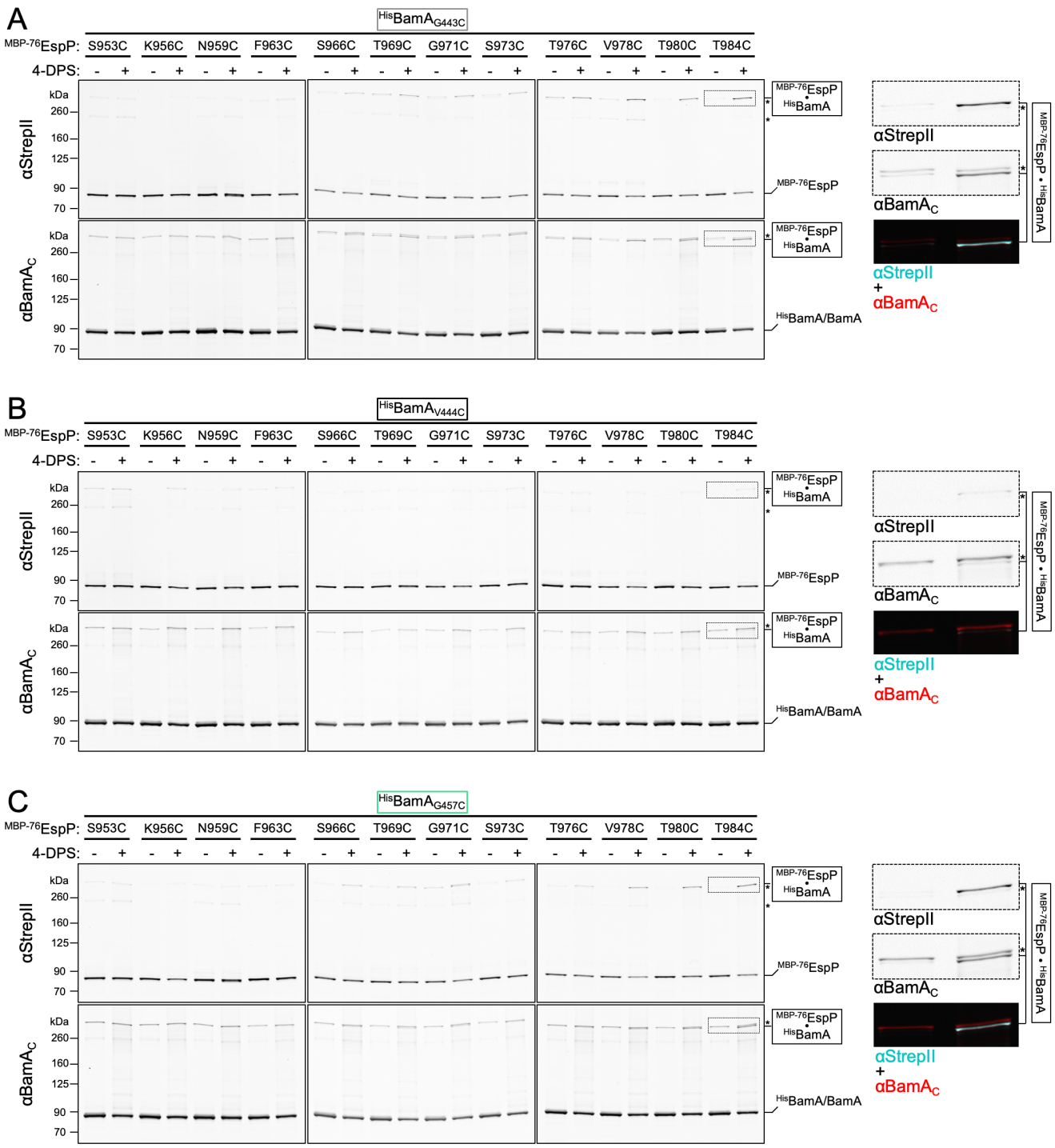
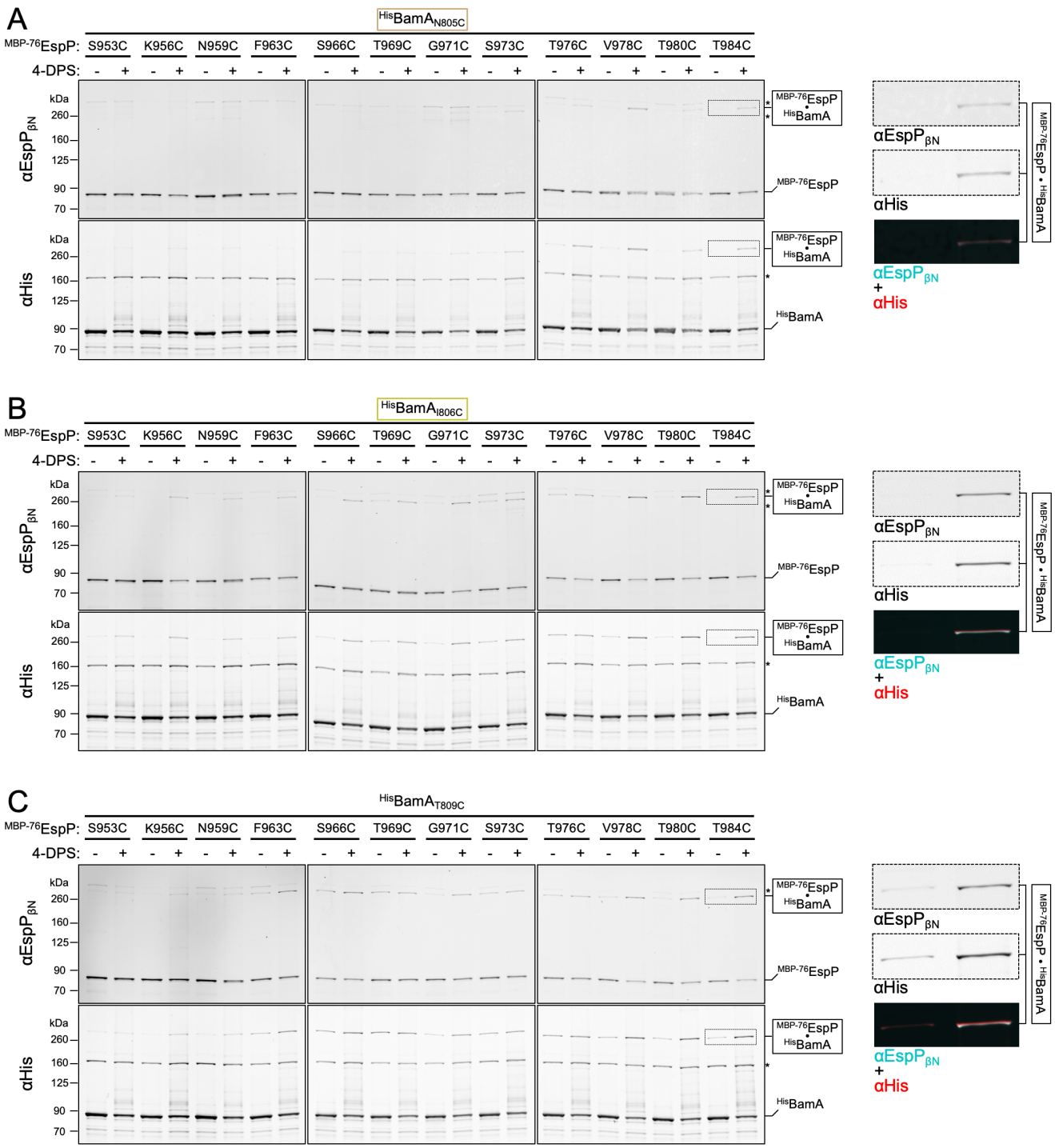


