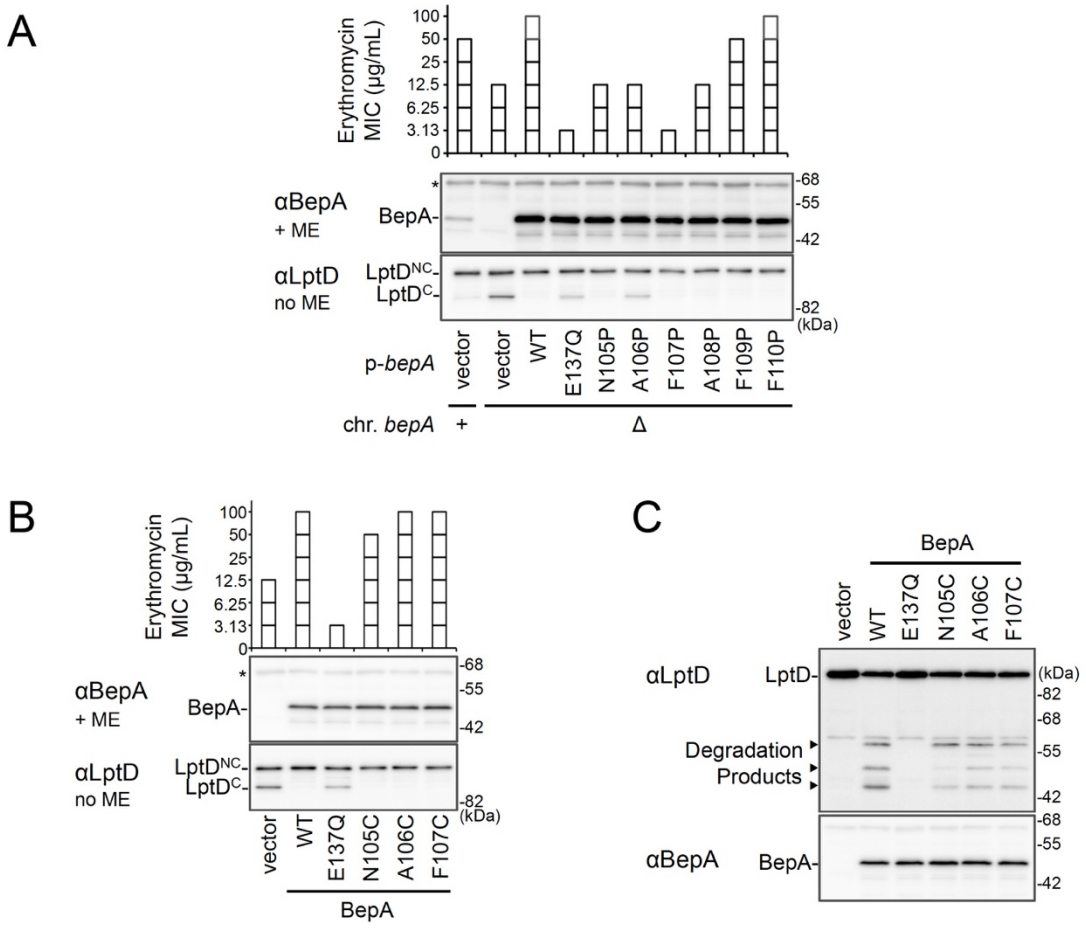

Fig. S2. The complementation and chaperon-like activities of the BepA edge strand mutants.

(*A, B*) Erythromycin sensitivity (*upper* panels) of the Δ*bepA* cells expressing the BepA mutants, and the accumulation of the BepA mutants (*middle* panels) and LptD^{C} in these cells (*lower* panel). The minimum inhibitory concentration (MIC) of erythromycin was determined as follow. 0.4 μL of overnight cultures of AD16 (*bepA*$^{+}$) carrying pSTD689 (vector) or SN56 (Δ*bepA*) carrying either pSTD689 or pSTD689-*bepA* plasmids were mixed with 250 μL of fresh L-broth, and 2.5 μL each of the cells were spotted on L-agar plate or L-agar plates supplemented with 0, 3.13, 6.25, 12.5, 25, 50 and 100 μg/mL erythromycin and 1 mM IPTG. Plates were incubated at 30˚C for 20 h. The MIC assay result shown is a representative of two independent experiments. For immunoblotting analysis, the above cells were grown at 30˚C in L medium until early log phase and induced with 1 mM IPTG for 1 h. Total cellular proteins were acid-precipitated, and analyzed by 10 or 7.5% Laemmli SDS-PAGE under a reducing (+ME) or non-reducing (no ME) condition and immunoblotting with anti-BepA (*middle* panels) or anti-LptD (*lower* panel) antibodies. (*C*) Protease activities of the BepA edge strand Cys mutants against overproduced LptD. Cells of SN56 (Δ*bepA*) carrying pTWV228-*lptD-his*$_{10}$ and either pSTD689 or pSTD689-*bepA* plasmids were grown at 30˚C in L-medium until early log phase and induced with 1 mM IPTG for 1 h. Total cellular proteins were analyzed as in Fig. 1B.

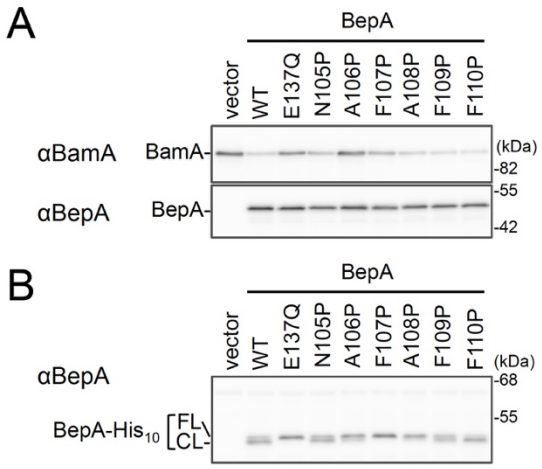

Fig. S3. Effects of the BepA edge strand Pro mutations on the BamA degradation and the BepA self-cleavage.

