A signal sequence suppressor mutant that stabilizes an assembled state of the twin arginine translocase

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SUPPORTING INFORMATION

SI APPENDIX

Clone	TatB substitution/s
BRE1	L9Q K103R
BRE2*	L9Q
BRN2	L9Q Q134V
BRN3*	L9Q
BRN4	L10P V12M
BRN5	L9P
BRQ1*	K30I K65R N99D
BRQ2*	K30I K65R N99D
BRQ3*	I36N S41T
BRQ4	L9Q T72A
BRQ5*	I36N S41T
BRH1	L9Q N73K
BRH2	E8K L71H
BRH4	F13Y P138L K159R
BRH5*	F13Y
BRH6*	F13Y
BRH7*	L9Q
BRH8	F6Y V32A A69V
BRH9*	F13Y
BKQ1*	F13Y

Table S1. Clones isolated from a *tatB* mutant library following screening for suppression of transport defects of inactive signal peptides. The BRE, BRN, BRQ, BRH, BHH, BKH or BKQ clone nomenclature signify substitutions isolated following screening against RE, RN or KQ variants of the AmiA signal peptide RR motif, respectively.

*identical clones

Clone	TatAB substitution/s
AB-1	TatA K23N,TatB I36N, TatB N119I, TatB S164C
AB-5	TatB L10P
AB-16	TatB L10P, TatB N73Y
AB-157	TatB F13Y
AB-172	TatA A60E, TatA A76R, TatB F13Y

Table S2. Clones isolated from a *tatAB* mutant library following screening for suppression of the transport defect arising from the TatC F94Q substitution.

Strain	Relevant genotype	Source
JM109	*end*A1, *rec*A1, *gyr*A96, *thi, hsd*R17 (r_k^-, m_k^+), *rel*A1, *sup*E44, Δ(*lac-pro*AB), [F´ *tra*D36, *pro*AB, *laq*I^{q}ZΔM15]	Promega
XL10-gold	Tet^{r}Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Hte* [F´ *proAB lacI*^{q}*ZDM15* Tn*10* (Tet^r) Amy Cam^r]	Agilent
MC4100	F-Δ*lacU169 araD139 rpsL150 relA1 ptsF rbs flbB5301*	(1)
DADE	As MC4100, Δ*tatABC*, Δ*tatE*	(2)
DADE-P	as DADE*, pcnB1 zad*-981*::*Tn*10*d (Kan^r)	(3)
MΔBC	MC4100 Δ*tatBC*	(4)
MCDSSAC Δ*tatABC*	MC4100, *amiA*Δ2-33 *amiC*Δ2-32, Δ*tatABC*::Apra	(5)
MC4100 Δ*amiA* Δ*amiC* Δ*tatABC*	MC4100, Δ*amiA*, Δ*amiC*, Δ*tatABC*::Apra	This work
AyBCE	MC4100 Δ*tatA*, *attB*::P$_{tatA}$*tatA-EAK-eyfp*$_{A206K}$	(4)
AyBCE (*tatB*$_{L9Q}$)	As AyBCE, *tatB*$_{L9Q}$	This work
AyBCE (*tatB*$_{L10P}$)	As AyBCE, *tatB*$_{L10P}$	This work
AyBCE (*tatB*$_{F13Y}$)	As AyBCE, *tatB*$_{F13Y}$	This work
AyBCE (*tatB*$_{I36N}$)	As AyBCE, *tatB*$_{I36N}$	This work
AyBCE (*tatC*$_{F94Q}$)	As AyBCE, *tatC*$_{F94Q}$	This work
AyBCE (*tatB*$_{L9Q}$ *tatC*$_{F94Q}$)	As AyBCE (*tatB*$_{L9Q}$, *tatC*$_{F94Q}$)	This work
AyBCE (*tatB*$_{L10P}$ *tatC*$_{F94Q}$)	As AyBCE (*tatB*$_{L10P}$, *tatC*$_{F94Q}$)	This work
AyBCE (*tatB*$_{F13Y}$ *tatC*$_{F94Q}$)	As AyBCE (*tatB*$_{F13Y}$, *tatC*$_{F94Q}$)	This work
AyBCE (*tatB*$_{I36N}$ *tatC*$_{F94Q}$)	As AyBCE (*tatB*$_{I36N}$, *tatC*$_{F94Q}$)	This work
BL21(DE3) Δ*tatABC*	BL21(DE3), Δ*tatABC*::Apra	This work
BW25113	*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBA D*$_{LD78}$	(6)
BW25113 Δ*glpF* Δ*tatABC*	BW25113, Δ*glpF*, Δ*tatABC*::Apra	This work

Table S3. Strains used and constructed in this study.

Plasmid	Relevant genotype	Source
pTAT101	Low copy number vector expressing TatABC under the control of *tat* promoter. Kan^r.	(7)
pTH19kr	Low copy-number cloning vector. Backbone of pTAT101.	(8)
pTAT101-BF6Y	As pTAT101, TatB F6Y exchange	This work
pTAT101-BE8K	As pTAT101, TatB E8K exchange	This work
pTAT101-BL9P	As pTAT101, TatB L9P exchange	This work
pTAT101-BL9Q	As pTAT101, TatB L9Q exchange	This work
pTAT101-BL10P	As pTAT101, TatB L10P exchange	This work
pTAT101-BF13Y	As pTAT101, TatB F13Y exchange	This work
pTAT101-BK30I	As pTAT101, TatB K30I exchange	This work
pTAT101-BI36N	As pTAT101, TatB I36N exchange	This work
pTAT101-CF94Q	As pTAT101, TatC F94Q exchange	This work
pTAT101-CF94A	As pTAT101, TatC F94A exchange	This work
pTAT101-CF94D	As pTAT101, TatC F94D exchange	This work
pTAT101-CF94G	As pTAT101, TatC F94G exchange	This work
pTAT101-CF94K	As pTAT101, TatC F94K exchange	This work
pTAT101-CF94P	As pTAT101, TatC F94P exchange	This work
pTAT101-CF94R	As pTAT101, TatC F94R exchange	This work
pTAT101-CF94S	As pTAT101, TatC F94S exchange	This work
pTAT101-CF94Q-BF6Y	As pTAT101-CF94Q, TatB F6Y exchange	This work
pTAT101-CF94Q-BE8K	As pTAT101-CF94Q, TatB E8K exchange	This work
pTAT101-CF94Q-BL9P	As pTAT101-CF94Q, TatB L9P exchange	This work
pTAT101-CF94Q-BL9Q	As pTAT101-CF94Q, TatB L9Q exchange	This work
pTAT101-CF94Q-BL10Q	As pTAT101-CF94Q, TatB L10P exchange	This work
pTAT101-CF94Q-BF13Y	As pTAT101-CF94Q, TatB F13Y exchange	This work
pTAT101-CF94Q-BK30I	As pTAT101-CF94Q, TatB K30I exchange	This work
pTAT101-CF94Q-BI36N	As pTAT101-CF94Q, TatB I36N exchange	This work
pTAT101-BL9Q F13Y	As pTAT101, TatB L9Q, F13Y exchange	This work
pTAT101-BL10P F13Y	As pTAT101, TatB L10P, F13Y exchange	This work
pTAT101-BL9Q I36N	As pTAT101, TatB L9Q, I36N exchange	This work
pTAT101-BL10P I36N	As pTAT101, TatB L10P, I36N exchange	This work
pTAT101-CE103A	As pTAT101, TatC E103A exchange	This work
pTAT101-CE103A-BL9Q	As pTAT101-CE103A, TatB L9Q exchange	This work
pTAT101-CE103A-BL10P	As pTAT101-CE103A, TatB L10P exchange	This work
pTAT101-CE103A-BF13Y	As pTAT101-CE103A, TatB F13Y exchange	This work
pTAT101-CE103A-BI36N	As pTAT101-CE103A, TatB I36N exchange	This work
pTAT101-CE103K	As pTAT101, TatC E103K exchange	(7)
pTAT101-CE103K-BL9Q	As pTAT101-CE103K, TatB L9Q exchange	This work
pTAT101-CE103K-BL10P	As pTAT101-CE103K, TatB L10P exchange	This work

pTAT101-CE103K-BF13Y	As pTAT101-CE103A, TatB F13Y exchange	This work
pTAT101-CE103K-BI36N	As pTAT101-CE103A, TatB I36N exchange	This work
pTAT101-CP48L	As pTAT101, TatC P48L exchange	(9)
pTAT101-CP48L- BL9Q	As pTAT101-CP48L, TatB L9Q exchange	This work
pTAT101-CP48L - BL10P	As pTAT101-CP48L, TatB L10P exchange	This work
pTAT101-CP48L - BF13Y	As pTAT101-CP48L, TatB F13Y exchange	This work
pTAT101-CM59K	As pTAT101, TatC M59K exchange	(9)
pTAT101-CM59K-BL9Q	As pTAT101-CM59K, TatB L9Q exchange	This work
pTAT101-CM59K - BL10P	As pTAT101-CM59K, TatB L10P exchange	This work
pTAT101-CM59K - BF13Y	As pTAT101-CM59K, TatB F13Y exchange	This work
pTAT101-CV145E	As pTAT101, TatC V145E exchange	(9)
pTAT101-CV145E-BL9Q	As pTAT101-CV145E, TatB L9Q exchange	This work
pTAT101-CV145E - BL10P	As pTAT101-CV145E, TatB L10P exchange	This work
pTAT101-CV145E - BF13Y	As pTAT101-CV145E, TatB F13Y exchange	This work
pTAT101-CD211K	As pTAT101, TatC D211K exchange	This work
pTAT101-CD211K-BL9Q	As pTAT101-CD211K, TatB L9Q exchange	This work
pTAT101-CD211K - BL10P	As pTAT101-CD211K, TatB L10P exchange	This work
pTAT101-CD211K - BF13Y	As pTAT101-CD211K, TatB F13Y exchange	This work
pTAT101-CQ215K	As pTAT101, TatC Q215K exchange	This work
pTAT101-CQ215K-BL9Q	As pTAT101-CQ215K, TatB L9Q exchange	This work
pTAT101-CQ215K - BL10P	As pTAT101-CQ215K, TatB L10P exchange	This work
pTAT101-CQ215K - BF13Y	As pTAT101-CQ215K, TatB F13Y exchange	This work
pTAT101 cys less	As pTAT101, All 4 cys codons in *tatC* substituted with ala	(9)
pTAT101 cys less CM205C	As pTAT101 cys less, TatC M205C exchange	(9)
pTAT101 cys less BL9Q CM205C	As pTAT101 cys less CM205C, TatB L9Q exchange	This work
pTAT101 cys less BL10P CM205C	As pTAT101 cys less CM205C, TatB L10P exchange	This work
pTAT101 cys less BF13Y CM205C	As pTAT101 cys less CM205C, TatB F13Y exchange	This work
pTAT101 cys less BI36N CM205C	As pTAT101 cys less CM205C, TatB I36N exchange	This work
pTAT101 cys less CF94Q M205C	As pTAT101 cys less CM205C, TatC F94Q	This work
pTAT101 cys less BL10P CF94Q M205C	As pTAT101 cys less CF94Q M205C, TatB L10P	This work

