

Supplemental Material, Baker & Postle

P0ABU7	1	---MGNNLMQTDLSVWGMVQHADIIVVVCVMIGLILASVVTWAIFFSKSVEFFNQKRRLLKR	57
Q5FQC2	8	S--ALGAVGATGLSPLDLFLHASIVVVKLVMLGLLLCAGVWAIIAEKIILIRRVNREATF	65
Q9A3H1	1	---MIAAAAAAPNFSFFALFMQADWVWVSVMIGLILASLGSWAVILDKLFRFQALNRAANR	57
B2Q373	1	-----MTDMNIVDLFLKASLLVQIIMFVLIGFSIASWAIIOQTILNAAAREAEA	51
A1VBP6	1	-----MEIIELYSHATPVAVAVMAVLVVMVSVWSIIVRRKALLFRSLEGRLDG	48
B5EL52	1	----M---NL-----QYLIHLANYSIGVLYVLGALLLVLSVIVDRFWFLRR-----TI	42
Q256H5	1	---ML---QLSNPIIQAYREADLFGKGIFFSLILSLCTWTWVLFHQKLAIQKFLKSGKS	54
A3SCQ3	1	-MDMF---SALIASFRQIAETGGPVVVVLMGVAVLTLAVA---IYVWQFWRSVAVGRHKA	53
Q1YK5	241	SRGMMLEQLANAPFLKDRLEHGGVVGKVLIGLLVIGAIIT---LFRGSKLFTIQ---QI	294
		:: :	
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Q5FQC2	66	FEDRFWS-----GGSLEDD---LYESDGARPTH--PMAAVFGAAMGEWRRSARI--	108
Q9A3H1	58	FEEQVSG-----GRSLED---VAGEAGANPRH--ALPRMLQAALKWWRDAKSK--	100
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A1VBP6	49	AIHRLA-----CDGIAEAMSEFKGH---ADN--TVWHLLETGYREYMLLPR--	91
B5EL52	43	LRGLIFVHELGSIGRLDRITLNQMA-----DGASDLPEAALLR--	80
Q256H5	55	LKDFLIKRRHAPLSLEIHPFLNPFALYFTIK-----RGALELL---KNR--	97
A3SCQ3	54	LTEAVE-----AWDEGDRPVA-----REALGRSKSYLAPVIKMA	87
Q1YK5	295	KAQLKR-----PNEPGNNPLG-----RIL---KVYSL-----	318
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Q5FQC2	109	GGIDLSRG---G-VREKVDRAIDITIMRENDRLTRRLI FLATIGPVAPFVGLFGTVWVGIM	164
Q9A3H1	101	GAMSETQA---GFLIARIDRILDTQIARETTVEEGLGSLAIVATASPFIGLFGTVWVGIM	157
B2Q373	96	--ANIHAP---DAVVTGASRAMRISMNRELSVAHIFPLGTVGSISPYIGLFGTVWVGIM	150
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Q256H5	98	QQAPDHGCVLSMEDIQSLEITLLGAIMPKYRAIMHENNFIPATTISLAPFLGLLGTVWVGIL	157
A3SCQ3	88	MVSNHGKS---GAGVE---RLQAFAETRFALLETGFRLLSVAQLAPLLGLFGTVLGM	140
Q1YK5	319	-GVNKKEQ---PSVEALRLRLELAIVDEQQEIKKGLSMLLLAALAPMLGLLGTVTGMI	374
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Q5FQC2	165	HSFASIAQMHN-TNSLVVAPGISEALFATAIGLVTAIPAYIAYNGLSNSFEKFAADRMEAF	223
Q9A3H1	158	HAFQNIASLKN-TSLAVVAPSIAEALFATAIGLIAAIPAYIAYNKFSTDAGKYAGRLEGF	216
B2Q373	151	HAFIALGAVKQ-ATLQMVAPGIAEALIAATAIGLFAAIPAVMAYNRLNQVVKLEQSYDNF	209
A1VBP6	145	DAFHKLAKAKT-AAIASVAPGISEALIAATAVGLGVAIPAAIGYNLHMTVRRVQARLISL	203
B5EL52	137	HAFSVLATPGH--APTAVTGGVADALVATATGLFIAMGLMAFNAFNNOVRQIILLQDSV	194
Q256H5	158	VAFSHISTGHA--GGTAMMGLATALGTTIVGLFVAIPSLIGFNYLVAHSSRLILEIEQT	215
A3SCQ3	141	EAFRLSQAAGSQVDP SILAGGIWVALLTAVGLVVAMPTALILSWLEQFMEAEERVIADKA	200
Q1YK5	375	ETFQVITQFGN-GDPKVMAGGISTALVTTVLGLISAMPLLAHNILSTQADAVNILEKQ	433
		: * : * * * * * : :	
P0ABU7	213	AAQVLLQSRDL-DLEA-----SAAHPVREVAQ--KLEAG-----	244
Q5FQC2	224	GTEFAAILSRQS-EERA-----DDTTGGKA-----	247
Q9A3H1	217	ADDLSTAIQRRL-AERV-----	232
B2Q373	210	MEEFLAILHRQA-FSAD-----KK-----	227
A1VBP6	204	AGLTINTIMLETAPLPAVENGKPSSTAGDEAPAT--ARRHDAASLTPAQPVTSITDAAL	261
B5EL52	195	KTMLNRMGQPMITPDNS-DKQRSEMLS-----VARAS-----	227
Q256H5	216	AYLLLNSEIVKYEQTNL-----	232
A3SCQ3	201	ILTVLNPSNDVP---A-----PAAATPAPTPEMVAAHG-----	230
Q1YK5	434	GISLVAEQAQKV---G-----SAA-----	449