(*A*) Degradation of BamA in a Δ*surA* strain expressing the BepA edge strand Pro mutants. Cells of SN259 (Δ*bepA*, Δ*surA*) carrying either pSTD689 or pSTD689-*bepA* plasmids were grown at 30˚C in M9-based medium supplemented with 1 mM IPTG for 4 h. Total cellular proteins were acid-precipitated and analyzed by 7.5 or 10% Laemmli SDS-PAGE and immunoblotting with the indicated antibodies. (*B*) C-terminal self-cleavages of the BepA edge strand mutants. Cells of SN56 (Δ*bepA*) carrying either pUC18 or pUC18-*bepA-his*$_{10}$ plasmids were grown at 30˚C in L medium until early log phase and induced with 1 mM IPTG for 1 h. Total cellular proteins were acid-precipitated and analyzed by 7.5% Laemmli SDS-PAGE and immunoblotting with an anti-BepA antibody.

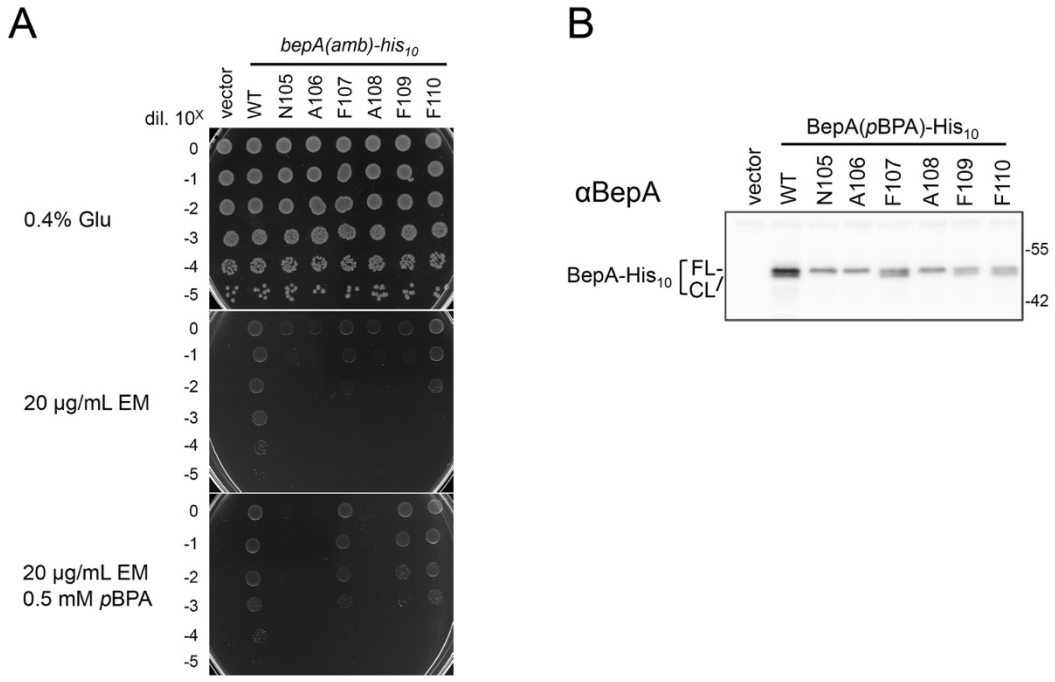

Fig. S4. Functionality of the BepA derivatives having *p*BPA in the edge strand.

(*A*) Complementation activity of the BepA(*p*BPA) derivatives. Cells from 30 μL of overnight cultures of SN56 (Δ*bepA*) carrying pEVOL-pBpF and either pUC18 or pUC18-*bepA(amb)-his*$_{10}$ plasmids were washed, suspended in saline, and serially diluted with saline (to about 10^9 cells/mL). 2.5 μL each of the diluted cells were spotted on L-agar plate or L-agar plates supplemented with 20 μg/mL erythromycin (EM) and 1 mM IPTG with or without 0.5 mM *p*BPA. Plates were incubated at 30˚C for 22 h. (*B*) Self-cleavage of the BepA*p*BPA derivatives. Cells of the same strains in *A* were grown at 30˚C in L medium supplemented with 0.02% arabinose and 0.5 mM *p*BPA until early log phase and induced with 1 mM IPTG for 1 h. Total cellular proteins were acid-precipitated and analyzed by 7.5% Laemmli SDS-PAGE and immunoblotting with an anti-BepA antibody.

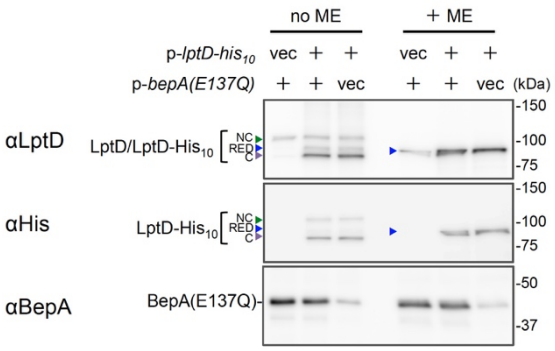

Fig. S5. Over-expressed LptD molecules mainly accumulate as LptD^{C}.

Cells of RM2243(*bepA(E137Q)*)/pEVOL-pBpF carrying either pMW118 or pMW118-*bepA(E137Q)* and either pRM294 or pRM294-*lptD-his*$_{10}$ were grown n L medium containing 0.02% arabinose and 0.5 mM *p*BPA until early log phase at 30˚C and induced with 1 mM IPTG for 3 h. Total cellular proteins were acid-precipitated and analyzed by 7.5 or 10% Laemmli SDS-PAGE under a reducing (+ME) or non-reducing (no ME) condition and immunoblotting with the indicated antibodies.