pTAT101 cys less BF13Y CF94Q M205C	As pTAT101 cys less CF94Q M205C, TatB F13Y	This work
pQE80-CueO	As pQE80, carrying *cueO*$_{his}$	(4)
pQE80-CueO^{KK}h	As pQE80-CueO, CueO R3K, R4K exchange	(4)
pTAT1d	Medium copy number vector expressing TatABC under the control of *tat* promoter. Amp^r.	(10)
pUNIPROM	pT7.5 vector carrying a *tat* promoter. Backbone of pTAT1d	(11)
pTAT1d-CF94Q	As pTAT1d, TatC F94Q exchange	This work
pTAT1d-CF94A	As pTAT1d, TatC F94A exchange	This work
pTAT1d-CF94D	As pTAT1d, TatC F94D exchange	This work
pTAT1d-CF94G	As pTAT1d, TatC F94G exchange	This work
pTAT1d-CF94K	As pTAT1d, TatC F94K exchange	This work
pTAT1d-CF94P	As pTAT1d, TatC F94P exchange	This work
pTAT1d-CF94R	As pTAT1d, TatC F94R exchange	This work
pTAT1d-CF94S	As pTAT1d, TatC F94S exchange	This work
pTAT1d-CF94Q-BL9Q	As pTAT1d-CF94Q, TatB L9Q exchange	This work
pTAT1d-CF94Q-BL10P	As pTAT1d-CF94Q, TatB L10P exchange	This work
pTAT1d-CF94Q-BF13Y	As pTAT1d-CF94Q, TatB F13Y exchange	This work
pTAT1d-CF94Q-BI36N	As pTAT1d-CF94Q, TatB I36N exchange	This work
pTATBC1d	pUNIPROM carrying *tatBC*	This work
pSUAmiA	pSU18 carrying *amiA*	(12)
pSUAmiA-RD	As pSUAmiA, R14D exchange	This work
pSUAmiA-RE	As pSUAmiA, R14E exchange	This work
pSUAmiA-RH	As pSUAmiA, R14H exchange	This work
pSUAmiA-RN	As pSUAmiA, R14N exchange	This work
pSUAmiA-RQ	As pSUAmiA, R14Q exchange	This work
pSUAmiA-KH	As pSUAmiA, R13K, R14H exchange	This work
pSUAmiA-KQ	As pSUAmiA, R13K, R14Q exchange	This work
pSUAmiA-HH	As pSUAmiA, R13H, R14H exchange	This work
pSUSufIss-mAmiA	pSU18, carrying SufIss-mAmiA	This work
pSUSufIss-mAmiA-RD	As pSUSufIss-mAmiA, SufI R6D exchange	This work
pSUSufIss-mAmiA-RE	As pSUSufIss-mAmiA, SufI R6E exchange	This work
pSUSufIss-mAmiA-RH	As pSUSufIss-mAmiA, SufI R6H exchange	This work
pSUSufIss-mAmiA-RN	As pSUSufIss-mAmiA, SufI R6N exchange	This work
pSUSufIss-mAmiA-RQ	As pSUSufIss-mAmiA, SufI R6Q exchange	This work
pSUSufIss-mAmiA-KH	As pSUSufIss-mAmiA, SufI R5K, R6H exchange	This work
pSUSufIss-mAmiA-KQ	As pSUSufIss-mAmiA, SufI R5K, R6Q exchange	This work
pSUSufIss-mAmiA-KK	As pSUSufIss-mAmiA, SufI R5K, R6K exchange	This work
pSUSufIss-mAmiA-HH	As pSUSufIss-mAmiA, SufI R5H, R6H exchange	This work
pSUSufIssnoH-mAmiA	As pSUSufIss-mAmiA, *sufI* Δ11-21	This work
pSUmAmiA	As pSUAmiA, *amiA* Δ2-34	This work
pFAT75ΔA-BC	As pQEABC, but with *tatA* gene in frame deleted	(13)
pFAT75ΔA-BC BE8K	As pFAT75ΔA-BC, TatB E8K exchange	This work
pFAT75ΔA-BC BF13Y	As pFAT75ΔA-BC, TatB F13Y exchange	This work
pFAT75ΔA-BC BI36N	As pFAT75ΔA-BC, TatB BI36N exchange	This work
pFAT75ΔA-BC	As pFAT75ΔA-BC, TatC F94Q exchange	This work

CF94Q		
pFAT75ΔA-BC BL9Q CF94Q	As pFAT75ΔA-BC CF94Q, TatB L9Q exchange	This work
pFAT75ΔA-BC BL10P CF94Q	As pFAT75ΔA-BC CF94Q, TatB L10P exchange	This work
pFAT75ΔA-BC BF13Y CF94Q	As pFAT75ΔA-BC CF94Q, TatB F13Y exchange	This work
pFAT75ΔA-BC BI36NC CF94Q	As pFAT75ΔA-BC CF94Q, TatB I36N exchange	This work
pFAT75ΔA-BC-AmiAhis	As pFAT75ΔA-BC also producing C-terminally his-tagged AmiA	This work
pFAT75ΔA-BC-AmiARDhis	As pFAT75ΔA-BC-AmiAhis, AmiA R14D exchange	This work
pFAT75ΔA-BC-AmiARNhis	As pFAT75ΔA-BC-AmiAhis, AmiA R14N exchange	This work
pFAT75ΔA-BC-AmiAKKhis	As pFAT75ΔA-BC-AmiAhis, AmiA R13K, R14K exchange	This work
pFAT75ΔA-BC-AmiAKQhis	As pFAT75ΔA-BC-AmiAhis, AmiA R13K, R14Q exchange	This work
pFAT75ΔA-BF13YC-AmiAhis	As pFAT75ΔA-BC, TatBF13Y exchange	This work
pFAT75ΔA-BF13YC-AmiARDhis	As pFAT75ΔA-BF13YC-AmiAhis, AmiA R14D exchange	This work
pFAT75ΔA-BF13YC-AmiARNhis	As pFAT75ΔA-BF13YC-AmiAhis, AmiA R14N exchange	This work
pFAT75ΔA-BF13YC-AmiAKKhis	As pFAT75ΔA-BF13YC-AmiAhis, AmiA R13K, R14K exchange	This work
pFAT75ΔA-BF13YC-AmiAKQhis	As pFAT75ΔA-BF13YC-AmiAhis, AmiA R13K, R14Q exchange	This work
pFAT75ΔA-BC-mAmiAhis	As pFAT75ΔA-BC also producing C-terminally his-tagged mature AmiA	This work
pFAT75ΔA-BF13YC-mAmiAhis	As pFAT75ΔA-BC-mAmiAhis, TatBF13Y exchange	This work
pQE70-mAmiA	pQE70 producing C-terminally his-tagged mature AmiA	This work
pQE70-mAmiC	pQE70 producing C-terminally his-tagged mature AmiC	This work
pSufIss-GFPhis	As pCDFDuet-1, carrying synthetic SufI signal sequence-fused GFPhis	This work
pSufIssRD-GFPhis	As pSufIss-GFPhis, SufI R6D exchange	This work
pSufIssRN-GFPhis	As pSufIss-GFPhis, SufI R6N exchange	This work
pSufIssKK-GFPhis	As pSufIss-GFPhis, SufI R5K, R6K exchange	This work
pQE80 suflhis	pQE80 carrying *suflhis*	This work
pQE80 RDsuflhis	pQE80 suflhis SufI R6D exchange	This work
pQE80 RNsuflhis	pQE80 suflhis SufI R6N exchange	This work
pQE80 KQsuflhis	pQE80 suflhis SufI R5K, R6Q exchange	This work
pMAK705	Cloning vector with a temperature-sensitive replicon	(14)
pMAK-AupBC	As pMAK705, carrying 500 bp upstream sequence of *tatA* and *tatBC* sequence	This work
pMAK-AupBC-BL9Q	As pMAK-AupBC, TatB L9Q exchange	This work
pMAK-AupBC-BL10P	As pMAK-AupBC, TatB L10P exchange	This work
pMAK-AupBC-BF13Y	As pMAK-AupBC, TatB F13Y exchange	This work

pMAK-AupBC-BI36N	As pMAK-AupBC, TatB I36N exchange	This work
pMAK-AupBC-CF94Q	As pMAK-AupBC, TatC F94Q exchange	This work
pMAK-AupBC- BL9Q CF94Q	As pMAK-AupBC, TatB L9Q, TatC F94Q exchange	This work
pMAK-AupBC- BL10P CF94Q	As pMAK-AupBC, TatB L10P, TatC F94Q exchange	This work
pMAK-AupBC- BF13Y CF94Q	As pMAK-AupBC, TatB F13Y, TatC F94Q exchange	This work
pMAK-AupBC- BI36N CF94Q	As pMAK-AupBC, TatB I36N, TatC F94Q exchange	This work
pBAD24	Arabinose-inducible protein expression vector	(15)
pBADTatABChis	As pBAD24, carrying *tatABC*his	This work
pBADTatABChis-BL9Q	As pBADTatABChis, TatB L9Q exchange	This work
pBADTatABChis-BL10P	As pBADTatABChis, TatB L10P exchange	This work
pBADTatABChis-BF13Y	As pBADTatABChis, TatB F13Y exchange	This work
pBADTatABChis-BI36N	As pBADTatABChis, TatB I36N exchange	This work
pBAD22SecY(Δplug)EG	pBAD22, producing SecY(Δcodons62-72)EG	Ian Collinson
p101C*TatBC	Low copy vector for expression of *tatBC* from the *tatA* promoter with a modified RBS	(4)
p101C*BCflag	p101C*BC derivative producing TatB and C-terminally flag-tagged TatC	This work
p101C*BCflag E8K	As p101C*BCflag, TatB E8K exchange	This work
p101C*BCflag F13Y	As p101C*BCflag, TatB F13Y exchange	This work