FIG. S1. Alignment of distantly related *ExbB* sequences. Distantly related *ExbB* sequences were aligned using clustal omega multiple sequence alignment program (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). Charged residues are highlighted in grey.

TOPCONS-predicted TMDs are indicated by a black bar above the sequence: TMD1 (22-42), TMD2 (132-152), TMD3 (178-198). Names of distantly related species, their corresponding accession numbers, and expect scores relative to ExbB from *E.coli* K12 are: *Escherichia coli* (P0ABU7, e^{-130}), *Gluconobacter oxydans* (Q5FQC2, $2e^{-28}$), *Caulobacter crescentus* (Q9A3H1, $9e^{-29}$), *Providencia stuartii* (B2Q373, $9e^{-21}$), *Desulfovibrio vulgaris* (A1VBP6, $1e^{-20}$), *Acidithiobacillus ferrooxidans* (B5EL52, $4e^{-7}$), *Chlamydophila felis* (Q256H5, $4e^{-7}$), *Sulfitobacter sp. EE-36* (A3SCQ3, $3e^{-7}$), *Photobacterium profundum* (Q1YYK5, $4e^{-7}$).

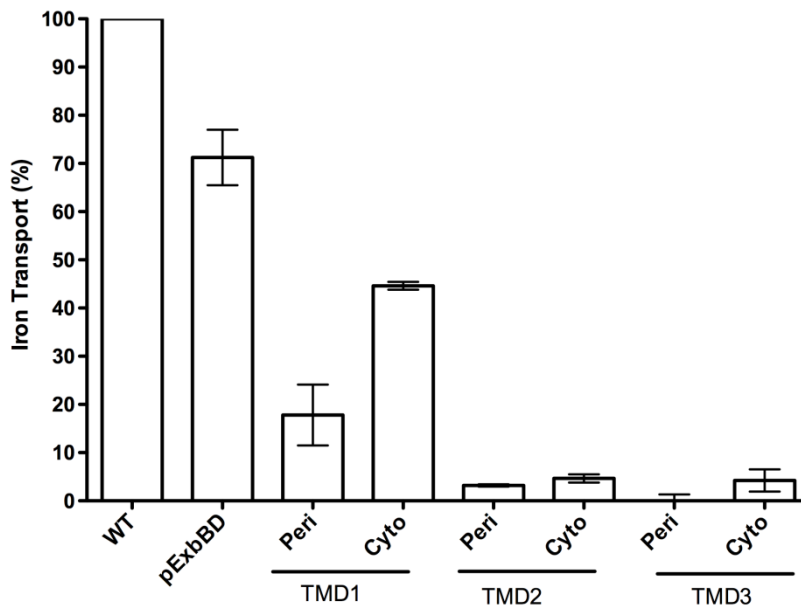


FIG. S2. *Most of the ExbB half-Ala substitutions are dominant.* ExbB half-Ala substitution mutants co-expressed with ExbD were induced at subculture with 0.01% L-arabinose in wild type (WT) strain W3110. All mutants were overexpressed greater than 100-fold as determined by immunoblot (data not shown). Initial rates of [^{55}Fe]-ferrichrome transport were determined from multiple triplicate experiments and normalized to W3110 (100%). Substituted portions and their corresponding plasmid numbers were: pExbBD (pKP660); TMD1 peri (pKP1459); TMD1 cyto (pKP1460); TMD2 peri (pKP1481), TMD2 cyto (pKP1482); TMD3 peri (pKP1483) ; TMD3 cyto (pKP1484).