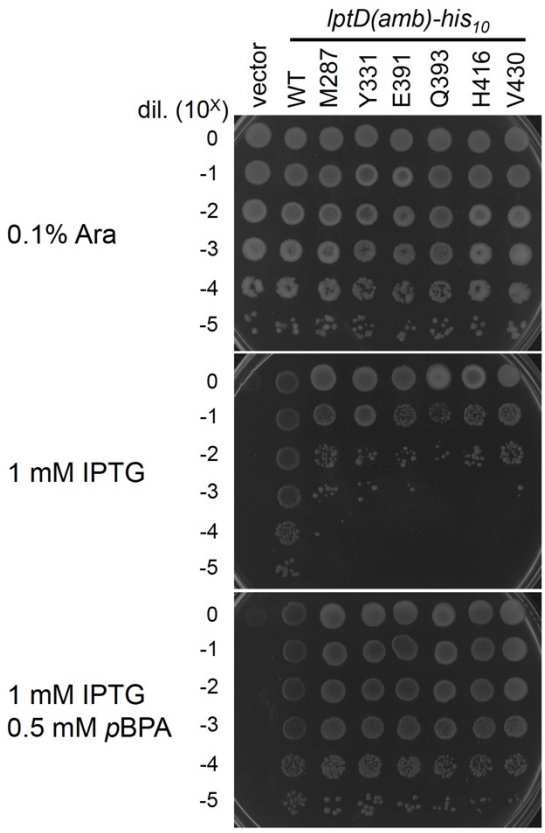

Fig. S6. Complementation activity of the LptD(*p*BPA) derivatives.

Cells of RM3588 (P_{ara}-*lptD*) carrying pEVOL-pBpF and either pRM294 or pRM294-*lptD(amb)-his*$_{10}$ plasmids were grown at 30˚C in L-medium supplemented with 0.1% arabinose for 2.5 h. Cells were washed, suspended in saline, and serially diluted with saline (to about 10^9 cells/mL). 2.5 μL each of the diluted cells were spotted on L-agar plate containing 0.1% arabinose or L medium-based agar plates supplemented with 1 mM IPTG with or without 0.5 mM *p*BPA. Plates were incubated at 30˚C for 22 h.

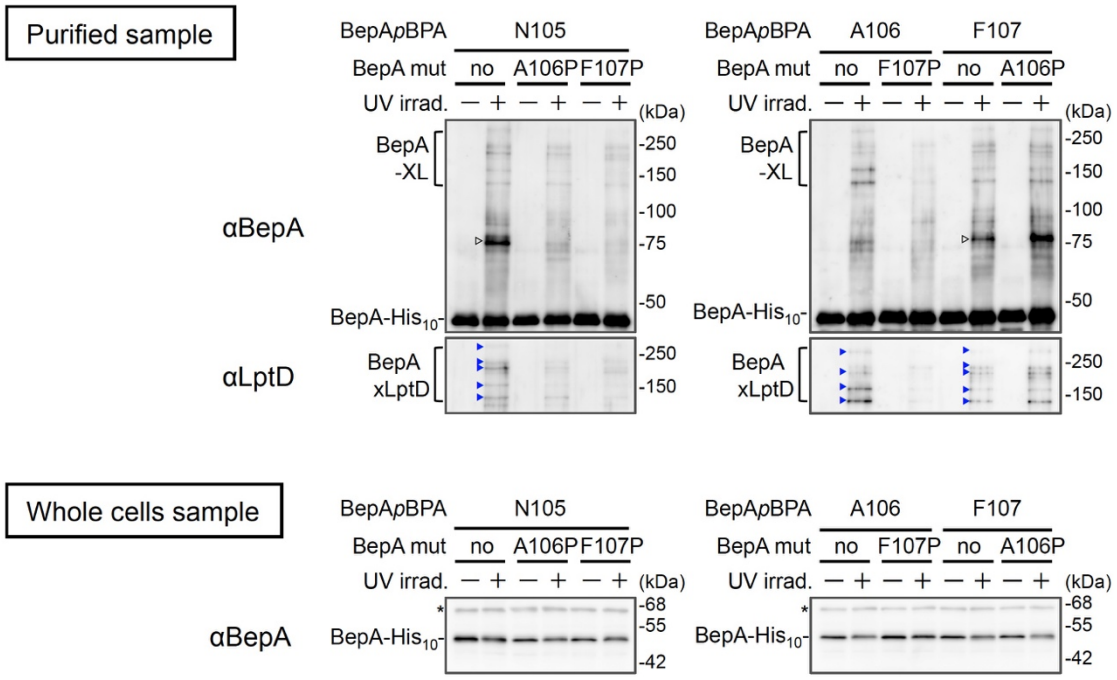

Fig. S7. Effects of the BepA edge strand mutations on the crosslinking of the BepA edge strand to LptD.

Cells SN56 (Δ*bepA*) carrying pEVOL-pBpF and pUC18-*bepA(E137Q, mut., amb)-his*$_{10}$ plasmids were grown in L medium containing 0.02% arabinose and 0.5 mM *p*BPA until early log phase at 30˚C and induced with 1 mM IPTG for 1 h to express the indicated BepA(*p*BPA) variants. The cultures were divided into two portions, each of which was treated with or without UV-irradiation for 10 min at 4˚C. Proteins of the total membrane fractions were subjected to pull-down with Ni-NTA agarose. Purified proteins were analyzed by 7.5% Laemmli SDS-PAGE and immunoblotting with the indicated antibodies. For analysis of the cellular accumulation of each BepA derivative, total cellular proteins of cells of the same cultures were acid-precipitated, and analyzed by 10% Laemmli SDS-PAGE and immunoblotting with the indicated antibodies. Open triangles indicate unknown crosslinked products.