Table S4. Plasmids used and constructed in this study

Primer name	Sequence (5'-3')
AmiARDf	CTCACTTCGCGCGACCAGGTGCTG
AmiARDr	CAGCACCTGGTCGCGCGAAGTGAG
AmiAREf	CTCACTTCGCGCGAACAGGTGCTG
AmiAREr	CAGCACCTGTTCGCGCGAAGTGAG
AmiARNf	CTCACTTCGCGCAACCAGGTGCTG
AmiARNr	CAGCACCTGGTTGCGCGAAGTGAG
AmiARQf	CTCACTTCGCGCCAACAGGTGCTG
AmiARQr	CAGCACCTGTTGGCGCGAAGTGAG
AmiARHf	CTCACTTCGCGCCACCAGGTGCTG
AmiARHr	CAGCACCTGGTGGCGCGAAGTGAG
AmiAHHf	CTCACTTCGCACCACCAGGTGCTG
AmiAHHr	CAGCACCTGGTGGTGCGAAGTGAG
AmiAKHf	CTCACTTCGAAACACCAGGTGCTG
AmiAKHr	CAGCACCTGGTGTTTCGAAGTGAG
AmiAKQf	CTCACTTCGAAACAACAGGTGCTG
suflssFE	CCGGAATTCGTTTTACATGGAGCAAATATG
suflssR	GTTCGTCTTTTGCGCTGGCCTTCAGGG
amiA-mF	GGCCAGCGCAAAAGACGAACTTTTAAAAACC
amiA-mRX	GACTCTAGATTATCGCTT TTTC
AmiAKQr	CAGCACCTGTTGTTTCGAAGTGAG
Suflss-RDf	GTCACTCAGTCGGGATCAGTTCATTCAGGC
Suflss-RDr	GCCTGAATGAACTGATCCCGACTGAGTGAC
Suflss-RHf	GTCACTCAGTCGGCATCAGTTCATTCAGGC
Suflss-RHr	GCCTGAATGAACTGATGCCGACTGAGTGAC
Suflss-RNf	GTCACTCAGTCGGAACCAGTTCATTCAGGC
Suflss-RNr	GCCTGAATGAACTGGTTCCGACTGAGTGAC
Suflss-RQf	GTCACTCAGTCGGCAGCAGTTCATTCAGGC
Suflss-RQr	GCCTGAATGAACTGCTGCCGACTGAGTGAC
Suflss-RKf	GTCACTCAGTCGGAAACAGTTCATTCAGGC
Suflss-RKr	GCCTGAATGAACTGTTTCCGACTGAGTGAC
Suflss-KHf	GTCACTCAGTAAACATCAGTTCATTCAGGC
Suflss-KHr	GCCTGAATGAACTGATGTTTACTGAGTGAC
Suflss-KQf	GTCACTCAGTAAACAGCAGTTCATTCAGGC
Suflss-KQr	GCCTGAATGAACTGCTGTTTACTGAGTGAC
Suflss-KKf	GTCACTCAGTAAAAAACAGTTCATTCAGGC
Suflss-KKr	GCCTGAATGAACTGTTTTTTACTGAGTGAC
Suflss-HHf	GTCACTCAGTCATCATCAGTTCATTCAGGC
Suflss-HHr	GCCTGAATGAACTGATGATGACTGAGTGAC
Sufl-noHF	CGGCGTCAGTTCATTCAGCCCCTGAAGGCCAGCGCA
Sufl-noHR	TGCGCTGGCCTTCAGGGGCTGAATGAACTGACGCCG
AmiA-nossFE	CCGGAATTCTATTACAACTCAGGCCGTATGAAAGACGAACTTTT AAAAACCAG
AmiAFATApaI-F	GCGCGGGCCCATTAAAGAGGAGAAATTAACCATGAGCACTTTT AAACCACTAAAAAC
mAmiAFATApaI-F	GCGCGGGCCCATTAAAGAGGAGAAATTAACCATGAAAGACGAA CTTTTAAAAACCAG

mAmiA-SphI-F	GCGCGCATGCGAAAAGACGAACTTTTAAAAACC
AmiAnostopBamHI-R	CGCGGATCCTCGCTTTTTCGAATGTGCTTTC
mAmiC-SphI-F	GCGCGCATGCGAGCGGTCAGCCAGGTCGTG
AmiCnostopBamHI-R	CGCGGATCCTCCCCTTCTCGCCAGCGTC
C-F94S1	GGTGTGGGCATCTATCGCCCCAG
C-F94S2	CTGGGGCGATAGATGCCCACACC
C-F94A1	GGTGTGGGCAGCGATCGCCCCAG
C-F94A2	CTGGGGCGATCGCTGCCCACACC
C-F94K1	GGTGTGGGCAAAAATCGCCCCAG
C-F94K2	CTGGGGCGATTTTTGCCCACACC
C-F94Q1	GGTGTGGGCACAGATCGCCCCAG
C-F94Q2	CTGGGGCGATCTGTGCCCACACC
C-F94R1	GGTGTGGGCACGCATCGCCCCAG
C-F94R2	CTGGGGCGATGCGTGCCCACACC
C-F94P1	GGTGTGGGCACCGATCGCCCCAG
C-F94P2	CTGGGGCGATCGGTGCCCACACC
C-F94D1	GGTGTGGGCAGATATCGCCCCAG
C-F94D2	CTGGGGCGATATCTGCCCACACC
C-F94G1	GGTGTGGGCAGGCATCGCCCCAG
C-F94G2	CTGGGGCGATGCCTGCCCACACC
C-F94C1	GGTGTGGGCATGCATCGCCCCAG
C-F94C2	CTGGGGCGATGCATGCCCACACC
B-F6Yf	GTTTGATATCGGTTATAGCGAACTGC
B-F6Yr	GCAGTTCGCTATAACCGATATCAAAC
B-L9Qf	GGTTTTAGCGAACAGCTATTGGTG
B-L9Qr	CACCAATAGCTGTTCGCTAAAACC
B-L9Pf	GGTTTTAGCGAACCGCTATTGGTG
B-L9Pr	CACCAATAGCGGTTCGCTAAAACC
B-L10Pf	GCGAACTGCCATTGGTGTTCATC
B-L10Pr	GATGAACACCAATGGCAGTTCGC
B-F13Yf	GCTATTGGTGTACATCATCGGCC
B-F13Yr	GGCCGATGATGTACACCAATAGC
B-K30If	GTGGCGGTAATTACGGTAGCGG
B-K30Ir	CCGCTACCGTAATTACCGCCAC
B-I36Nf	GTAGCGGGCTGGAATCGCGCGTTGC
B-I36Nr	GCAACGCGCGATTCCAGCCCGCTAC
tatB E8K 1	GATATCGGTTTTAGCAAACTGCTATTGG
tatB E8K 2	CCAATAGCAGTTTGCTAAAACCGATATC
C-E103A1	CGCTGTATAAGCATGCGCGTCGCCTGGTGGTGC
C-E103A2	GCACCACCAGGCGACGCGCATGCTTATACAGCG
C-P48L1	GGTATCCGCGCTGTTGATCAAGC
C-P48L2	GCTTGATCAACAGCGCGGATACC
C-M59K1	GGTTCAACGAAGATCGCCACCG
C-M59K2	CGGTGGCGATCTTCGTTGAACC

C-V145E1	CGGAAGGGGAACAGGTATCCAC
C-V145E2	GTGGATACCTGTTCCCCTTCCG
C-D211K1	CTGACGCCGCCGAAAGTCTTCTCGCAAAC
C-D211K2	GTTTGCGAGAAGACTTTCGGCGGCGTCAG
C-Q215K1	GTCTTCTCGAAAACGCTGTTG
C-Q215K2	CAACAGCGTTTTCGAGAAGAC
TatB-L9QL10P-F	GGTTTTAGCGAACAGCCATTGGTGTTCATC
TatB-L9QL10P-R	GATGAACACCAATGGCTGTTCGCTAAAACC
TatB-L10PF13Y-F	GCGAACTGCCATTGGTGTACATCATCGG
TatB-L10PF13Y-R	CCGATGATGTACACCAATGGCAGTTCGC
TatA-FB	CAGAGGAGGATCCATGGG
TatB-RS	GTATCGTCGACAGACATGC
TatCR1d	CTTGGGCTGCAGCCTTATTCTTC
TatC93R	TGCCCACACCTGATAGAG
TatCm6	CTTCCTCGAGTGATAAACCTTAAGCATG
TatC95F	ATCGCCCCAGCGCTGTAT
TatAup1-Xba I	CGCTCTAGAGAAAACCCTGCTCTACGTC
TatAup2-ClaI	GCGCATCGATAAGCTTGATATCGAAT
TatA6B7-ClaI	GCGCATCGATGATAAAGAGCAGGTGTAATCCGTGTTTGATATC GGTTTTAGC
TatCrev-KpnI	CGGGGTACCTTATTCTTCAGTTTTTTCGCTTTC
TatANcoI	GCGCCCATGGGTGGTATCAGTATTTGG
HisXba	GCGCTCTAGATTAGTGATGGTGATGGTG
STIPE-ISH	GCGGATACGAATCAGGAACAG
pT7.5R	CGCTGAGATAGGTGCC.
p101C*BCflag_F	GAAAGCGAAAAAACTGAAGAAGACTACAAGGACGATGACAAGT AAGGCTGCAGGCATGCAAG
p101C*BCflag_R	CTTGCATGCCTGCAGCCTTACTTGTCATCGTCCTTGTAGTCTTC TTCAGTTTTTTCGCTTTC

Table S5. Oligonucleotides used in this study

Supplementary References

1. Casadaban MJ, Cohen SN (1979) Lactose genes fused to exogenous promoters in one step using a Mu-lac bacteriophage: *in vivo* probe for transcriptional control sequences. *Proc Natl Acad Sci USA* 76(9):4530-4533.