Supplementary Figure 1 (legend next page)

Supplementary Figure 1: Identification of disulfide-bonds between ^{His}BamA G443C, V444C, or G457C and $^{MBP\text{-}76}$EspP passenger domain cysteine mutants, relating to Figure 2.

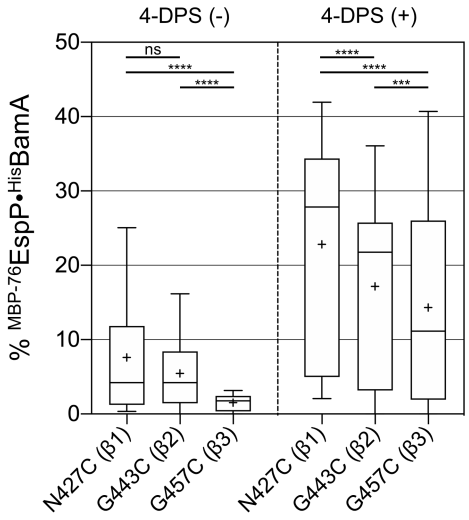
E. coli BL21(DE3) transformed with a plasmid expressing $^{MBP\text{-}76}$EspP with a single cysteine substitution at the indicated residue and a plasmid expressing ^{His}BamA$_{G443C}$BCDE (**A**), ^{His}BamA$_{V444C}$BCDE (**B**), or ^{His}BamA$_{G457C}$BCDE (**C**) were mock treated (-) or treated with 4-DPS (+), N = 3. Proteins were then detected by double-immunoblot using αStrepII antibodies (top) and αBamA$_C$ (bottom) antiserum to monitor disulfide-bond formation between cysteine pairs *in vivo* (•). Right, magnification of boxed regions of immunoblots showing examples of disulfide-bonds detected between ^{His}BamA cysteine positions and $^{MBP\text{-}76}$EspP$_{T984C}$. In the bottom panel, the αStrepII (cyan) and αBamA$_C$ (red) signals were overlaid. Non-specific side-reactions are denoted (*).



Supplementary Figure 2 (legend next page)