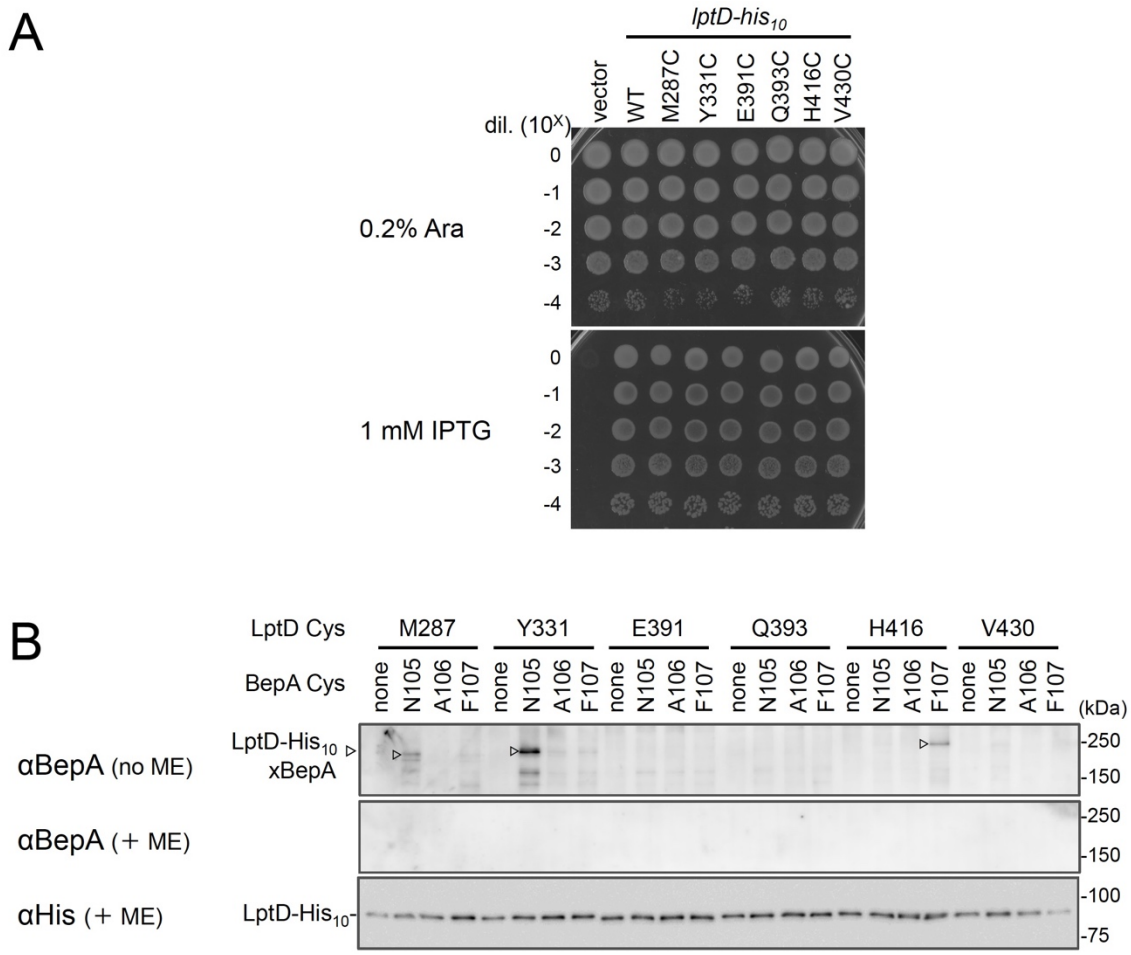

Fig. S8. Disulfide crosslinking between BepA and LptD.

(*A*) Complementation activity of the LptD derivatives with an engineered Cys residue. Cells of RM3588 (P_{ara}-*lptD*) carrying pRM294 or pRM294-*lptD(Cys)-his*$_{10}$ plasmids were grown at 30˚C in L medium supplemented with 0.1% arabinose for 2.5 h. Cells were washed, suspended in saline, and serially diluted with saline (to about 10^9 cells/mL). 2.5 μL each of the diluted cells were spotted on L-agar plate containing 0.1% arabinose or 1 mM IPTG. Plates were incubated at 30˚C for 22 h. (*B*) BepA–LptD disulfide crosslinking. Cells of SN56 (Δ*bepA*) carrying a combination of plasmids encoding WT or a Cys-mutant form of BepA and LptD-His_{10} as indicated were grown in L-medium and induced with 1 mM IPTG for 3 h to express BepA(Cys) and LptD(Cys)-His_{10}. Total cellular proteins were acid-precipitated, solubilized with SDS buffer containing NEM and subjected to pull-down with Ni-NTA agarose. The purified proteins were treated with or without 2-mercaptoethanol (ME) and analyzed by 7.5% Laemmli SDS-PAGE and immunoblotting with the indicated antibodies.

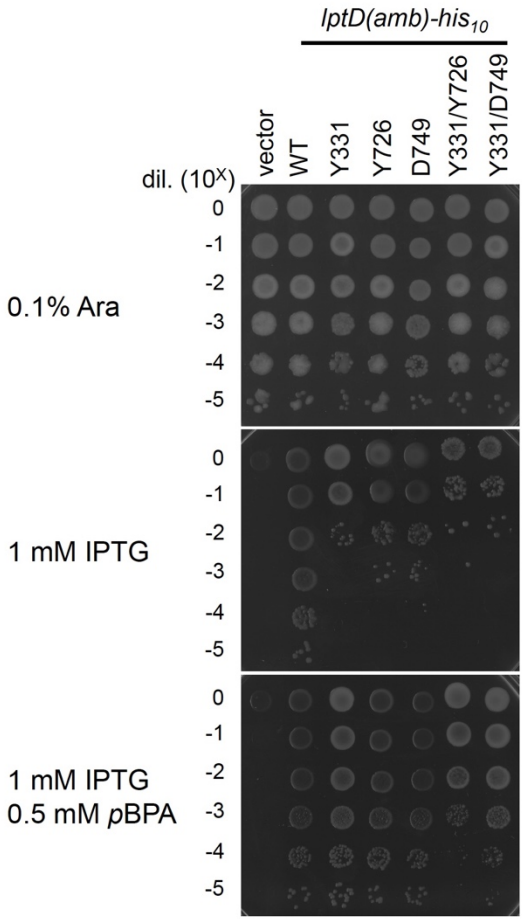

Fig. S9. Complementation activity of the LptD derivatives having *p*BPA at one or two positions.