2. Wexler M*, et al.* (2000) TatD is a cytoplasmic protein with DNase activity. No requirement for TatD family proteins in sec-independent protein export. *J Biol Chem* 275(22):16717-16722.

3. Lee PA*, et al.* (2006) Cysteine-scanning mutagenesis and disulfide mapping studies of the conserved domain of the twin-arginine translocase TatB component. *J Biol Chem* 281(45):34072-34085.

4. Alcock F*, et al.* (2013) Live cell imaging shows reversible assembly of the TatA component of the twin-arginine protein transport system. *Proc Natl Acad Sci USA* 110(38):E3650-3659.

5. Keller R, de Keyzer J, Driessen AJ, Palmer T (2012) Co-operation between different targeting pathways during integration of a membrane protein. *Journal Cell Biol* 199(2):303-315.

6. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* 97(12):6640-6645.

7. Kneuper H*, et al.* (2012) Molecular dissection of TatC defines critical regions essential for protein transport and a TatB-TatC contact site. *Mol Microbiol* 85(5):945-961.

8. Hashimoto-Gotoh T*, et al.* (2000) A set of temperature sensitive-replication/-segregation and temperature resistant plasmid vectors with different copy numbers and in an isogenic background (chloramphenicol, kanamycin, *lacZ*, *repA*, *par*, *polA*). *Gene* 241(1):185-191.

9. Cléon F*, et al.* (2015) The TatC component of the twin-arginine protein translocase functions as an obligate oligomer. *Mol Microbiol* 98(1):111-129.

10. Maldonado B*, et al.* (2011) Characterisation of the membrane-extrinsic domain of the TatB component of the twin arginine protein translocase. *FEBS Lett* 585(3):478-484.

11. Jack RL*, et al.* (2004) Coordinating assembly and export of complex bacterial proteins. *EMBO J* 23(20):3962-3972.

12. Ize B, Stanley NR, Buchanan G, Palmer T (2003) Role of the *Escherichia coli* Tat pathway in outer membrane integrity. *Mol Microbiol* 48(5):1183-1193.

13. Tarry MJ*, et al.* (2009) Structural analysis of substrate binding by the TatBC component of the twin-arginine protein transport system. *Proc Natl Acad Sci USA* 106(32):13284-13289.

14. Hamilton CM, Aldea M, Washburn BK, Babitzke P, Kushner SR (1989) New method for generating deletions and gene replacements in *Escherichia coli*. *J Bacteriol* 171(9):4617-4622.

15. Guzman LM, Belin D, Carson MJ, Beckwith J (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. *J Bacteriol* 177(14):4121-4130.

Supplementary Figure Legends

Figure S1. Substitutions of the twin arginines in the AmiA signal peptide prevent growth in the presence of SDS. Strain MCDSSAC Δ*tatABC* producing wild type *tatABC* from plasmid pTAT1d and either wild type ('RR') or signal peptide point-substituted AmiA, as indicated, from pSUAmiA. The strain and plasmid combinations were cultured overnight in LB medium supplemented with chloramphenicol and ampicillin (for plasmid selection), after which they were streaked onto LB agar containing the same antibiotics, with and without the addition of 2% SDS and incubated for 16 hr at 37°C.

Figure S2. TatB variants are able to restore Tat transport to a range of defective twin arginine substitutions in the AmiA signal sequence. Growth of MCDSSAC Δ*tatABC* coproducing the indicated TatB variants (with wild type *tatA* and *tatC*) from pTAT101, or the empty plasmid pTH19kr (indicated by '*Δtat*') alongside signal peptide variants of AmiA, on LB agar supplemented with chloramphenicol and kanamycin, with and without the addition of 2% SDS as indicated. An 8μl aliquot of each strain/plasmid combination following aerobic growth to an OD_{600} of 1.0 was spotted and incubated for 16 hr at 37°C. A. Individual signal peptide substitutions of AmiA (indicated to the left of each panel) were tested against the TatB suppressors F6Y, L9P, L9Q, L10P, F13Y, K30I and I36N. B. The TatB E8K suppressor was tested for the ability to suppress the indicated AmiA signal peptide substitutions.

Figure S3. TatB variants are able to restore Tat transport to a range of defective twin arginine substitutions in the SufI signal sequence. Growth of MCDSSAC Δ*tatABC* coproducing the indicated TatB variants (with wild type *tatA* and *tatC*) from pTAT101, or the empty plasmid pTH19kr (indicated by '*Δtat*') alongside signal peptide variants of SufI fused to the AmiA mature domain, on LB agar supplemented with chloramphenicol and kanamycin, with and without the addition of 2% SDS as indicated. An 8μl aliquot of each strain/plasmid combination following aerobic growth to an OD_{600} of 1.0 was spotted and incubated for 16 hr at 37°C. A. Individual signal peptide substitutions of AmiA (indicated to the left of each panel) were tested against the TatB suppressors F6Y, L9P, L9Q, L10P, F13Y, K30I and I36N. B. The

TatB E8K suppressor was tested for the ability to suppress the indicated AmiA signal peptide substitutions

Figure S4. A subset of amino acid substitutions at TatCF94 abolish Tat activity when produced at medium and low copy number. A and C. Growth of DADE coproducing either wild type TatABC (Tat^+), wild type TatAB alongside F94-substituted TatC or the cognate empty plasmid (Tat^-) on LB agar containing 2% SDS. A single colony of each strain/plasmid combination was resuspended in 30μl of PBS and an 8μl aliquot was spotted onto LB agar supplemented with appropriate antibiotics, along with 2% SDS as indicated. Plates were incubated for 16 hr at 37°C. B and D. Detection of TatC protein present in membrane fractions of the same strain and plasmid combinations as in A. and C., respectively, by Western immunoblot with anti-TatC antiserum. A total of 5μg membranes was loaded per lane for TatC produced from pTAT1d (B) and 20μg per lane for membranes produced from strains harboring pTAT101 derivatives (D).

Figure S5. TatB variants cannot supress TatC inactivating substitutions outside of the signal peptide binding site. Growth of DADE (Δ*tatABCD*, Δ*tatE*) coproducing wild type TatA alongside and the indicated substitution in TatB alongside either of TatC P48L, TatC M59K, TatC V145E, TatC D211K or TatC Q215K as indicated, from plasmid pTAT101 on LB agar or LB agar containing 2% SDS. A single colony of each strain/plasmid combination was resuspended in 30μl of PBS and an 8μl aliquot was spotted onto LB agar supplemented with appropriate antibiotics, along with 2% SDS as indicated, and incubated for 16 hr at 37°C.

Figure S6. The suppressive effect of the TatB variants is not additive and mature AmiC is not exported in the presence of the TatB F13Y suppressor. A. Growth of DADE coproducing either wild type TatABC (Tat^+), wild type TatAB alongside F94-substituted TatC or the cognate empty plasmid (Tat^-) on LB agar or LB agar containing 2% SDS. B. Growth of MCDSSAC Δ*tatABC* coproducing the indicated TatB variants (with wild type *tatA* and *tatC*) from pTAT101, or the empty plasmid pTH19kr (indicated by '*Δtat*') alongside the RN or KK

signal peptide variants of SufI fused to the AmiA mature domain, as indicated, on LB agar with or without the addition of 2% SDS. C. Strain MC4100 Δ*ami*A Δ*amiC* Δ*tatABC* coproducing either wild-type TatB or TatB F13Y (with wild type *tatA* and *tatC*) from pTAT101 and the AmiA or AmiC mature domains (from pQE70-mAmiA or pQE70-mAmiC, respectively) on LB agar or LB agar containing 2% SDS. In each case a single colony of each strain/plasmid combination was resuspended in 30μl of PBS and an 8μl aliquot was spotted onto LB agar supplemented with appropriate antibiotics, along with 2% SDS where indicated. Plates were incubated for 16 hr at 37°C.

Figure S7. The TatB suppressors support export of his-tagged SufI with its native signal peptide. A. and B. *E. coli* strain DADE producing wild type TatA and TatC and the indicated TatB variants alongside wild-type SufI-his or the indicated signal-peptide variants were fractionated into whole cell (upper panels) and periplasm (lower panels) fractions, then analysed by Western blot with anti-6X His tag® or anti-RNA polymerase β subunit antibodies (cytoplasmic control protein). wc – whole cell.

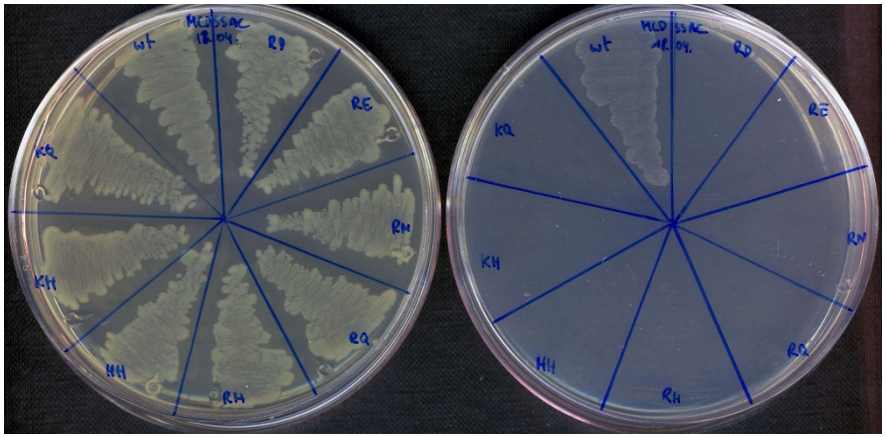
Figure S8. TatBC and SufIss-GFP-His twin-arginine variants are detectable in whole cell samples. A. and B. Cells producing SufIss-GFP-His with the wild type (RR) or twin-arginine substituted SufI signal peptide, as indicated, alongside TatC and either wild type TatB or the E8K, F13Y or I36N substituted variants, or C. and D. Cells producing SufIss-GFP-His with the wild type SufI signal peptide along with either wild type TatBC, the TatC F94Q allele along with either wild type TatB or the L9Q, L10P, F13Y or I36N substituted variants, or the TatC E103K allele along with either wild type TatB or the L9Q, L10P, F13Y or I36N substituted variants, as indicated were harvested and resuspended in PBS. A. and C. The fluorescence intensity and OD_{600} of the samples were measured using a plate reader and the Fluorescence/OD_{600} plotted for each sample. B. and D. 20 µl of each cell suspension was taken, all samples were normalized to the same OD_{600} and then analysed by SDS-PAGE followed by western blot using a TatB-TatC mixed antibody.