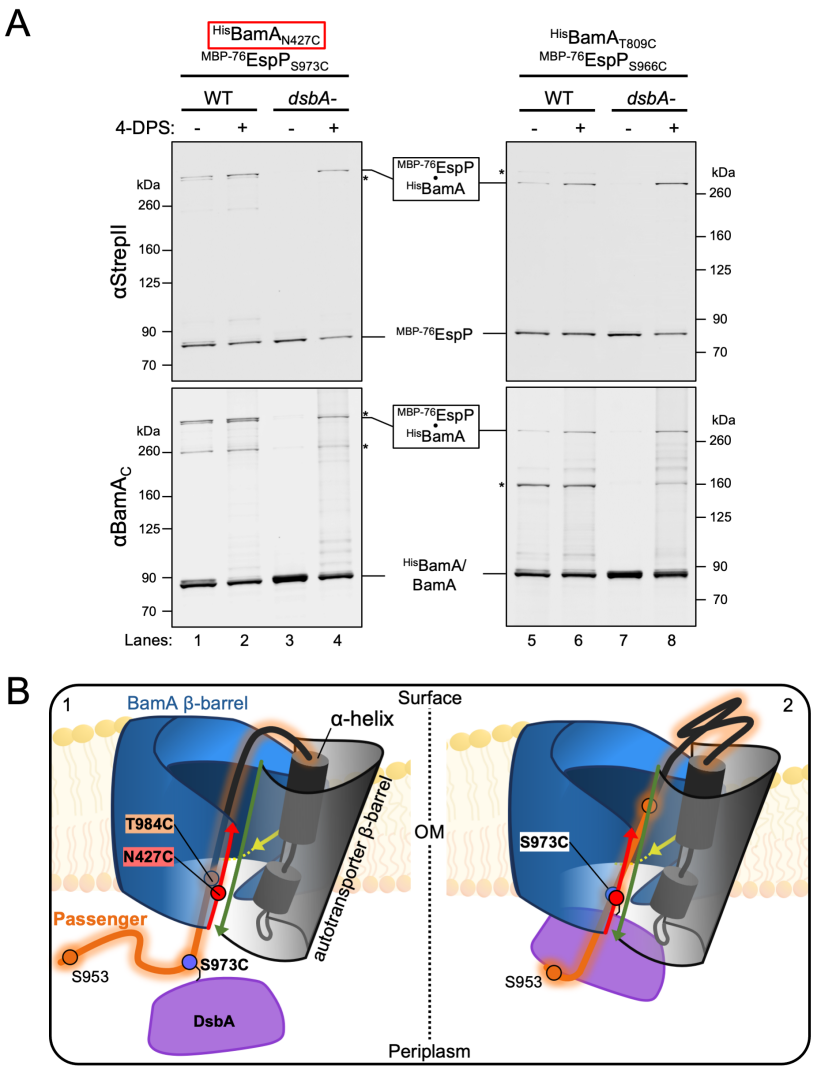
Supplementary Figure 2: Identification of disulfide-bonds between ^{His}BamA N805C, I806C, or T809C and $^{MBP\text{-}76}$EspP passenger domain cysteine mutants, relating to Figure 2.

E. coli BL21(DE3) transformed with a plasmid expressing $^{MBP\text{-}76}$EspP with a single cysteine substitution at the indicated residue and a plasmid expressing ^{His}BamA$_{N805C}$BCDE (**A**), ^{His}BamA$_{I806C}$BCDE (**B**), or ^{His}BamA$_{T809C}$BCDE (**C**) were mock treated (-) or treated with 4-DPS (+), N = 3. Proteins were then detected by double-immunoblot using an antiserum against EspP$_{\beta N}$ (top) and antibodies against the N-terminus of ^{His}BamA (αHis) (bottom) to monitor disulfide-bond formation between cysteine pairs *in vivo* (•). Right, magnification of boxed regions of immunoblots showing examples of disulfide-bonds detected between ^{His}BamA cysteine positions and $^{MBP\text{-}76}$EspP$_{T984C.}$ In the bottom panel the αEspP$_{\beta N}$ (cyan) and αHis (red) signals were overlaid. Non-specific side-reactions are denoted (*).



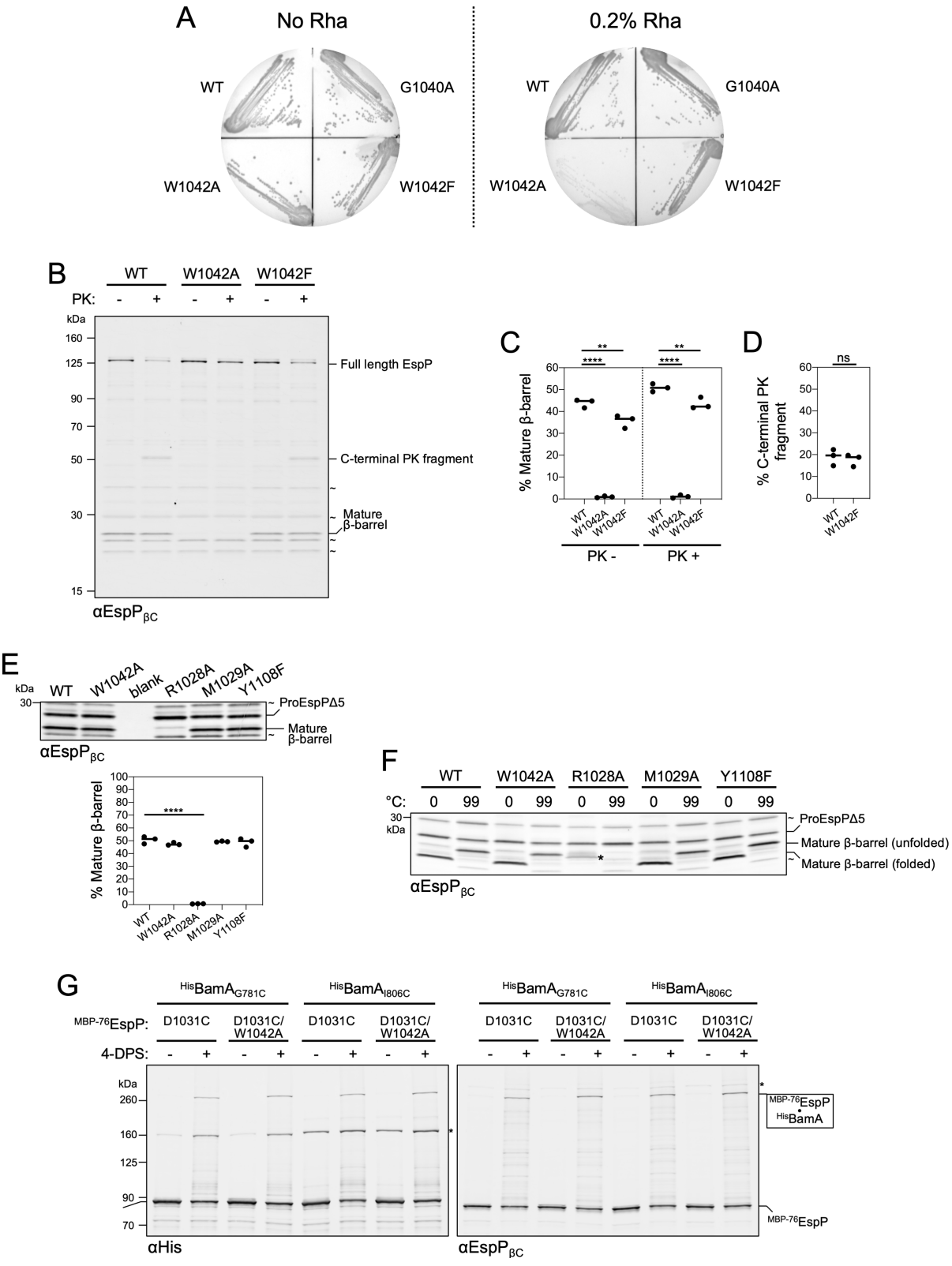
Supplementary Figure 3: The EspP passenger domain forms disulfide-bonds most efficiently with BamAβ1 and least efficiently with BamAβ3, relating to Figure 2B.