Cells of RM3588 (P_{ara}-*lptD*) carrying pEVOL-pBpF and either pRM294 or pRM294-*lptD(amb)-his*$_{10}$ plasmids were grown and analyzed as in Fig. S6.

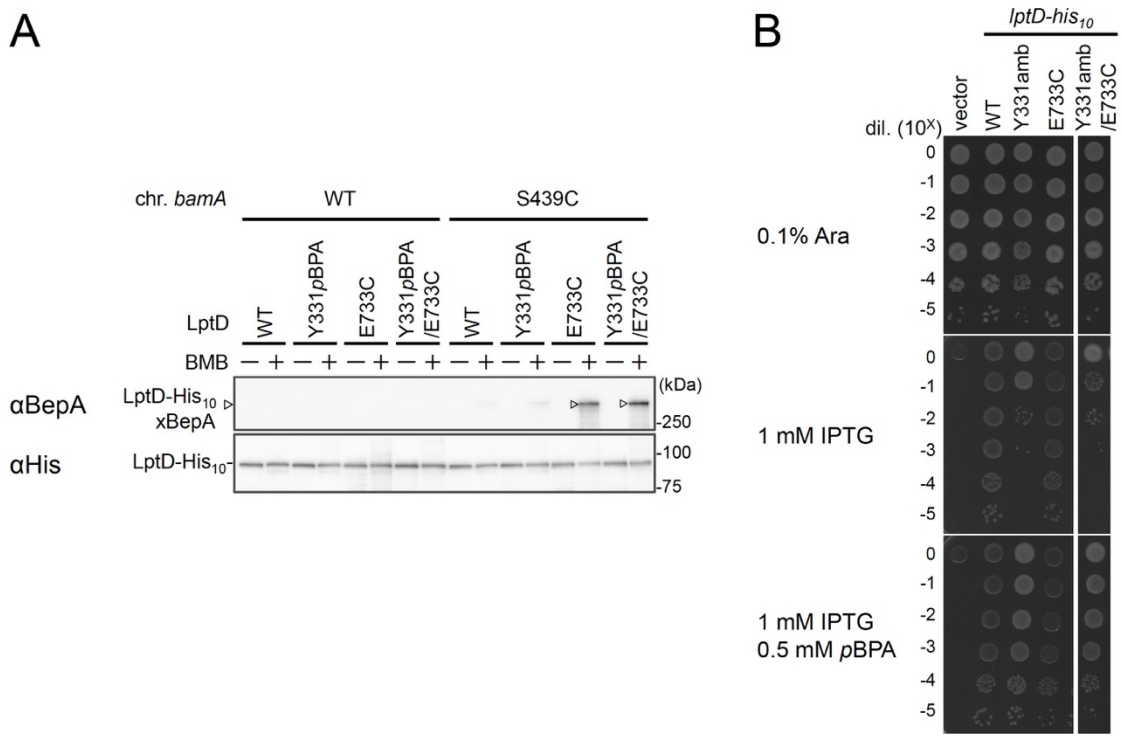

Fig. S10. BMB crosslinking between LptD(Y331*p*BPA/E733C) and BamA(S439C).

(*A*) BMB crosslinking. Cells of RM3654 (*bamA*$^{+}$, Δ*bepA*)/pEVOL-pBpF/pMW118-*bepA(E137Q)* or RM3655 (*bamA(S439C)*, Δ*bepA*)/pEVOL-pBpF/pMW118-*bepA(E137Q)* carrying each pRM294-*lptD-his*$_{10}$ derivative were grown at 30˚C in L medium containing 0.5 mM *p*BPA until early log phase and induced with 1 mM IPTG for 3 h to express the indicated LptD variants. Cell cultures were treated with TCEP to reduce possible artificially-formed disulfide bonds for E733C in LptD and S439C in BamA. The cultures were divided into two portions, each of which was treated with or without BMB. After quenching of BMB by addition of excess cysteine, total cellular proteins were acid-precipitated, solubilized with SDS-buffer containing NEM and subjected to pull-down with Ni-NTA agarose. The purified proteins were analyzed by 7.5% Laemmli SDS-PAGE and immunoblotting with the indicated antibodies. (*B*) Complementation activity of LptD derivatives. Cells of RM3588 (P$_{ara}$-*lptD*) carrying pEVOL-pBpF and pRM294 or each pRM294-*lptD-his*$_{10}$ derivative were grown are analyzed as in Fig. S6.

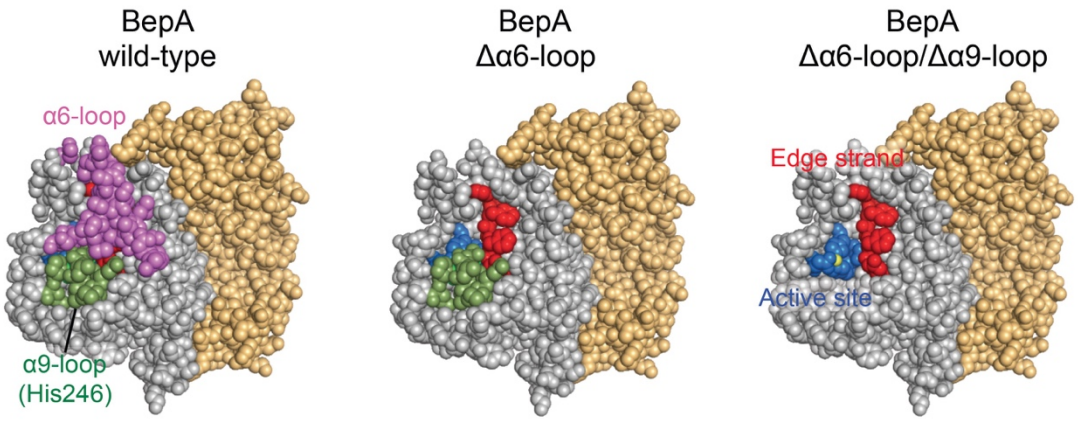

Fig. S11. α6 and α9-loop regions shield the intramolecular active stie and edge strand of BepA.