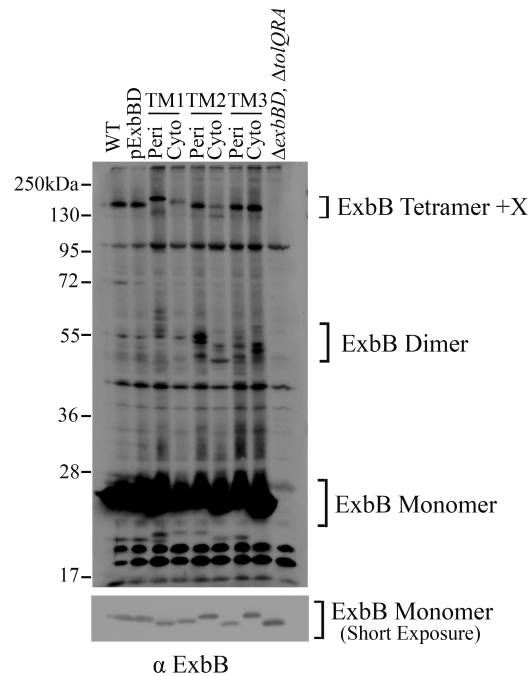
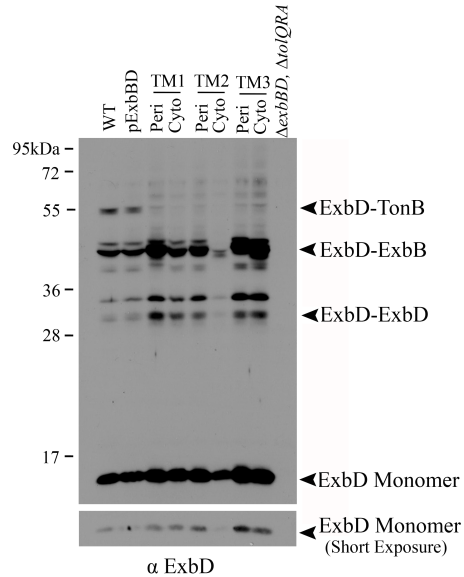


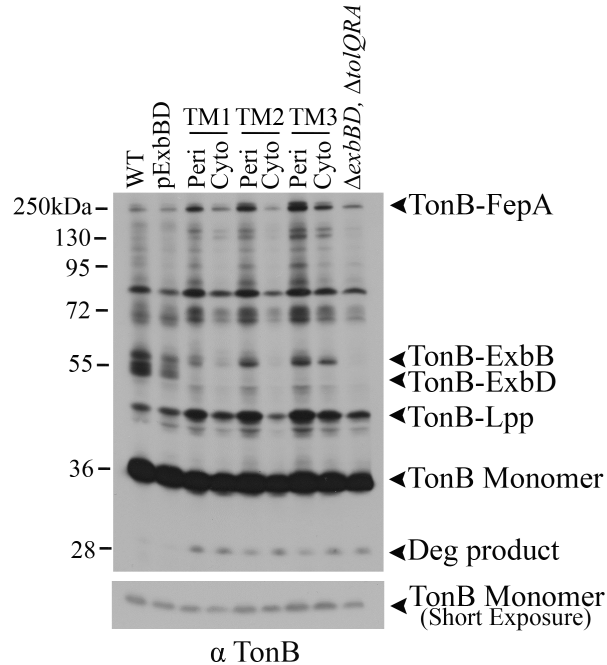
Fig. S3A. *ExbB* half-Ala TMDs do not form the *TonB-ExbD* complex. *ExbB* half-Ala mutants and the plasmid encoded (pExbBD) control were expressed to near chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$) and parent strain W3110 served as the wild type (WT) chromosomal control. The corresponding locations of the half-Ala substitutions are indicated above each lane as TMD1, TMD2 or TMD3 and Peri or Cyto. Cultures grown to mid-exponential phase were cross-linked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 11% or 13% SDS-polyacrylamide gels and immunoblotted with (A) anti-*ExbB*, (B) anti-*ExbD* or (C) anti-*TonB* antibodies. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels. Corresponding plasmid names and percentages of L-arabinose used to induce chromosomal level expression are as follows: pExbBD (pKP660) 0.0004%; TMD1-peri (pKP1459) 0.0005%; TMD1-cyto

(pKP1460) 0.0005%; TMD2-peri (pKP1481) 0.0005%, TMD2-cyto (pKP1482)
0.0009%; TMD3-peri (pKP1483) 0.0005%; TMD3-cyto (pKP1484) *0.0008%*.