Box and whisker plots of mean disulfide-bond formation levels (•) of all $^{MBP\text{-}76}$EspP passenger domain cysteine substitution mutants with ^{His}BamA$_{N427C}$, ^{His}BamA$_{G443C}$, and ^{His}BamA$_{G457C}$ substitution mutants in Figure 2B. Whiskers: data range; plus symbols on boxes: mean-of-means. ANOVA and multiple comparisons tests are provided in Table S1.



Supplementary Figure 4: Spatially-restricted disulfide-bond formation in the absence of 4-DPS is DsbA-dependent, relating to Figure 2.

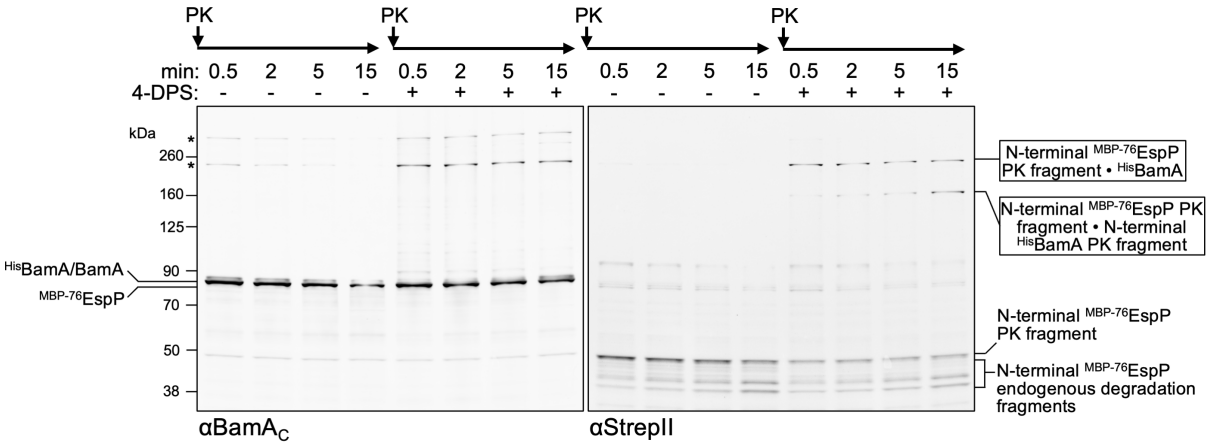
(A) *E. coli* AD202 and RI2 (AD202 *dsbA*$^{-}$) that expressed $^{MBP\text{-}76}EspP_{S973C}/^{His}BamA_{N427C}$ or $^{MBP\text{-}76}EspP_{S966C}/^{His}BamA_{T809C}$ were mock treated (-) or treated with 4-DPS (+). Proteins were then detected by double-immunoblot using αStrepII (top) and $\alpha BamA_C$ (bottom) to monitor disulfide-bond formation between cysteine pairs *in vivo* (•), N = 2. Non-specific side-reactions are denoted (*). **(B)** Model of DsbA-dependent disulfide bond formation that explains 4-DPS (-) data in Figure 2b. A cysteine in DsbA initially forms a disulfide bond with the exposed passenger in the periplasm (1) that is exchanged with a cysteine in BamA when the passenger enters the channel (2). Steric hinderance by the channel inhibits disulfide bonding between the C-terminal region of the passenger and DsbA. The MBP moiety immediately upstream of S953 prevents entry of the N-terminus of the passenger into the channel. Relevant $^{MBP\text{-}76}EspP$ and $^{His}BamA$ cysteine substitutions are depicted.



Supplementary Figure 5 (legend next page)

Supplementary Figure 5: Characterization of W1040 mutant phenotypes, and assembly and structural effects, relating to Figures 4 and 5.