The space-filling models of wild type BepA (*left*), BepA without the α6-loop (*middle*) and BepA without the α6- and α9-loops (*right*). The peptidase and the TPR domains of the BepA crystal structure (PDB code: 6AIT) are shown in gray and orange, respectively. The α6-loop, the α9-loop, the proteolytic active site (the HExxH motif and the third zinc ligand, Glu-201) and the edge strand in the peptidase domain are shown in magenta, green, blue and red, respectively.

Table S1. Strains used in this study.

Strain	Genotype	References
AD16	Δ*pro-lac thi*/F' *lacI*^{q} *ZΔM15 Y*$^{+}$ *pro*$^{+}$	(4)
SN56	AD16, Δ*bepA*	(6)
SN259	AD16, Δ*bepA ΔsurA*::*kan*	(6)
RM2243	AD16, *bepA(E137Q) purC80*::Tn*10*	This study
RM3654	AD16, Δ*bepA bamA*$^{+}$ *zae502*::Tn*10*	This study
RM3655	AD16, Δ*bepA bamA(S439C) zae502*::Tn*10*	This study
JE6631	*Hfr:str thi polA1*	(3)
RM2091	JE6631, *purC80*::Tn*10*	This study
YH188	JE6631, *zae502*::Tn*10*	(5)
MC4100	F^{-} *araD139 Δ(argF-lac)U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*	(12)
CU141	MC4100/F' *lacI*^{q} *lacZ*$^{+}$, *Y*$^{+}$, *A*$^{+}$	(13)
HM1742	CU141 *ara*$^{+}$	(14)
RM3588	HM1742, *kan araC*-P$_{araBAD}$-*lptD*	This study
DY330	W3110, Δ*lacU169 gal490 λcI857* Δ(*cro-bioA*)	(8)
RM3444	DY330, *kan araC*-P$_{araBAD}$-*lptD*	This study

Table S2. Plasmids used in this study.

Plasmid	Vector	Encoded protein and description	Reference or source
pEVOL-pBpF		p15A-derivative encoding an evolved *M. jannaschii* aminoacyl-tRNA synthetase/ suppressor tRNA pair for incorporation of *p*BPA; Cm^R	(15)
pSTD689		pACYC184-derived expression vector; P_{lac}, Spc^R	(16)
pRM290	pSTD689	BepA	(9)
pRM291	pSTD689	BepA(E137Q)	(9)
pRM512	pSTD689	BepA(N105P)	This study
pRM513	pSTD689	BepA(A106P)	This study
pRM514	pSTD689	BepA(F107P)	This study
pRM515	pSTD689	BepA(A108P)	This study
pRM516	pSTD689	BepA(F109P)	This study
pRM517	pSTD689	BepA(F110P)	This study
pRM833	pSTD689	BepA(N105C)	This study
pRM786	pSTD689	BepA(N105C, E137Q)	This study
pRM834	pSTD689	BepA(A106C)	This study
pRM787	pSTD689	BepA(A106C, E137Q)	This study
pRM835	pSTD689	BepA(F107C)	This study
pRM788	pSTD689	BepA(F107C, E137Q)	This study
pUC18		Expression vector; P_{lac}, Amp^R	Takara Bio
pUC-bepA(E137Q)	pUC18	BepA(E137Q)	(6)
pUC-bepA-his_{10}	pUC18	BepA-His_{10}	(6)
pUC-bepA(E137Q)-his_{10}	pUC18	BepA(E137Q)-His_{10}	(6)
pYN333	pUC18	BepA(N105P)-His_{10}	This study
pYN334	pUC18	BepA(A106P)-His_{10}	This study
pYN335	pUC18	BepA(F107P)-His_{10}	This study
pYN336	pUC18	BepA(A108P)-His_{10}	This study
pYN337	pUC18	BepA(F109P)-His_{10}	This study
pYN338	pUC18	BepA(F110P)-His_{10}	This study
pYN339	pUC18	BepA(N105*amb*)-His_{10}	This study
pRM536	pUC18	BepA(N105*amb*, E137Q)-His_{10}	This study
pRM778	pUC18	BepA(N105*amb*, A106P, E137Q)-His_{10}	This study
pRM779	pUC18	BepA(N105*amb*, F107P, E137Q)-His_{10}	This study
pYN340	pUC18	BepA(A106*amb*)-His_{10}	This study