Figure S9. TatBC complexes containing the TatB F13Y suppressor do not co-purify with signal peptide variants of AmiA. C-terminally his-tagged wild type AmiA, twin-arginine substituted AmiA or signal sequence-less AmiA, as indicated was co-produced alongside wild type TatBC or TatBF13Y/TatC and purified using nickel beads from digitonin–treated cell extracts. Aliquots of the load and elution fractions were subject to SDS-PAGE followed by Western blot using either anti-His, anti-TatB and TatC antibodies.

Figure S10. TatB variants are extracted from the membrane with digitonin. Membrane suspensions (containing equivalent amounts of total protein) from strain DADE coproducing either wild type TatABC or wild type TatA and TatC alongside the indicated amino acid variant of TatB were solubilized by addition of 2% digitonin and incubation on ice for 30 min. Samples total membranes and digitonin solubilized material (each containing 10μg protein) were analysed by SDS-PAGE followed by western blotting with anti-TatA, anti-TatB or anti-TatC antibodies as indicated.

Figure S11. Constitutive oligomerisation of TatA is not promoted by the TatB L9Q, L10P or I36N substitutions. Fluorescence images of TatA-YFP in representative cells of A. strains AyBCE or AyBC_{F94Q}E (encoding chromosomal TatC F94Q) in the presence (pAmiA) or absence of plasmid-encoded wild type AmiA, as indicated (reproduced from Fig 5A). B. strains AyB_{L9Q}CE (encoding chromosomal TatB L9Q), AyB_{L10P}CE (encoding chromosomal TatB L10P) and AyB_{I36N}CE (encoding chromosomal TatB I36N) or the same strains additionally harboring the chromosomally-encoded TatC F94Q substitution. Scale bar: 1 μm. Note that the pictures in panel A are identical to those in Fig 5A and were included here to provide a direct comparison with panel B.

LB LB + 2% SDS



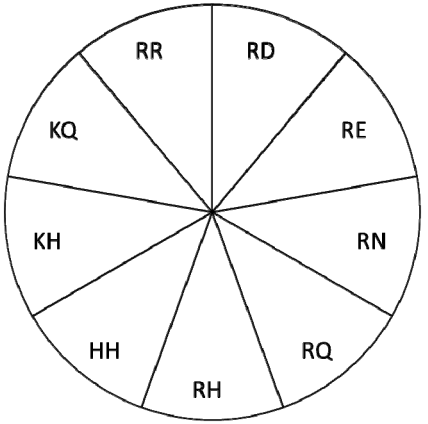
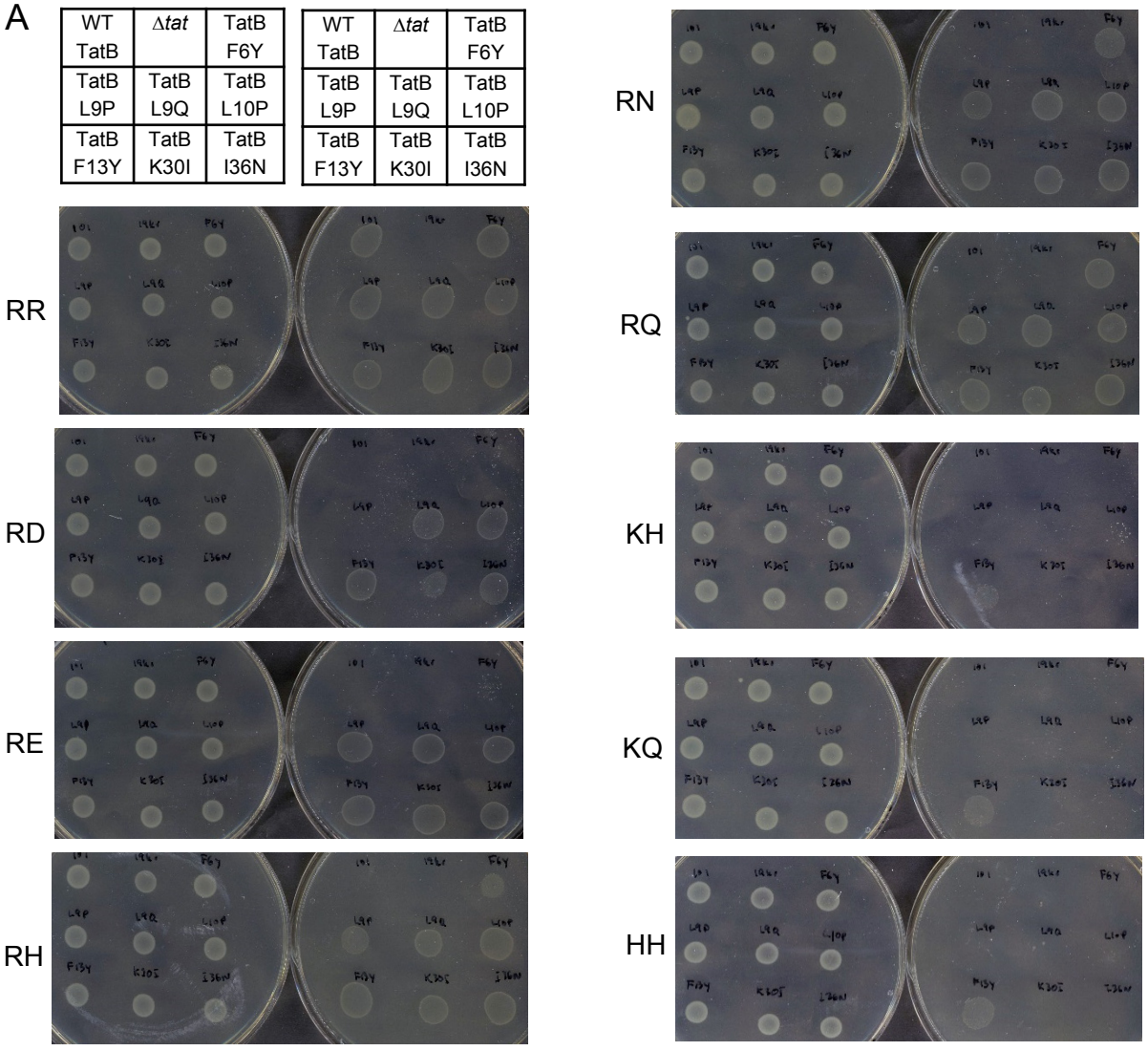


Fig S1

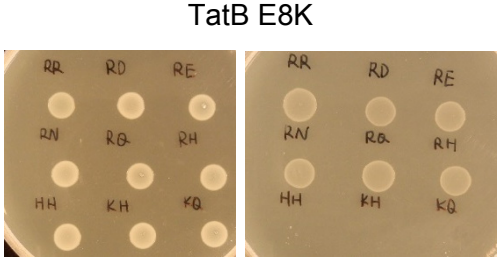
A

WT TatB	Δ*tat*	TatB F6Y
TatB L9P	TatB L9Q	TatB L10P
TatB F13Y	TatB K30I	TatB I36N

WT TatB	Δ*tat*	TatB F6Y
TatB L9P	TatB L9Q	TatB L10P
TatB F13Y	TatB K30I	TatB I36N