S3B. *ExbB* half-Ala TMDs do not form the TonB-ExbD complex. *ExbB* half-Ala mutants and the plasmid encoded (pExbBD) control were expressed to near chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$) and parent strain W3110 served as the wild type (WT) chromosomal control. The corresponding locations of the half-Ala substitutions are indicated above each lane as TMD1, TMD2 or TMD3 and Peri or Cyto. Cultures grown to mid-exponential phase were cross-linked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 11% or 13% SDS-polyacrylamide gels and immunoblotted with (A) anti-ExbB, (B) anti-ExbD or (C) anti-TonB antibodies. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels. Corresponding plasmid names and percentages of L-arabinose used to induce chromosomal level expression are as follows: pExbBD (pKP660) 0.0004%; TMD1-peri (pKP1459) 0.0005%; TMD1-cyto (pKP1460) 0.0005%;

TMD2-peri (pKP1481) 0.0005%, TMD2-cyto (pKP1482) 0.0009%; TMD3-peri (pKP1483) 0.0005%; TMD3-cyto (pKP1484) *0.0008%*.



S3C. *ExbB* half-Ala TMDs do not form the *TonB-ExbD* complex. *ExbB* half-Ala mutants and the plasmid encoded (pExbBD) control were expressed to near chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$) and parent strain W3110 served as the wild type (WT) chromosomal control. The corresponding locations of the half-Ala substitutions are indicated above each lane as TMD1, TMD2 or TMD3 and Peri or Cyto. Cultures grown to mid-exponential phase were cross-linked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 11% or 13% SDS-polyacrylamide gels and immunoblotted with specific (A) anti-ExbB, (B) anti-ExbD or (C) anti-TonB antibodies. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels. Corresponding plasmid names and percentages of L-arabinose used to induce chromosomal level expression are as follows: pExbBD (pKP660) 0.0004%; TMD1-peri (pKP1459) 0.0005%; TMD1-cyto (pKP1460)

0.0005%; TMD2-peri (pKP1481) 0.0005%, TMD2-cyto (pKP1482) 0.0009%; TMD3-peri (pKP1483) 0.0005%; TMD3-cyto (pKP1484) *0.0008%*.

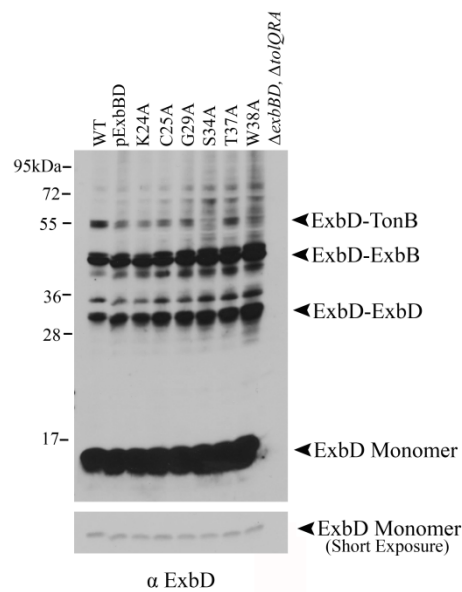


FIG. S4A. *Formaldehyde crosslinking profile of ExbB Ala-substituted mutants using anti-ExbD antibody.* ExbB Ala substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$). W3110 served as the wild type (WT) chromosomal control. ExbB TMD mutants are indicated above each lane. Cultures were grown to mid-exponential phase, crosslinked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 13% SDS-polyacrylamide gels and immunoblotted with anti-ExbD antibody. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels. (A) TMD1 ExbB Ala substitutions. (B) ExbB TMD2 Ala substitutions (C) ExbB TMD3 Ala substitutions. Aberrant migration of the ExbD-ExbB complex of ExbB E176A and ExbB P190A corresponds to the faster migration of the ExbB monomer (refer to Fig. 6)

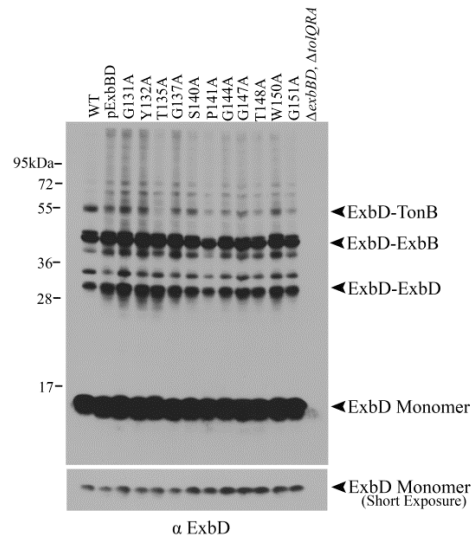


FIG. S4B. *Formaldehyde crosslinking profile of ExbB Ala substituted mutants using anti-ExbD antibody.* ExbB Ala substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$). W3110 served as the wild type (WT) chromosomal control. ExbB TMD mutants are indicated above each lane. Cultures were grown to mid-exponential phase, crosslinked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 13% SDS-polyacrylamide gels and immunoblotted with anti-ExbD antibody. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels. (A) TMD1 ExbB Ala substitutions. (B) ExbB TMD2 Ala substitutions (C) ExbB TMD3 Ala substitutions. Aberrant migration of the ExbD-ExbB complex of ExbB E176A and ExbB P190A corresponds to the faster migration of the ExbB monomer (refer to Fig. 6)