pRM537	pUC18	BepA(A106*amb*, E137Q)-His_{10}	This study
pYN345	pUC18	BepA(A106*amb*, F107P, E137Q)-His_{10}	This study
pYN341	pUC18	BepA(F107*amb*)-His_{10}	This study
pRM538	pUC18	BepA(F107*amb*, E137Q)-His_{10}	This study
pYN346	pUC18	BepA(F107*amb*, A106P, E137Q)-His_{10}	This study
pYN342	pUC18	BepA(A108*amb*)-His_{10}	This study
pRM539	pUC18	BepA(A108*amb*, E137Q)-His_{10}	This study
pYN343	pUC18	BepA(F109*amb*)-His_{10}	This study
pRM540	pUC18	BepA(F109*amb*, E137Q)-His_{10}	This study
pYN344	pUC18	BepA(F110*amb*)-His_{10}	This study
pRM541	pUC18	BepA(F110*amb*, E137Q)-His_{10}	This study
pMW118		pSC101-derived expression vector; P_{lac}, Amp^R	Nippon Gene
pNB91	pMW118	BepA(E137Q)	This study
pRM807	pMW118	BepA(A106P, E137Q)	This study
pRM808	pMW118	BepA(F107P, E137Q)	This study
pTWV228		pBR322-derived expression vector; P_{lac}, Amp^R	Takara Bio
pRM267	pTWV228	LptD-His_{10} (using the native SD sequence)	(9)
pRM816	pTWV228	LptD-His_{10}^{SD} (using a strong SD sequence)	This study
pRM810	pTWV228	LptD(M287C)-His_{10}^{SD}	This study
pRM811	pTWV228	LptD(Y331C)-His_{10}^{SD}	This study
pRM812	pTWV228	LptD(E391C)-His_{10}^{SD}	This study
pRM813	pTWV228	LptD(Q393C)-His_{10}^{SD}	This study
pRM814	pTWV228	LptD(H416C)-His_{10}^{SD}	This study
pRM815	pTWV228	LptD(V430C)-His_{10}^{SD}	This study
pRM294		pBR322-derived expression vector; P_{lac}, Spc^R	(10)
pRM309	pRM294	LptD-His_{10}^{SD} (using a strong SD sequence)	(10)
pNB22	pRM294	LptD(R40*amb*)-His_{10}^{SD}	This study
pNB23	pRM294	LptD(N55*amb*)-His_{10}^{SD}	This study
pNB24	pRM294	LptD(T70*amb*)-His_{10}^{SD}	This study
pNB25	pRM294	LptD(D85*amb*)-His_{10}^{SD}	This study
pNB26	pRM294	LptD(P100*amb*)-His_{10}^{SD}	This study
pNB27	pRM294	LptD(N115*amb*)-His_{10}^{SD}	This study
pNB28	pRM294	LptD(T130*amb*)-His_{10}^{SD}	This study
pNB29	pRM294	LptD(R145*amb*)-His_{10}^{SD}	This study
pNB30	pRM294	LptD(N160*amb*)-His_{10}^{SD}	This study
pNB31	pRM294	LptD(P175*amb*)-His_{10}^{SD}	This study
pNB49	pRM294	LptD(D190*amb*)-His_{10}^{SD}	This study

pNB50	pRM294	LptD(V205*amb*)-His_{10}^{SD}	This study
pNB51	pRM294	LptD(V220*amb*)-His_{10}^{SD}	This study
pNB52	pRM294	LptD(Y235*amb*)-His_{10}^{SD}	This study
pNB53	pRM294	LptD(N250*amb*)-His_{10}^{SD}	This study
pRM618	pRM294	LptD(T258*amb*)-His_{10}^{SD}	This study
pRM619	pRM294	LptD(I259*amb*)-His_{10}^{SD}	This study
pNB54	pRM294	LptD(H265*amb*)-His_{10}^{SD}	This study
pRM620	pRM294	LptD(E273*amb*)-His_{10}^{SD}	This study
pNB55	pRM294	LptD(S280*amb*)-His_{10}^{SD}	This study
pRM621	pRM294	LptD(L286*amb*)-His_{10}^{SD}	This study
pRM622	pRM294	LptD(M287*amb*)-His_{10}^{SD}	This study
pNB56	pRM294	LptD(S295*amb*)-His_{10}^{SD}	This study
pNB57	pRM294	LptD(R310*amb*)-His_{10}^{SD}	This study
pRM623	pRM294	LptD(Y314*amb*)-His_{10}^{SD}	This study
pNB58	pRM294	LptD(W325*amb*)-His_{10}^{SD}	This study
pRM624	pRM294	LptD(F327*amb*)-His_{10}^{SD}	This study
pRM625	pRM294	LptD(D330*amb*)-His_{10}^{SD}	This study
pRM626	pRM294	LptD(Y331*amb*)-His_{10}^{SD}	This study
pNB59	pRM294	LptD(F340*amb*)-His_{10}^{SD}	This study
pNB60	pRM294	LptD(A355*amb*)-His_{10}^{SD}	This study
pRM627	pRM294	LptD(K358*amb*)-His_{10}^{SD}	This study
pNB61	pRM294	LptD(A370*amb*)-His_{10}^{SD}	This study
pRM628	pRM294	LptD(T371*amb*)-His_{10}^{SD}	This study
pNB62	pRM294	LptD(T385*amb*)-His_{10}^{SD}	This study
pRM629	pRM294	LptD(E391*amb*)-His_{10}^{SD}	This study
pRM630	pRM294	LptD(Q393*amb*)-His_{10}^{SD}	This study
pNB63	pRM294	LptD(Q400*amb*)-His_{10}^{SD}	This study
pNB64	pRM294	LptD(V415*amb*)-His_{10}^{SD}	This study
pRM631	pRM294	LptD(H416*amb*)-His_{10}^{SD}	This study
pRM632	pRM294	LptD(R429*amb*)-His_{10}^{SD}	This study
pNB65	pRM294	LptD(V430*amb*)-His_{10}^{SD}	This study
pRM633	pRM294	LptD(H431*amb*)-His_{10}^{SD}	This study
pNB66	pRM294	LptD(G445*amb*)-His_{10}^{SD}	This study
pRM634	pRM294	LptD(H457*amb*)-His_{10}^{SD}	This study
pRM635	pRM294	LptD(Y458*amb*)-His_{10}^{SD}	This study
pNB67	pRM294	LptD(Q460*amb*)-His_{10}^{SD}	This study
pNB68	pRM294	LptD(D475*amb*)-His_{10}^{SD}	This study
pRM636	pRM294	LptD(V478*amb*)-His_{10}^{SD}	This study