B



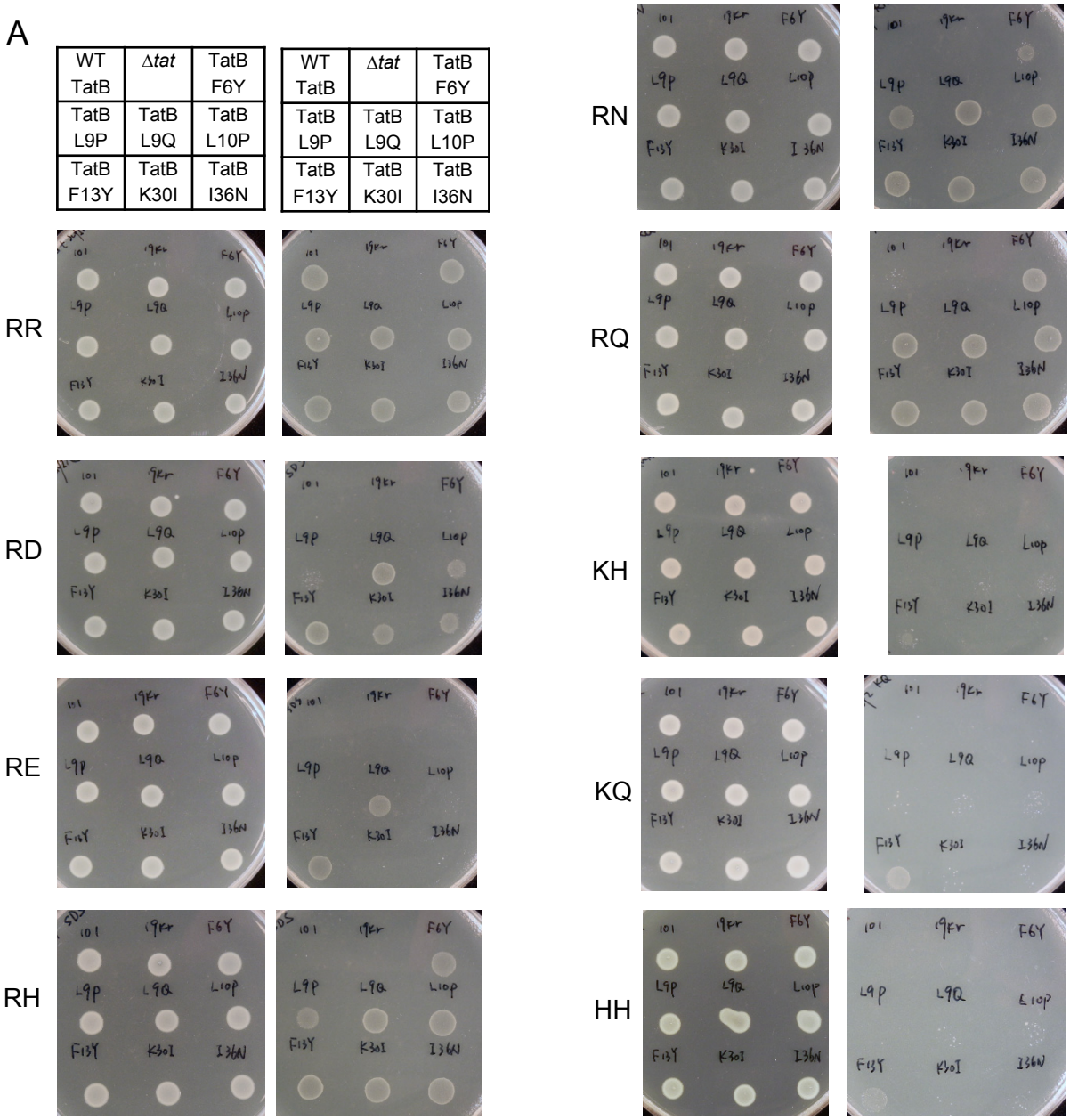
AmiA RR	AmiA RD	AmiA RE
AmiA RN	AmiA RQ	AmiA RH
AmiA HH	AmiA KH	AmiA KQ

Fig S2

A

WT TatB	Δ*tat*	TatB F6Y
TatB L9P	TatB L9Q	TatB L10P
TatB F13Y	TatB K30I	TatB I36N

WT TatB	Δ*tat*	TatB F6Y
TatB L9P	TatB L9Q	TatB L10P
TatB F13Y	TatB K30I	TatB I36N



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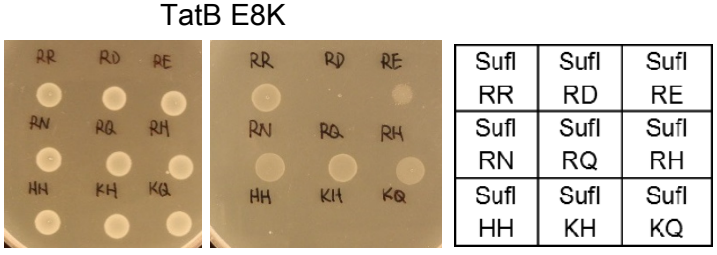
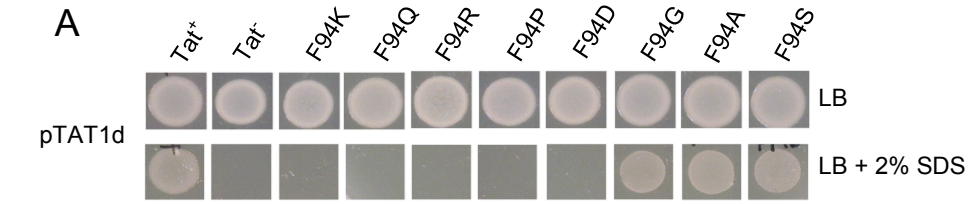


SufI RR	SufI RD	SufI RE
SufI RN	SufI RQ	SufI RH
SufI HH	SufI KH	SufI KQ

Fig S3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Tat+
Tat-
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F94Q
F94R
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F94G
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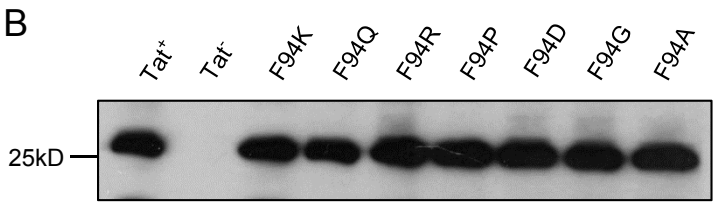
F94S
pTAT1d
LB
LB + 2% SDS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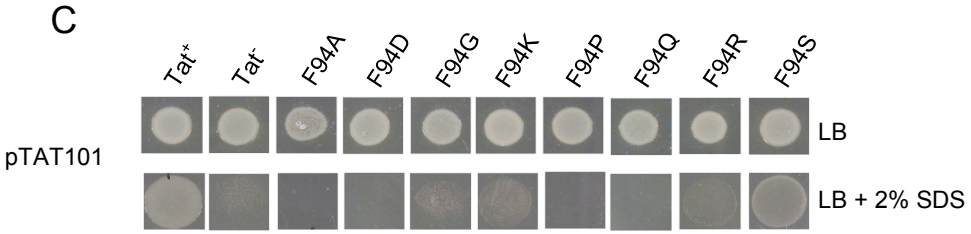
F94G
F94A
25kD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Tat-
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F94R
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pTAT101
LB
LB + 2% SDS

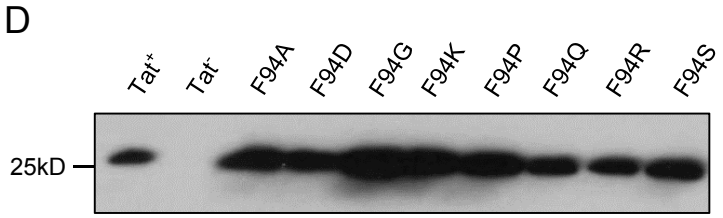
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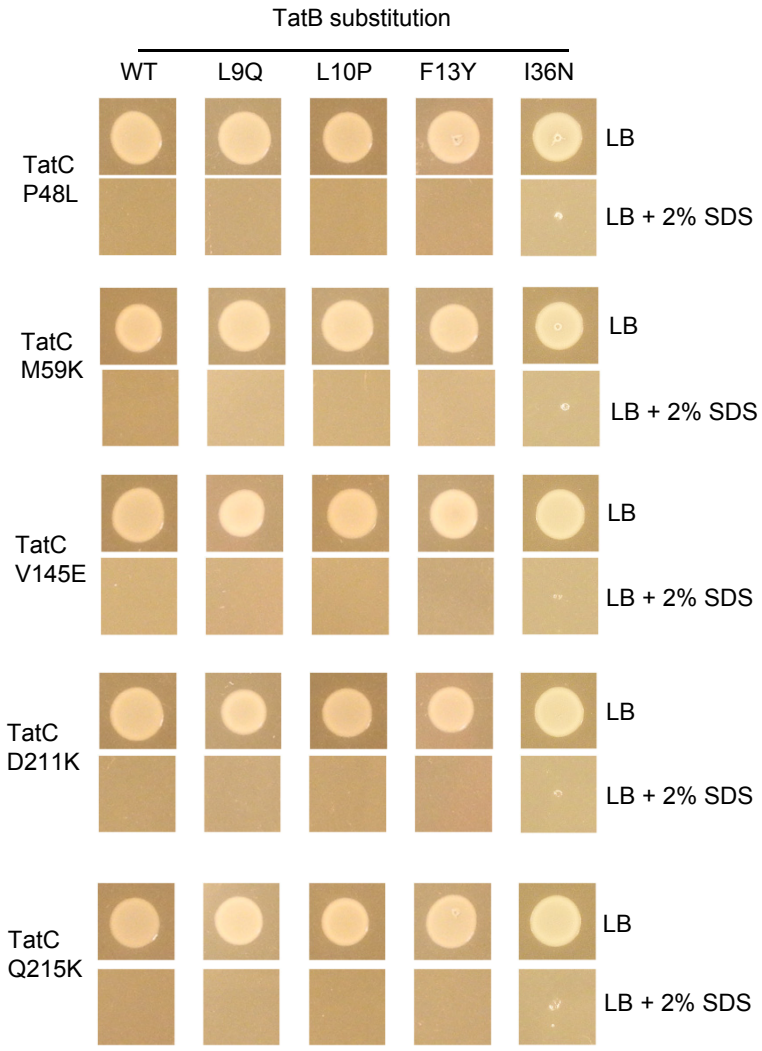
Fig S4