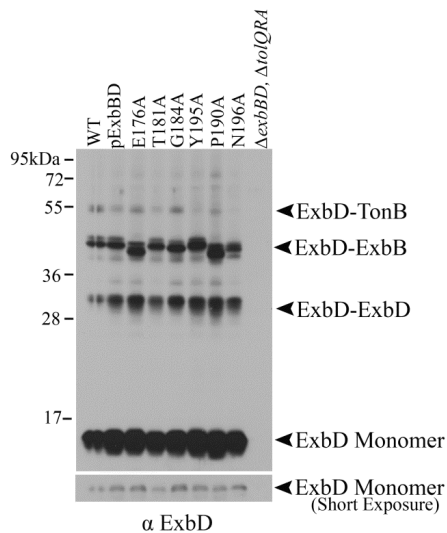


FIG. S4C. *Formaldehyde crosslinking profile of ExbB Ala substituted mutants using anti-ExbD antibody.* ExbB Ala substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$). W3110 served as the wild type (WT) chromosomal control. ExbB TMD mutants are indicated above each lane. Cultures were grown to mid-exponential phase, crosslinked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 13% SDS-polyacrylamide gels and immunoblotted with anti-ExbD antibody. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels. (A) TMD1 ExbB Ala substitutions. (B) ExbB TMD2 Ala substitutions (C) ExbB TMD3 Ala

substitutions. Aberrant migration of the ExbD-ExbB complex of ExbB E176A and ExbB P190A corresponds to the faster migration of the ExbB monomer (refer to Fig. 6)

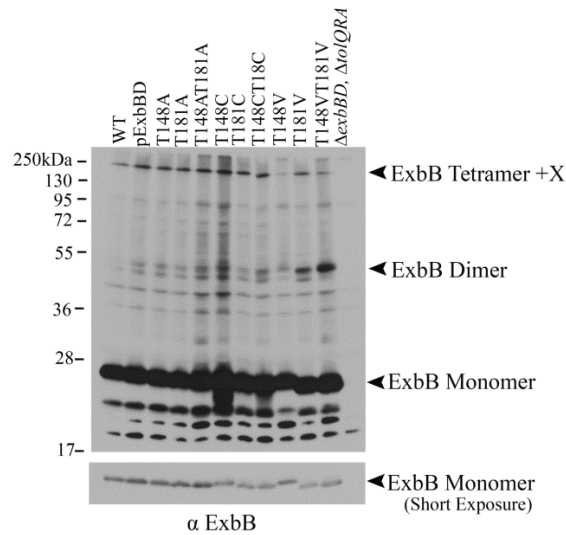


FIG. S5A. *Formaldehyde crosslinking profile of ExbB T148 and T181 substitutions.*

Substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$) and parent strain W3110 (WT) served as the wild type chromosomal control. Plasmids expressing ExbB substitutions are indicated above each lane. Cultures were grown to mid-exponential phase and crosslinked with monomeric formaldehyde then solubilized in LSB at 60°C. Samples were resolved on two 13% and one 11% SDS-polyacrylamide gels then immunoblotted with (A) anti-ExbB (B) anti-ExbD (C) anti-TonB antibodies. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels.

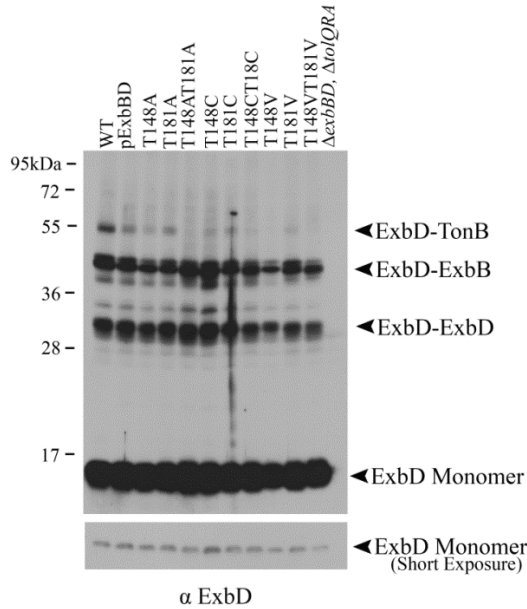


FIG. S5B. *Formaldehyde crosslinking profile of ExbB T148 and T181 substitutions.*

Substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta\text{exbBD} \Delta\text{tolQRA}$) and parent strain W3110 (WT) served as the wild type chromosomal control. Plasmids expressing ExbB substitutions are indicated above each lane. Cultures were grown to mid-exponential phase and crosslinked with monomeric formaldehyde then solubilized in LSB at 60°C. Samples were resolved on two 13% and one 11% SDS-polyacrylamide gels then immunoblotted with (A) anti-ExbB (B) anti-ExbD (C) anti-TonB antibodies. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels.