(A) *E. coli* AD202 transformed with a plasmid that expresses EspPΔ1, EspPΔ1$_{G1040A}$, EspPΔ1$_{W1042A}$, or EspPΔ1$_{W1042F}$ were streaked on LB agar with or without 0.2% L-rhamnose and incubated at 37 °C for 16 h. N = 2. **(B,C,D)** *E. coli* AD202 that expressed wild-type EspP, EspP$_{W1042A}$ or EspP$_{W1042F}$ were either mock treated or treated with PK. Assembly of the protein into a mature cleaved β-barrel was monitored by immunoblotting **(B)** using an antiserum against the EspP β-barrel C-terminus (αEspP$_{βC}$). Non-specific bands are denoted (~). Levels of mature β-barrel (**C**) and C-terminal PK fragments (**D**) were quantified (lines at median, N = 3). For ANOVA and multiple comparisons tests see Table S1. **(E)** Experiment conducted as in **B** except bacteria expressed EspPΔ5 or an EspPΔ5 substitution mutant. Example immunoblot (top) and quantified (bottom). Lines at median, N = 3. ANOVA and multiple comparisons tests are in Supplementary Table S1. Non-specific bands are denoted (~). **(F)** Experiment conducted as in **B** and **E** except that cells were lysed and lysates were either not heated (0 °C) or heated (99 °C) before proteins were resolved by cold-SDS-PAGE. N = 2. The folded form of proEspPΔ5$_{R1028A}$ (*) and non-specific bands (~) are denoted. **(G)** *E. coli* BL21(DE3) expressing ^{His}BamA$_{G781C}$BCDE or ^{His}BamA$_{I806C}$BCDE and $^{MBP\text{-}76}$EspP$_{D1031C}$ with or without the W1042A substitution were mock treated (-) or treated with 4-DPS (+) (N = 2). Proteins were then detected by double-immunoblot using αHis (left) and αEspP$_{βC}$ (right) to monitor disulfide-bond formation between cysteine pairs (•). Side-reactions are denoted (*).



Supplementary Figure 6: Arrest-release assembly assay samples double-immunoblotted with αBamA$_C$ and αStrepII, related to Figure 6.

E. coli BL21(DE3) transformed with plasmids expressing ^{His}BamA$_{N427C}$BCDE and $^{MBP\text{-}76}$EspP$_{T984C}$ were mock treated (-) or treated with 4-DPS (+) and subsequently incubated with PK for up to 15 min. Proteins were detected by double-immunoblot using αBamA (left) antiserum and αStrepII (right) antibodies. Combined with data presented in Figure 6, these results show that the ~ 160 kDa fragment contains N-terminal PK fragments of both $^{MBP\text{-}76}$EspP$_{T984C}$ and ^{His}BamA$_{N427C}$ that are disulfide bonded. Side-reactions are denoted (*).