TatB substitution
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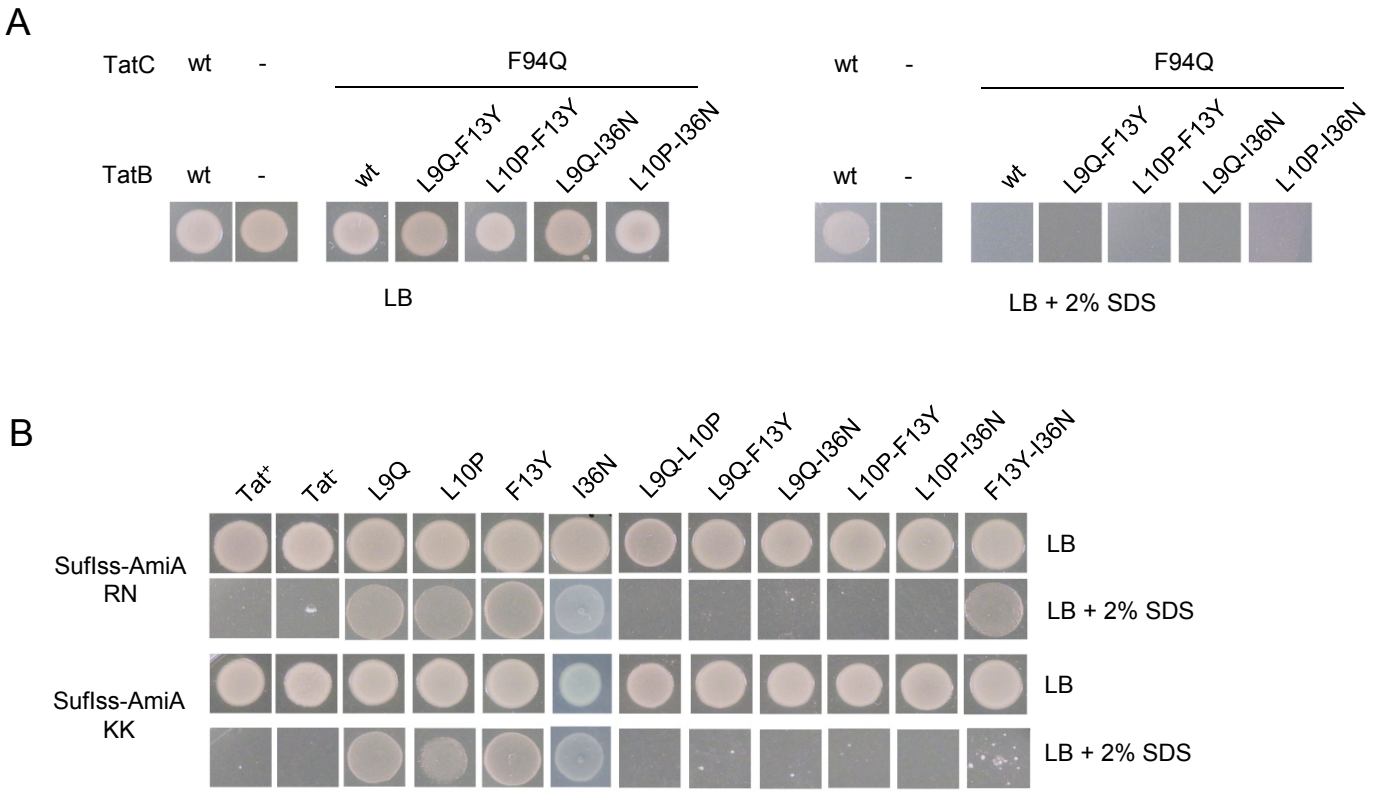
L9Q
L10P
F13Y
I36N
TatC P48L
TatC M59K
TatC V145E
TatC D211K
TatC Q215K
LB
LB + 2% SDS

Fig S5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-
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L10P-I36N
LB
LB + 2% SDS
B
Tat+
Tat-
L9Q
L10P
F13Y
I36N
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L9Q-F13Y
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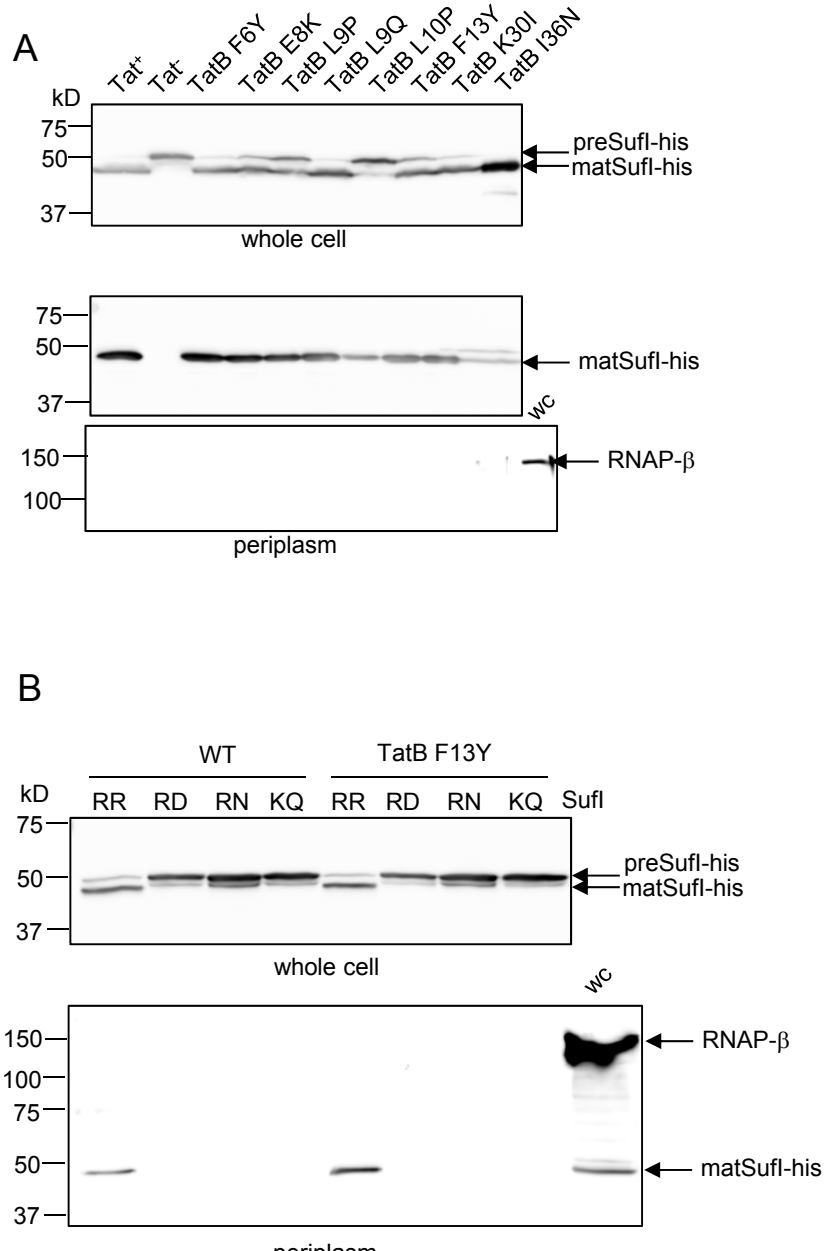
Fig S6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Tat-
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TatB K30I
TatB I36N
kD
75
50
37
preSufI-his
matSufI-his
whole cell
75
50
37
matSufI-his
WC
150
100
RNAP-β
periplasm
B
WT
TatB F13Y
kD
RR
RD
RN
KQ
RR
RD
RN
KQ
SufI
75
50
37
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150
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RNAP-β
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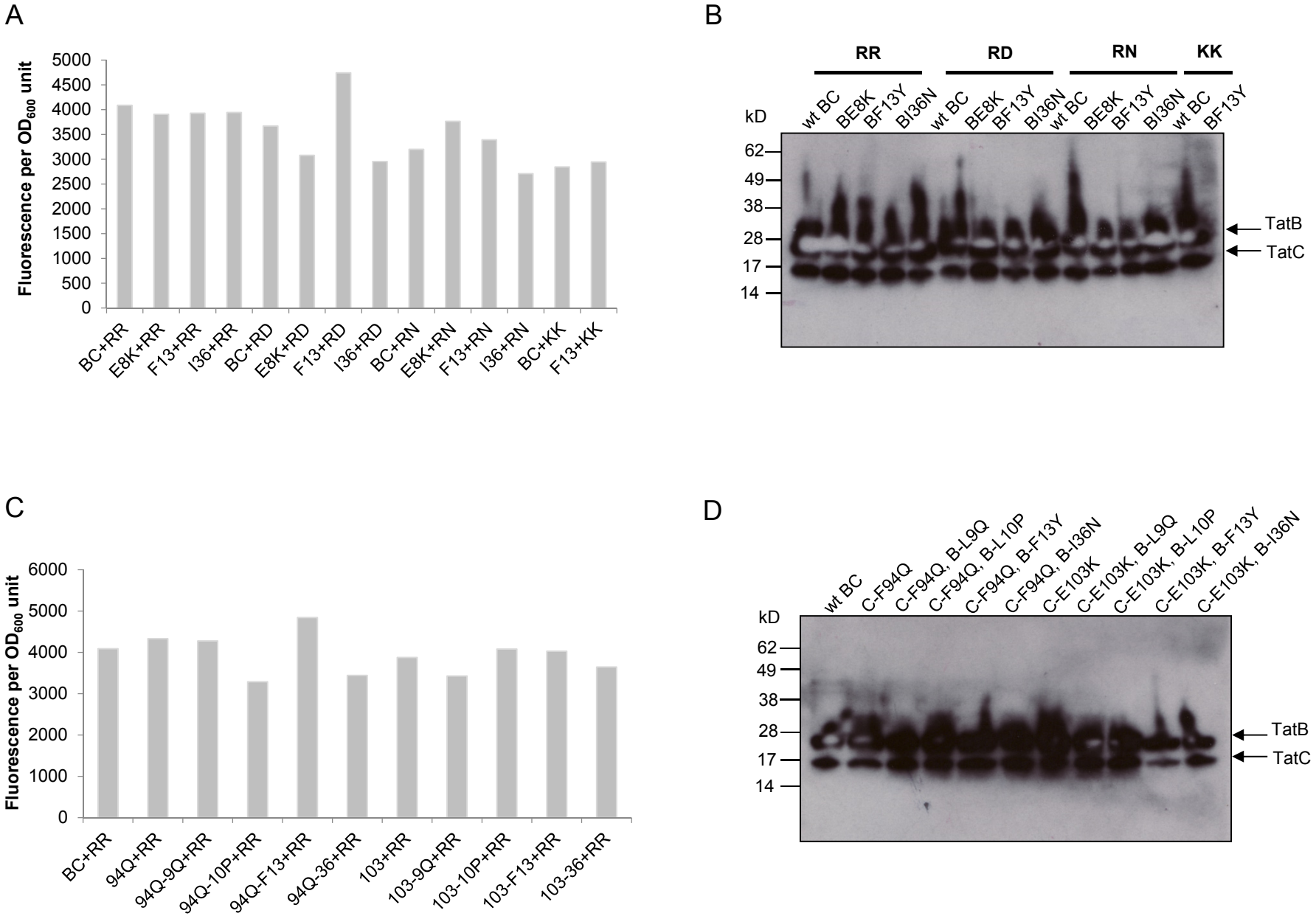
periplasm