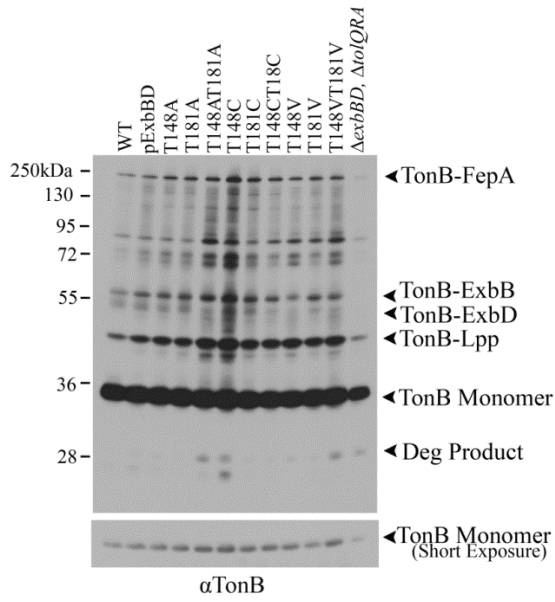


FIG. S5C. *Formaldehyde crosslinking profile of ExbB T148 and T181 substitutions.*

Substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$) and parent strain W3110 (WT) served as the wild type chromosomal control. Plasmids expressing ExbB substitutions are indicated above each lane. Cultures were grown to mid-exponential phase and crosslinked with monomeric formaldehyde then solubilized in LSB at 60°C. Samples were resolved on two 13% and one 11% SDS-polyacrylamide gels then immunoblotted with (A) anti-ExbB (B) anti-ExbD (C) anti-TonB antibodies. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels.

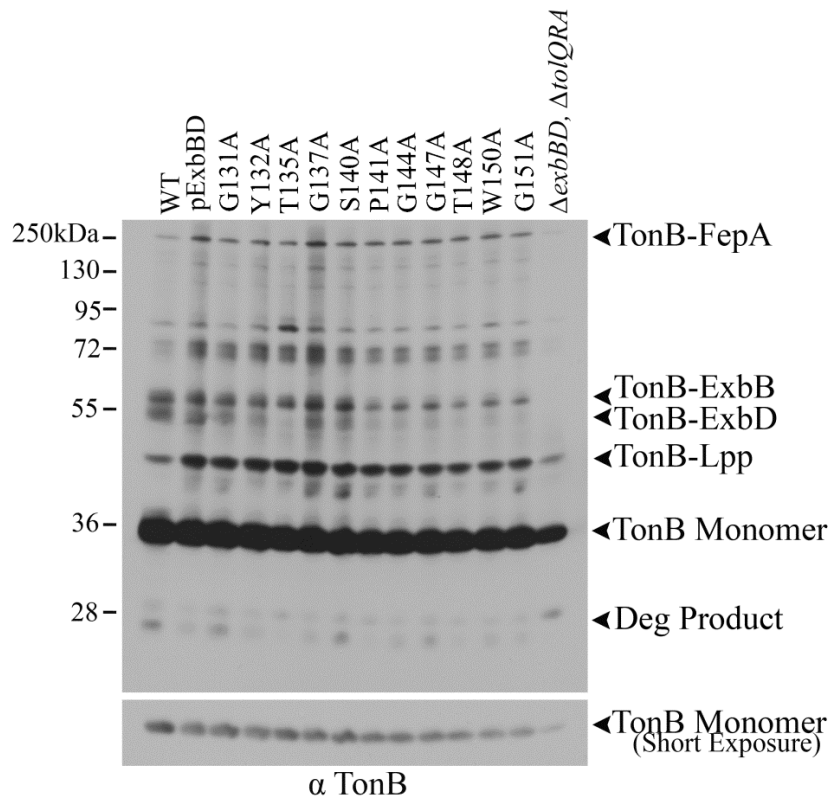


FIG. S6A. *Formaldehyde crosslinking profile of ExbB Ala substituted mutants using anti-TonB antibody.* ExbB Ala substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$) and parent strain W3110 (WT) served as the wild type chromosomal control. ExbB TMD mutants are indicated above each lane. Cultures grown to mid-exponential phase were crosslinked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 11% SDS-polyacrylamide gels and immunoblotted with anti-TonB antibody. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels. (A) ExbB TMD2 Ala substitutions (B) ExbB TMD3 Ala substitutions.