Plasmid	Notes	Source
pRha-MBP-EspPΔ1	pSCrhaB2::*espP*$_{SS}$-*malE*-*espP*$_{909\text{-}1300}$, P_{rhaB}, Tmp^R	1
pMTD372	pTrc99a::^{His8}*bamABCDE*, P_{Trc}, Amp^R	1
pMTD607	'pRha$^{MBP\text{-}76}$EspP' (pSCrhaB2::*espP*$_{SS}$-*TS*-*malE*-*espP*$_{948\text{-}984}$-*tev*-*espP*$_{985\text{-}1300}$), P_{rhaB}, Tmp^R	1
pMTD710	pTrc99a::^{His8}*bamA*$_{S425C}$-*BCDE*	1
pMTD712	pRha$^{MBP\text{-}76}$EspP$_{S1299C}$	1
pMTD792	pRha$^{MBP\text{-}76}$EspP$_{R1297C}$	1
pMTD820	pTrc99a::^{His8}*bamA*$_{T809C}$-*BCDE*	1
pMTD829	pTrc99a::^{His8}*bamA*$_{N427C}$-*BCDE*	1
pMTD893	pTrc99a::^{His8}*bamA*$_{G781C}$-*BCDE*	1
pMTD896	pTrc99a::^{His8}*bamA*$_{N805C}$-*BCDE*	1
pMTD957	pTrc99a::^{His8}*bamA*$_{I806C}$-*BCDE*	1
pMTD1029	pTrc99a::^{His8}*bamA*$_{G443C}$-*BCDE*	1
pJH207	'pRhaEspPΔ5' [pSCrhaB2::*ompA*$_{SS}$-*espP*$_{998\text{-}1300}$ (*espPΔ5*)], P_{rhaB}, Tmp^R	2
pWK21	'pRhaEspP' [pSCrhaB2:: *espP*], P_{rhaB}, Tmp^R	3
pMTD405	'pRhaEspPΔ1'[pSCrhaB2::*espP*$_{SS}$-*TS*-*espP*$_{909\text{-}1300}$ (*espPΔ1*)]	This work
pMTD802	pRha$^{MBP\text{-}76}$EspP$_{S953C}$ (*espP*$_{S953C}$ substitution via QC on pMTD607 with mtd224/225 primers)	This work
pMTD804	pRha$^{MBP\text{-}76}$EspP$_{K956C}$ (*espP*$_{K956C}$ substitution via QC on pMTD607 with mtd226/227 primers)	This work
pMTD806	pRha$^{MBP\text{-}76}$EspP$_{N959C}$ (*espP*$_{N959C}$ substitution via QC on pMTD607 with mtd228/229 primers)	This work
pMTD808	pRha$^{MBP\text{-}76}$EspP$_{F963C}$ (*espP*$_{F963C}$ substitution via QC on pMTD607 with mtd230/231 primers)	This work
pMTD810	pRha$^{MBP\text{-}76}$EspP$_{S966C}$ (*espP*$_{S966C}$ substitution via QC on pMTD607 with mtd232/233 primers)	This work
pMTD812	pRha$^{MBP\text{-}76}$EspP$_{T976C}$ (*espP*$_{T976C}$ substitution via QC on pMTD607 with mtd238/239 primers)	This work
pMTD813	pRha$^{MBP\text{-}76}$EspP$_{T980C}$ (*espP*$_{T980C}$ substitution via QC on pMTD607 with mtd240/241 primers)	This work
pMTD886	pRha$^{MBP\text{-}76}$EspP$_{S973C}$ (*espP*$_{S973C}$ substitution via QC on pMTD607 with mtd236/237 primers)	This work
pMTD899	pRha$^{MBP\text{-}76}$EspP$_{T969C}$ (*espP*$_{T969C}$ substitution via QC on pMTD607 with mtd234/235 primers)	This work
pMTD1115	pRha$^{MBP\text{-}76}$EspP$_{W1042A/S1299C}$ (*espP*$_{W1042A/S1299C}$ substitution via QC on pMTD712 with mtd295/296 primers)	This work
pMTD1144	pRhaEspPΔ1$_{W1042A}$ (*espPΔ1*$_{W1042A}$ substitution via QC on pMTD405 with mtd295/296 primers)	This work
pMTD1166	pRhaEspPΔ5$_{W1042A}$ (*espPΔ5*$_{W1042A}$ substitution via QC on pJH207 with mtd295/296 primers)	This work
pMTD1293	pRha$^{MBP\text{-}76}$EspP$_{V978C}$ (*espP*$_{V978C}$ substitution via QC on pMTD607 with mtd313/314 primers)	This work
pMTD1294	pRha$^{MBP\text{-}76}$EspP$_{G971C}$ (*espP*$_{G971C}$ substitution via QC on pMTD607 with mtd309/310 primers)	This work
pMTD1316	pTrc99a::^{His8}*bamA*$_{Y444C}$-*BCDE* (*bamA*$_{Y444C}$ substitution via QC on pMTD372 with mtd322/323 primers)	This work
pMTD1317	pTrc99a::^{His8}*bamA*$_{G457C}$-*BCDE* (*bamA*$_{G457C}$ substitution via QC on pMTD372 with mtd324/325 primers)	This work
pMTD1369	pRhaEspPΔ1$_{G1040A}$ (*espPΔ1*$_{G1040A}$ substitution via QC on pMTD405 with mtd293/294 primers)	This work
pMTD1370	pRhaEspPΔ1$_{W1042F}$ (*espPΔ1*$_{W1042F}$ substitution via QC on pMTD405 with mtd297/298 primers)	This work
pMTD1373	pRha$^{MBP\text{-}76}$EspP$_{T984C}$ (*espP*$_{T984C}$ substitution via QC on pMTD607 with mtd330/331 primers)	This work
pMTD1423	pRha$^{MBP\text{-}76}$EspP$_{T984C/W1042A}$ (*espP*$_{W1042A}$ substitution via QC on pMTD1373 with mtd295/296 primers)	This work
pMTD1443	pRha$^{MBP\text{-}76}$EspP$_{S966C/W1042A}$ (*espP*$_{W1042A}$ substitution via QC on pMTD810 with mtd295/296 primers)	This work
pMTD1446	pRha$^{MBP\text{-}76}$EspP$_{R1028A}$ (*espP*$_{R1028A}$ substitution via QC on pMTD607 with mtd341/342 primers)	This work
pMTD1449	pRha$^{MBP\text{-}76}$EspP$_{D1031C}$ (*espP*$_{D1031C}$ substitution via QC on pMTD607 with mtd345/346 primers)	This work
pMTD1450	pRha$^{MBP\text{-}76}$EspP$_{Y1108F}$ (*espP*$_{Y1108F}$ substitution via QC on pMTD607 with mtd347/348 primers)	This work
pMTD1464	pRha$^{MBP\text{-}76}$EspP$_{M1029A}$ (*espP*$_{M1029A}$ substitution via QC on pMTD607 with mtd343/344 primers)	This work
pMTD1472	pRha$^{MBP\text{-}76}$EspP$_{N1026C}$ (*espP*$_{N1026C}$ substitution via QC on pMTD607 with mtd339/340 primers)	This work
pMTD1479	pRha$^{MBP\text{-}76}$EspP$_{W1042A}$ (*espP*$_{W1042A}$ substitution via QC on pMTD607 with mtd295/296 primers)	This work
pMTD1481	pRha$^{MBP\text{-}76}$EspP$_{D1031C/W1042A}$ (*espP*$_{D1031C/W1042A}$ substitution via QC on pMTD1449 with mtd295/296 primers)	This work
pMTD1498	pRha$^{MBP\text{-}76}$EspP$_{N1026C/W1042A}$ (*espP*$_{N1026C/W1042A}$ substitution via QC on pMTD1472 with mtd295/296 primers)	This work
pMTD1505	pRha$^{MBP\text{-}76}$EspP$_{W1042F}$ (*espP*$_{W1042F}$ substitution via QC on pMTD607 with mtd297/298 primers)	This work
pMTD1510	pRhaEspP$_{W1042A}$ (*espP*$_{W1042A}$ substitution via QC on pWK21 with mtd295/296 primers)	This work
pMTD1511	pRhaEspP$_{W1042F}$ (*espP*$_{W1042F}$ substitution via QC on pWK21 with mtd297/298 primers)	This work

Supplementary Table 2: Plasmids used in this study, related to STAR Methods.