Fig S7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2500
2000
1500
1000
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I36+RR
BC+RD
E8K+RD
F13+RD
I36+RD
BC+RN
E8K+RN
F13+RN
I36+RN
BC+KK
F13+KK
B
RR
RD
RN
KK
kD
wt BC
BE8K
BF13Y
BI36N
wt BC
BE8K
BF13Y
BI36N
wt BC
BE8K
BF13Y
BI36N
wt BC
BF13Y
62
49
38
28
17
14
TatB
TatC
C
Fluorescence per OD600 unit
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4000
3000
2000
1000
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94Q+RR
94Q-9Q+RR
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94Q-F13+RR
94Q-36+RR
103+RR
103-9Q+RR
103-10P+RR
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C-F94Q, B-L9Q
C-F94Q, B-L10P
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17
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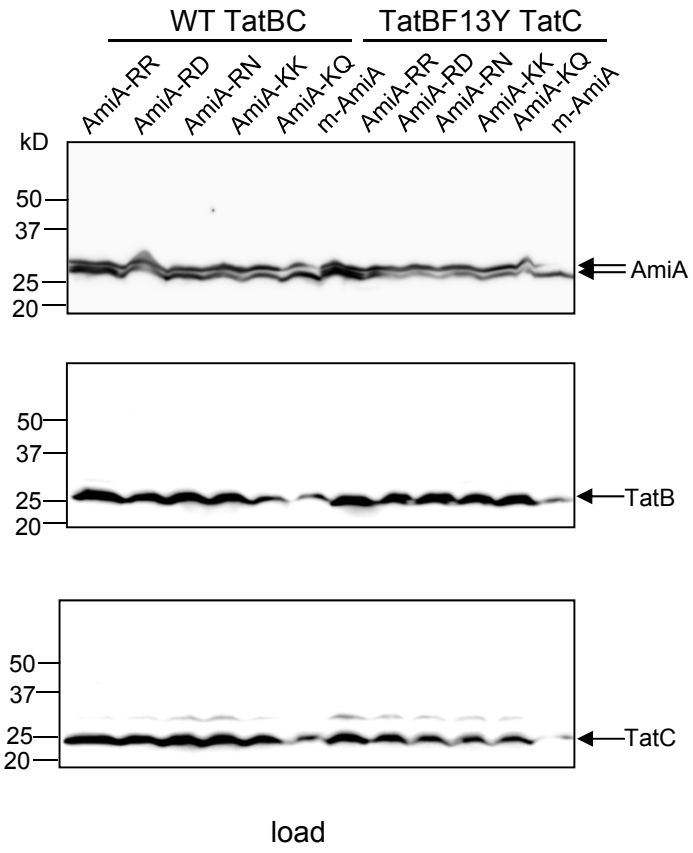
TatB
TatC

Fig S8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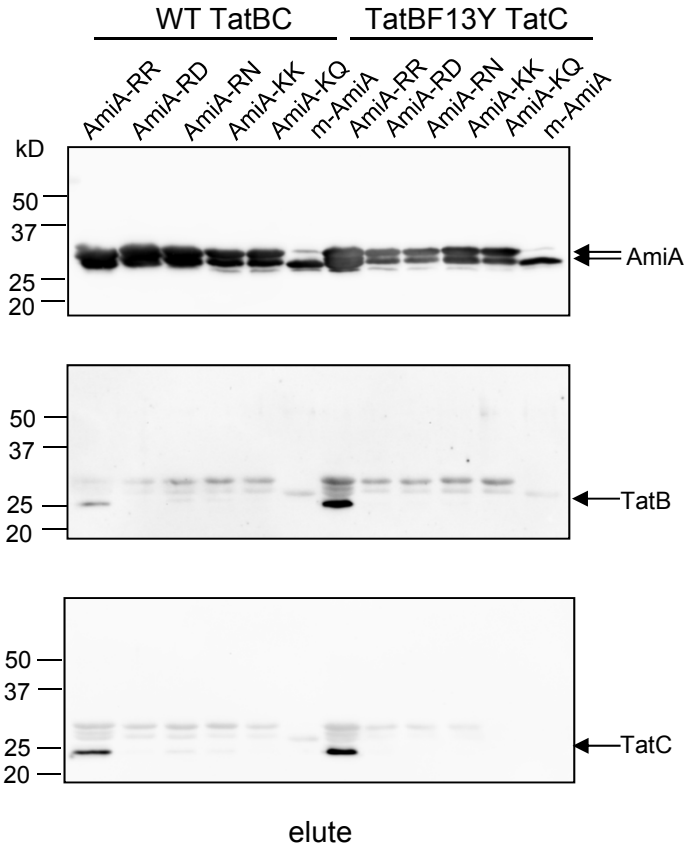
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TatB
TatC
elute

Fig S9

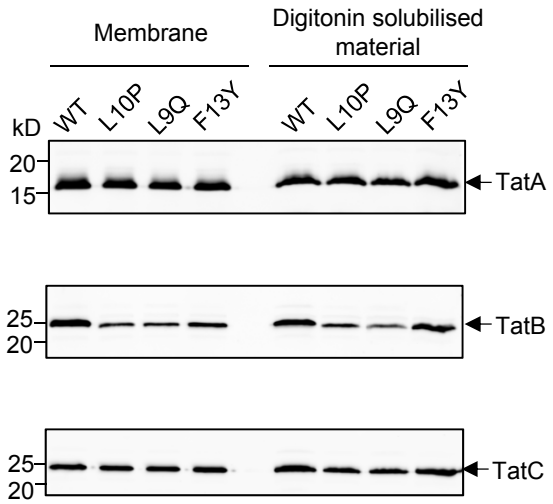
Membrane
Digitonin solubilised material
kD
WT
L10P
L9Q
F13Y
WT
L10P
L9Q
F13Y
20
15
TatA
25
20
TatB
25
20
TatC

Fig S10

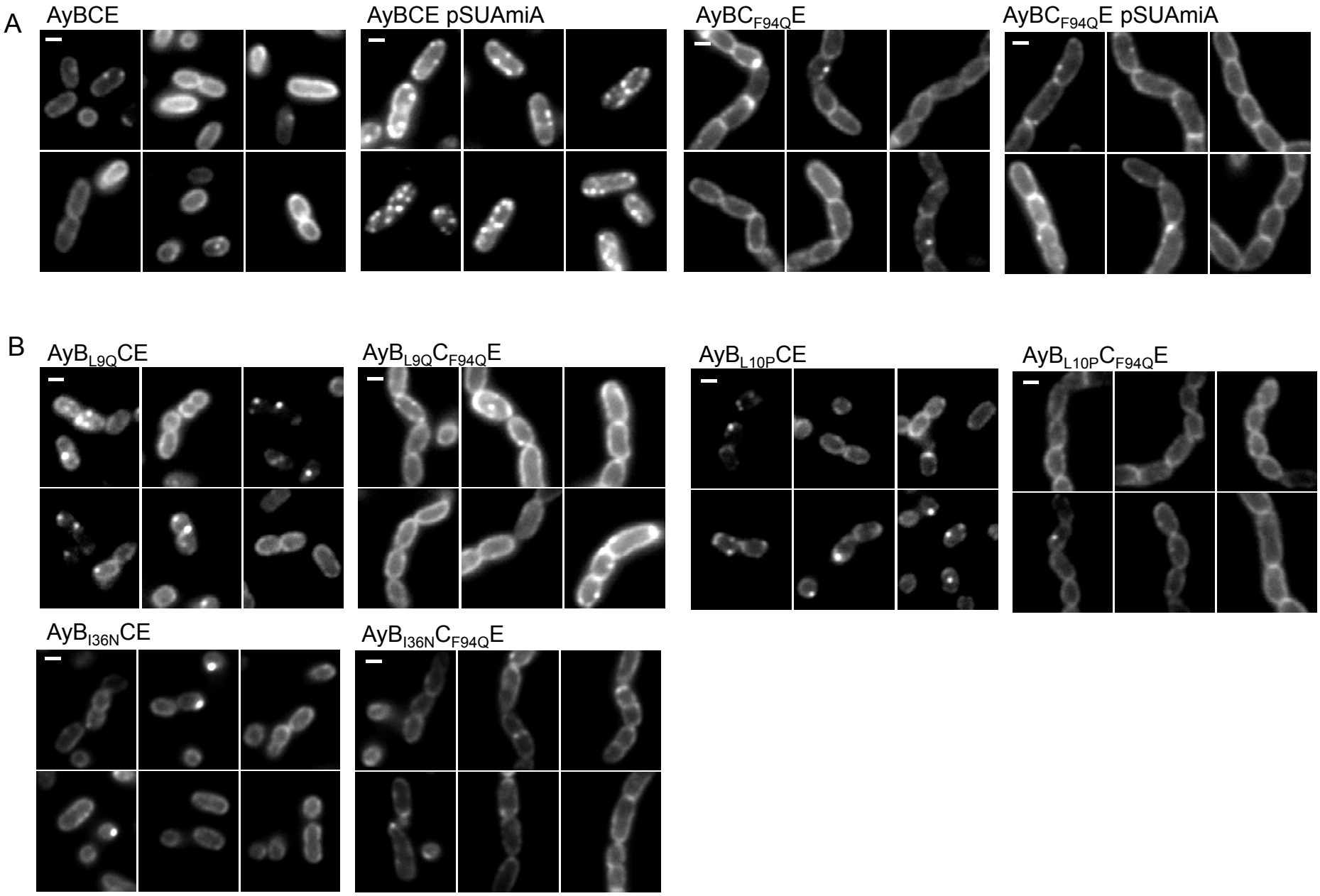
A
AyBCE
AyBCE pSUAmiA
AyBC$_{F94Q}$E
AyBC$_{F94Q}$E pSUAmiA
B
AyB$_{L9Q}$CE
AyB$_{L9Q}$C$_{F94Q}$E
AyB$_{L10P}$CE
AyB$_{L10P}$C$_{F94Q}$E
AyB$_{I36N}$CE
AyB$_{I36N}$C$_{F94Q}$E

Fig S11