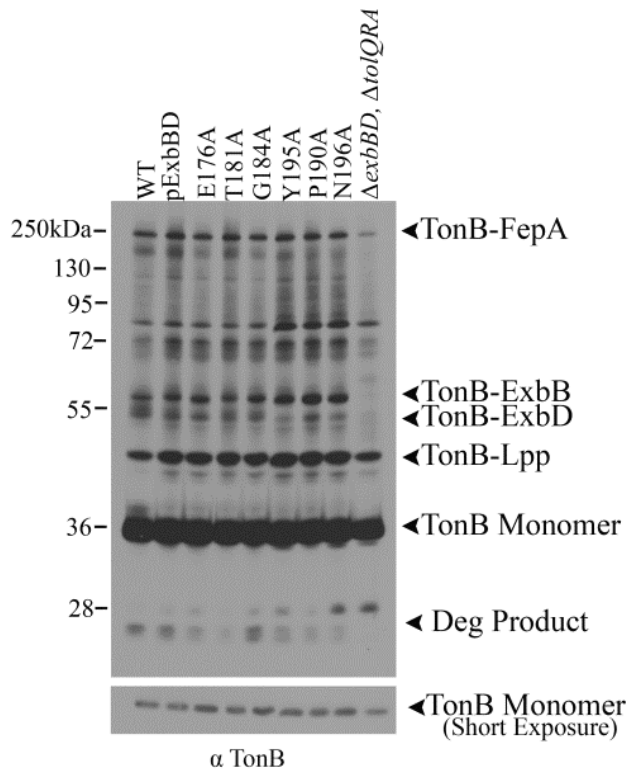


FIG. S6B. *Formaldehyde crosslinking profile of ExbB Ala substituted mutants using anti-TonB antibody.* ExbB Ala substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$) and parent strain W3110 (WT) served as the wild type chromosomal control. ExbB TMD mutants are indicated above each lane. Cultures grown to mid-exponential phase were crosslinked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 11% SDS-polyacrylamide gels and immunoblotted with anti-TonB antibody. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower

panel for monomer levels. (A) ExbB TMD2 Ala substitutions (B) ExbB TMD3 Ala substitutions.

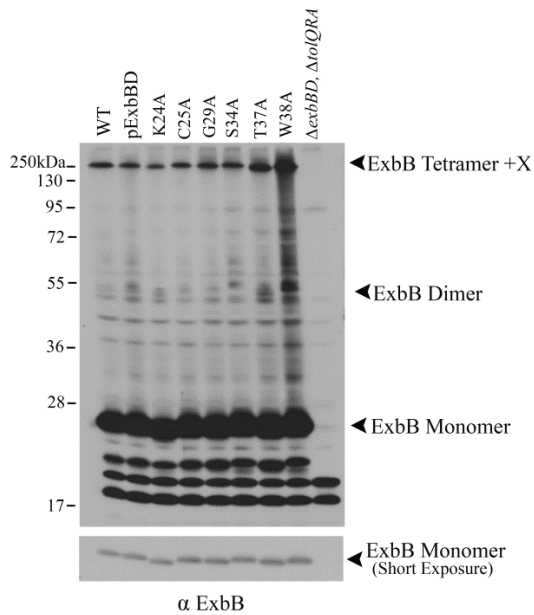


FIG. S7A. Formaldehyde crosslinking profile of ExbB Ala substituted mutants using anti-ExbB antibody. ExbB Ala substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$) and W3110 (WT) served as the wild type chromosomal control. ExbB TMD mutants are indicated above each lane. Cultures were grown to mid-exponential phase, crosslinked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 13% SDS-polyacrylamide gels and immunoblotted with anti-ExbB antibody. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels. (A) TMD1 ExbB Ala substitutions. (B) ExbB TMD2 Ala substitutions.