Abbreviations: SS = signal sequence, *malE* = *malE* codons *26-392*, MBP = maltose binding protein (26-392), Amp^R = Ampicillin resistance, Tmp^R = Trimethoprim resistance, TS = TwinStrepII tag, His/His8 = His x 8 tag, QC = QuikChange site directed mutagenesis, GA = Gibson Assembly.

(1) Doyle, M.T., and Bernstein, H.D. (2019) Bacterial outer membrane proteins assemble via asymmetric interactions with the BamA β-barrel. Nat Commun 10, 3358.

(2) Peterson, J.H., Plummer, A.M., Fleming, K.G., Bernstein, H.D. (2017) Selective pressure for rapid membrane integration constrains the sequence of bacterial outer membrane proteins. Mol Microbiol *106*, 777-792.

(3) Kang'ethe, W., and Bernstein, H.D. (2013) Charge-dependent secretion of an intrinsically disordered protein via the autotransporter pathway. Proc Natl Acad Sci USA *110*, E4246-E4255.

(Supplementary Table 3)

DNA	Sequence	Notes
ssDNA oligos		
mtd1	TAATCATCCGGCTCGTATAATGTG	F, sequencing primer, pTrc99a
mtd20	GGCATGGGGTCAGGTGG	R, sequencing primer, pTrc99a
mtd42	CATCACGTTCATCTTTCCCTGG	F, sequencing primer, pSCrhaB2
mtd43	CGGCGCTACGGCGTTTCAC	R, sequencing primer, pSCrhaB2
mtd96	GGCCGCAAAAGAATATGAGG	R, primer to linearize pMTD294 for TwinStrepII insertion (mtd95) between *espP(C)$_{SP}$* and *malE* via GA
mtd100	GCTGAGCGCAGGTATTAACGC	F, sequencing primer, *malE* middle coding region
mtd110	ACCCAGGTCAGTCTGACGCCAGAT	F, sequencing primer, *bamA* second (pTrc99a::^{His8}*bamABCDE*)
mtd111	TGGTACAGACGTGACGTTGGGCTT	F, sequencing primer, *bamA* third (pTrc99a::^{His8}*bamABCDE*)
mtd112	CTGGTGTTCCAGGAAGGTGTGTCA	F, sequencing primer, *bamA* first (pTrc99a::^{His8}*bamABCDE*)
mtd113	CGGGTATCGCATTACAATGGATGTC	F, sequencing primer, *bamA* into *bamB* (pTrc99a::^{His8}*bamABCDE*)
mtd114	GGTGTTAATCCACACCAGTAACGG	F, sequencing primer, *bamB* second (pTrc99a::^{His8}*bamABCDE*)
mtd115	CGTCGAAGATGGTCGTTTCGTTGCC	F, sequencing primer, *bamB* into *bamC* (pTrc99a::^{His8}*bamABCDE*)
mtd116	CCCAACGTGATGATGCTGGTCAGAC	F, sequencing primer, *bamC* second (pTrc99a::^{His8}*bamABCDE*)
mtd117	TGACGCGCATGAAATATCTGGTGGC	F, sequencing primer, *bamD* first (pTrc99a::^{His8}*bamABCDE*)
mtd118	TACTCCGTGGCCGAGTACTATACAG	F, sequencing primer, *bamD* into *bamE* (pTrc99a::^{His8}*bamABCDE*)
mtd119	GATGAGATCCAGCTGCACCTGCTGC	R, sequencing primer, *bamD* backwards into *bamC* (pTrc99a::^{His8}*bamABCDE*)
mtd120	CGCATCAGGCCAGTAATTATGATC	F, sequencing primer, *bamA* fourth (pTrc99a::^{His8}*bamABCDE*)
mtd127	GCGATGCGTACAAACCTTTC	F, sequencing primer, *espP* starting at codon 909
mtd143	GTGCGTACTGCGGTGATCAAC	F, sequencing primer, *malE* C-terminus coding region
mtd224	GAATATTGAACTGGTATGCGCGCCAAAAGACACC	F, QC primer for *espP S953C* substitution
mtd225	GGTGTCTTTTGGCGCGCATACCAGTTCAATATTC	R, QC primer for *espP S953C* substitution
mtd226	GAATATTGAACTGGTAAGCGCGCCATGCGACACCAATGAAAATGTC	F, QC primer for *espP K956C* substitution
mtd227	GACATTTTCATTGGTGTCGCATGGCGCGCTTACCAGTTCAATATTC	R, QC primer for *espP K956C* substitution
mtd228	CTGGTAAGCGCGCCAAAAGACACCTGTGAAAATGTCTTTAAAGCC	F, QC primer for *espP N959C* substitution
mtd229	GGCTTTAAAGACATTTTCACAGGTGTCTTTTGGCGCGCTTACCAG	R, QC primer for *espP N959C* substitution
mtd230	GACACCAATGAAAATGTCTGTAAAGCCAGTAAACAAACCATTGG	F, QC primer for *espP F963C* substitution
mtd231	CCAATGGTTTGTTTACTGGCTTTACAGACATTTTCATTGGTGTC	R, QC primer for *espP F963C* substitution
mtd232	CACCAATGAAAATGTCTTTAAAGCCTGTAAACAAACCATTGGTTTC	F, QC primer for *espP S966C* substitution
mtd233	GAAACCAATGGTTTGTTTACAGGCTTTAAAGACATTTTCATTGGTG	R, QC primer for *espP S966C* substitution
mtd234	GTCTTTAAAGCCAGTAAACAATGCATTGGTTTCAGTGATGTAACGC	F, QC primer for *espP T969C* substitution
mtd235	GCGTTACATCACTGAAACCAATGCATTGTTTACTGGCTTTAAAGAC	R, QC primer for *espP T969C* substitution
mtd236	CCAGTAAACAAACCATTGGTTTCTGTGATGTAACGCC	F, QC primer for *espP S973C* substitution
mtd237	GGCGTTACATCACAGAAACCAATGGTTTGTTTACTGG	R, QC primer for *espP S973C* substitution
mtd238	CCATTGGTTTCAGTGATGTATGCCCGGTCATTACAACCAGGG	F, QC primer for *espP T976C* substitution
mtd239	CCCTGGTTGTAATGACCGGGCATACATCACTGAAACCAATGG	R, QC primer for *espP T976C* substitution
mtd240	GATGTAACGCCGGTCATTTGCACCAGGGAAACCGATGAC	F, QC primer for *espP T980C* substitution