pRM637	pRM294	LptD(N479*amb*)-His_{10}^{SD}	This study
pNB69	pRM294	LptD(K490*amb*)-His_{10}^{SD}	This study
pNB70	pRM294	LptD(T505*amb*)-His_{10}^{SD}	This study
pNB71	pRM294	LptD(R520*amb*)-His_{10}^{SD}	This study
pNB72	pRM294	LptD(S535*amb*)-His_{10}^{SD}	This study
pNB73	pRM294	LptD(D550*amb*)-His_{10}^{SD}	This study
pNB74	pRM294	LptD(R565*amb*)-His_{10}^{SD}	This study
pNB75	pRM294	LptD(G580*amb*)-His_{10}^{SD}	This study
pNB76	pRM294	LptD(I595*amb*)-His_{10}^{SD}	This study
pNB77	pRM294	LptD(G610*amb*)-His_{10}^{SD}	This study
pNB78	pRM294	LptD(G625*amb*)-His_{10}^{SD}	This study
pNB79	pRM294	LptD(S640*amb*)-His_{10}^{SD}	This study
pNB80	pRM294	LptD(N655*amb*)-His_{10}^{SD}	This study
pNB81	pRM294	LptD(K670*amb*)-His_{10}^{SD}	This study
pNB82	pRM294	LptD(V685*amb*)-His_{10}^{SD}	This study
pNB83	pRM294	LptD(V700*amb*)-His_{10}^{SD}	This study
pNB84	pRM294	LptD(S715*amb*)-His_{10}^{SD}	This study
pNB32	pRM294	LptD(Y726*amb*)-His_{10}^{SD}	This study
pNB85	pRM294	LptD(V730*amb*)-His_{10}^{SD}	This study
pNB86	pRM294	LptD(H745*amb*)-His_{10}^{SD}	This study
pRM809	pRM294	LptD(D749*amb*)-His_{10}^{SD}	This study
pNB87	pRM294	LptD(G760*amb*)-His_{10}^{SD}	This study
pNB88	pRM294	LptD(S775*amb*)-His_{10}^{SD}	This study
pRM821	pRM294	LptD(Y331*amb*, D749*amb*)-His_{10}^{SD}	This study
pRM822	pRM294	LptD(Y331*amb*, Y726*amb*)-His_{10}^{SD}	This study
pRM829	pRM294	LptD(E733C)-His_{10}^{SD}	This study
pRM831	pRM294	LptD(Y331*amb*, E733C)-His_{10}^{SD}	This study
pUC118		Expression vector; P_{lac}, Amp^R	Takara Bio
pRM320	pUC118	*bepA-yfgD*	This study
pRM324	pUC118	*bepA(E137Q)-yfgD*	This study
pRM823	pUC118	*bamA*	This study
pRM836	pUC118	*bamA(S439C)*	This study
pK18mobsacB		pBR322-derived vector carrying *sacB* for chromosomal gene replacement; Km^R	(11)
pRM330	pK18mobsacB	*bepA(E137Q)-yfgD*	This study
pRM845	pK18mobsacB	*bamA(S439C)*	This study

SI References

1. J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory Press, 1972).
2. B. P. Nichols, O. Shafiq, V. Meiners, Sequence analysis of Tn*10* insertion sites in a collection of *Escherichia coli* strains used for genetic mapping and strain construction. *J. Bacteriol.* **180**, 6408–6411 (1998).
3. H. Mori, Y. Shimizu, K. Ito, Superactive SecY variants that fulfill the essential translocation function with a reduced cellular quantity. *J. Biol. Chem.* **277**, 48550–48557 (2002).
4. A. Kihara, Y. Akiyama, K. Ito, FtsH is required for proteolytic elimination of uncomplexed forms of SecY, an essential protein translocase subunit. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 4532–4536 (1995).
5. Y. Hizukuri, Y. Akiyama, PDZ domains of RseP are not essential for sequential cleavage of RseA or stress-induced σ^E activation *in vivo*. *Mol. Microbiol.* **86**, 1232–1245 (2012).
6. S. Narita, C. Masui, T. Suzuki, N. Dohmae, Y. Akiyama, Protease homolog BepA (YfgC) promotes assembly and degradation of β-barrel membrane proteins in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* **110**, E3612–E3621 (2013).
7. R. Miyazaki, Y. Akiyama, H. Mori, Fine interaction profiling of VemP and mechanisms responsible for its translocation-coupled arrest-cancelation. *eLife* **9**, e62623 (2020).
8. D. Yu, *et al.*, An efficient recombination system for chromosome engineering in *Escherichia coli. Proc. Natl. Acad. Sci. U. S. A.* **97**, 5978–5983 (2000).
9. Y. Daimon, *et al.*, The TPR domain of BepA is required for productive interaction with substrate proteins and the β-barrel assembly machinery complex. *Mol. Microbiol.* **106**, 760–776 (2017).
10. R. Miyazaki, N. Myougo, H. Mori, Y. Akiyama, A photo-cross-linking approach to monitor folding and assembly of newly synthesized proteins in a living cell. *J. Biol. Chem.* **293**, 677–686 (2018).
11. A. Schäfer, *et al.*, Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene* **145**, 69–73 (1994).
12. M. J. Casadaban, Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J. Mol. Biol.* **104**, 541–555 (1976).
13. Y. Akiyama, T. Ogura, K. Ito, Involvement of FtsH in protein assembly into and through the membrane. I. Mutations that reduce retention efficiency of a cytoplasmic reporter. *J. Biol. Chem.* **269**, 5218–5224 (1994).
14. H. Mori, K. Ito, The long α-helix of SecA is important for the ATPase coupling of translocation. *J. Biol. Chem.* **281**, 36249–36256 (2006).
15. T. S. Young, I. Ahmad, J. A. Yin, P. G. Schultz, An enhanced system for unnatural amino acid mutagenesis in *E. coli*. *J. Mol. Biol.* **395**, 361–374 (2010).
16. K. Kanehara, K. Ito, Y. Akiyama, YaeL proteolysis of RseA is controlled by the PDZ domain of YaeL and a Gln-rich region of RseA. *EMBO J.* **22**, 6389–6398 (2003).