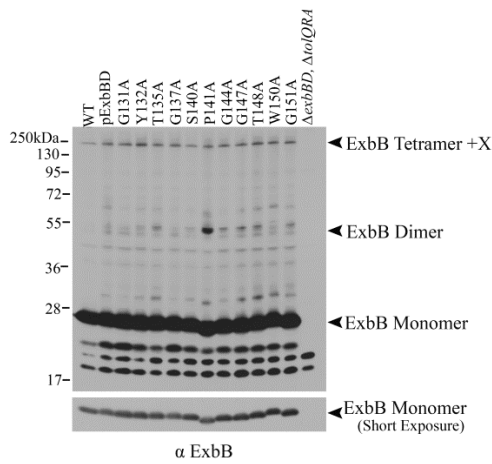


FIG. S7B. *Formaldehyde crosslinking profile of ExbB Ala substituted mutants using anti-ExbB antibody.* ExbB Ala substituted TMD mutants were expressed at chromosomal levels in strain RA1017 ($\Delta exbBD \Delta tolQRA$) and W3110 (WT) served as the wild type chromosomal control. ExbB TMD mutants are indicated above each lane. Cultures were grown to mid-exponential phase, crosslinked with monomeric formaldehyde and solubilized in LSB at 60°C. Samples were resolved on 13% SDS-polyacrylamide gels and immunoblotted with anti-ExbB antibody. Positions and composition of complexes are indicated on the right. Molecular mass standards are indicated on the left. A shorter exposure of the same immunoblot is shown in the lower panel for monomer levels. (A) TMD1 ExbB Ala substitutions. (B) ExbB TMD2 Ala substitutions.

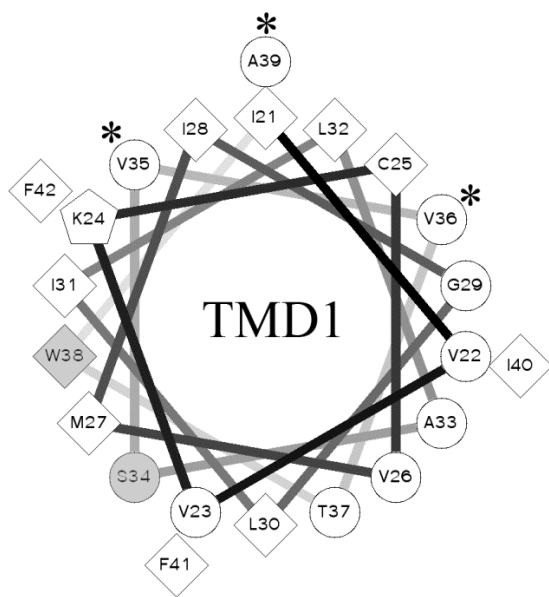


FIG. S9A. *Helical Wheel projection of ExbB TMDs*. ExbB TMD α -helices are projected as helical wheels from periplasm to cytoplasm, using the helical wheel projection program: (<http://rzlab.ucr.edu/scripts/wheel/wheel.cgi>). Functionally important residues are marked in gray. Hydrophilic residues are presented as circles, hydrophobic as diamonds, negatively and positively charged residues as triangles and pentagons respectively. (A) TMD1. The location of the TonB TMD ExbB suppressors, V35E, V36D and A39E, are indicated by asterisks (1, 2). (B) TMD2. Conserved Gly residues are indicated by asterisks. (C) TMD3.

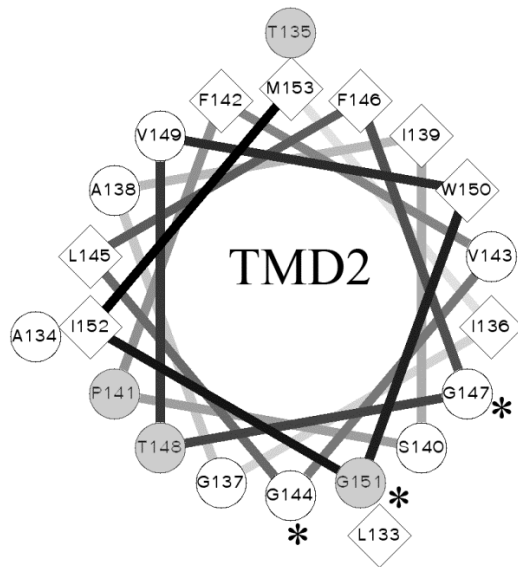


FIG. S9B. *Helical Wheel projection of ExbB TMDs.* ExbB TMD α -helices are projected as helical wheels from periplasm to cytoplasm, using the helical wheel projection program: (<http://rzlab.ucr.edu/scripts/wheel/wheel.cgi>). Functionally important residues are marked in gray. Hydrophilic residues are presented as circles, hydrophobic as diamonds, negatively and positively charged residues as triangles and pentagons respectively. (A) TMD1. The location of the TonB TMD ExbB suppressors, V35E, V36D and A39E, are indicated by asterisks (1, 2). (B) TMD2. Conserved Gly residues are indicated by asterisks. (C) TMD3.

References

1. **Larsen RA, Thomas MG, Postle K.** 1999. Protonmotive force, ExbB and ligand-bound FepA drive conformational changes in TonB. *Mol. Microbiol.* **31**:1809-1824.
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