Continued next page

mtd241	GTCATCGGTTTCCCTGGTGCAAATGACCGGCGTTACATC	R, QC primer for *espP T980C* substitution
mtd293	CAACGGCGAAGCCGCTGCATGGGCACGCATC	F, QC primer for *espP G1040A* substitution
mtd294	GATGCGTGCCCATGCAGCGGCTTCGCCGTTG	R, QC primer for *espP G1040A* substitution
mtd295	CGAAGCCGGTGCAGCGGCACGCATCATG	F, QC primer for *espP W1042A* substitution
mtd296	CATGATGCGTGCCGCTGCACCGGCTTCG	R, QC primer for *espP W1042A* substitution
mtd297	CGAAGCCGGTGCATTTGCACGCATCATGAGCGG	F, QC primer for *espP W1042F* substitution
mtd298	CCGCTCATGATGCGTGCAAATGCACCGGCTTCG	R, QC primer for *espP W1042F* substitution
mtd309	GCCAGTAAACAAACCATTTGTTTCAGTGATGTAACGCC	F, QC primer for *espP G971C* substitution
mtd310	GGCGTTACATCACTGAAACAAATGGTTTGTTTACTGGC	R, QC primer for *espP G971C* substitution
mtd313	CAGTGATGTAACGCCGTGCATTACAACCAGGGAAACCG	F, QC primer for *espP V978C* substitution
mtd314	CGGTTTCCCTGGTTGTAATGCACGGCGTTACATCACTG	R, QC primer for *espP V978C* substitution
mtd322	GCGTGAGCTTCCAGGCTGGTTGCCAGCAGGATAACTGG	F, QC primer for BamA *V444C* substitution
mtd323	CCAGTTATCCTGCTGGCAACCAGCCTGGAAGCTCACGC	R, QC primer for BamA *V444C* substitution
mtd324	GGTTAGGTACAGGTTATGCTGTTTGTATCAACGGGACC	F, QC primer for BamA *G457C* substitution
mtd325	GGTCCCGTTGATACAAACAGCATAACCTGTACCTAACC	R, QC primer for BamA *G457C* substitution
mtd330	CCGGTCATTACAACCAGGGAATGCGGCGAAAACCTGTATTTTCAG	F, QC primer for *espP T984C* substitution
mtd331	CTGAAAATACAGGTTTTCGCCGCATTCCCTGGTTGTAATGACCGG	R, QC primer for *espP T984C* substitution
mtd339	CGAGGTCAACAACCTGTGCAAACGTATGGGTGACC	F, QC primer for *espP N1026C* substitution
mtd340	GGTCACCCATACGTTTGCACAGGTTGTTGACCTCG	R, QC primer for *espP N1026C* substitution
mtd341	GGTCAACAACCTGAACAAAGCTATGGGTGACCTGCG	F, QC primer for *espP R1028A* substitution
mtd342	CGCAGGTCACCCATAGCTTTGTTCAGGTTGTTGACC	R, QC primer for *espP R1028A* substitution
mtd343	GGTCAACAACCTGAACAAACGTGCGGGTGACCTGCGTGATATC	F, QC primer for *espP M1029A* substitution
mtd344	GATATCACGCAGGTCACCCGCACGTTTGTTCAGGTTGTTGACC	R, QC primer for *espP M1029A* substitution
mtd345	CCTGAACAAACGTATGGGTTGCCTGCGTGATATCAACGG	F, QC primer for *espP D1031C* substitution
mtd346	CCGTTGATATCACGCAGGCAACCCATACGTTTGTTCAGG	R, QC primer for *espP D1031C* substitution
mtd347	GTGGGGGCTGGCCTGTTTGCTTCCGCCATGTTTGATTCC	F, QC primer for *espP Y1108F* substitution
mtd348	GGAATCAAACATGGCGGAAGCAAACAGGCCAGCCCCCAC	R, QC primer for *espP Y1108F* substitution
Linear dsDNA fragments		
mtd126	CTTGCATTATGTTTTTTAGGCTTATTACAATCCTCATATTCTTTTGC GGCCTGGTCTCATCCGCAGTTTGAAAAGGGTGGCGGGAGCGGTGGCG GTAGCGGTGGCTCCGCGTGGAGCCATCCGCAGTTTGAAAAGGGTGGC TATGCGATGCGTACAAACCTTTCTGAATCAGACAAACTGGAGGTCAA AAAAC	Fragment containing TwinStrepII-tag to assemble with pMTD607 after amplification with mtd96/127

Supplementary Table 3: Oligonucleotides and double-stranded DNA fragments used in this study, related to STAR Methods.
F = forward strand, R = reverse strand, SP = signal peptide, GA = Gibson assembly, QC = QuikChange