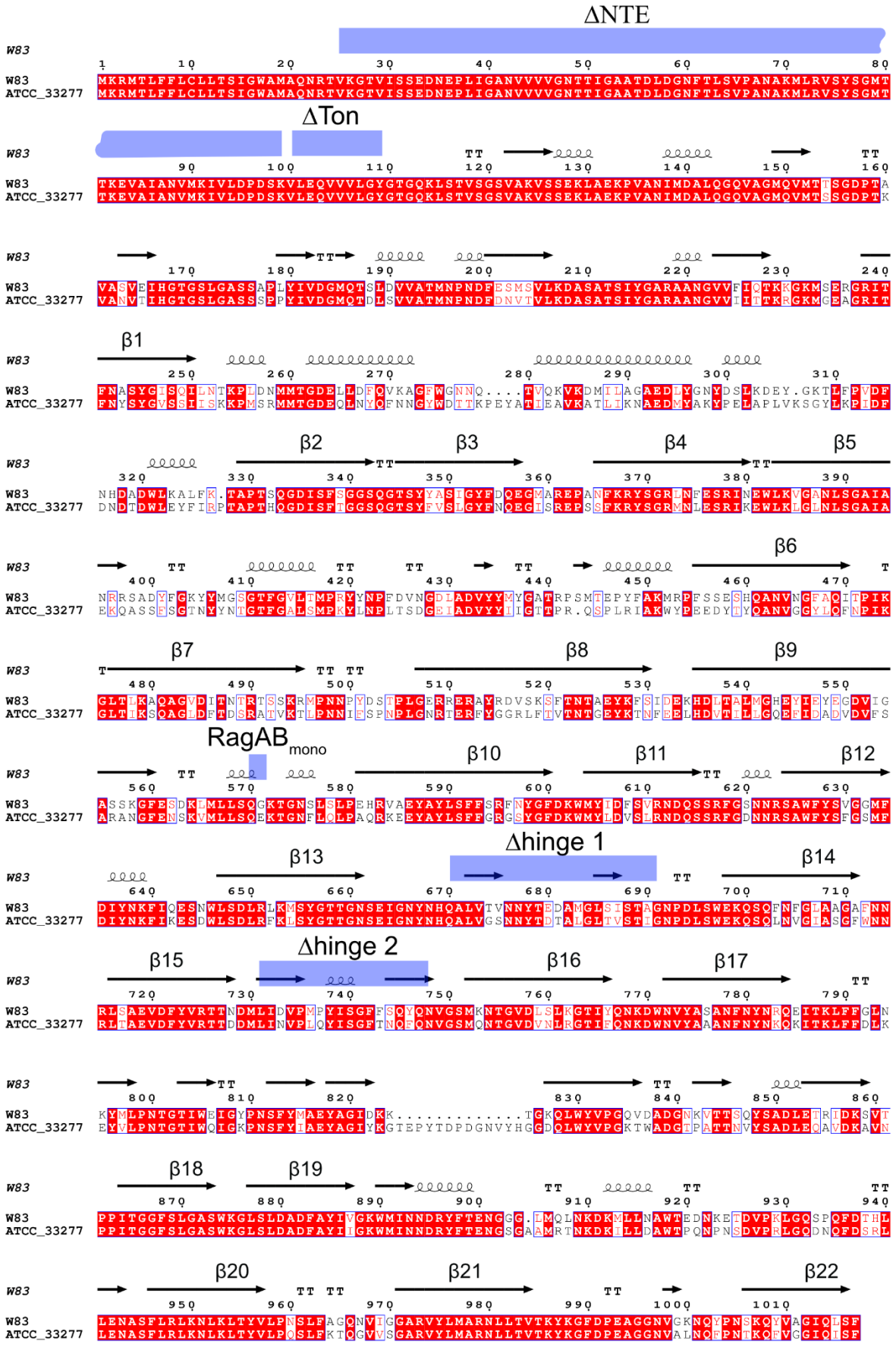
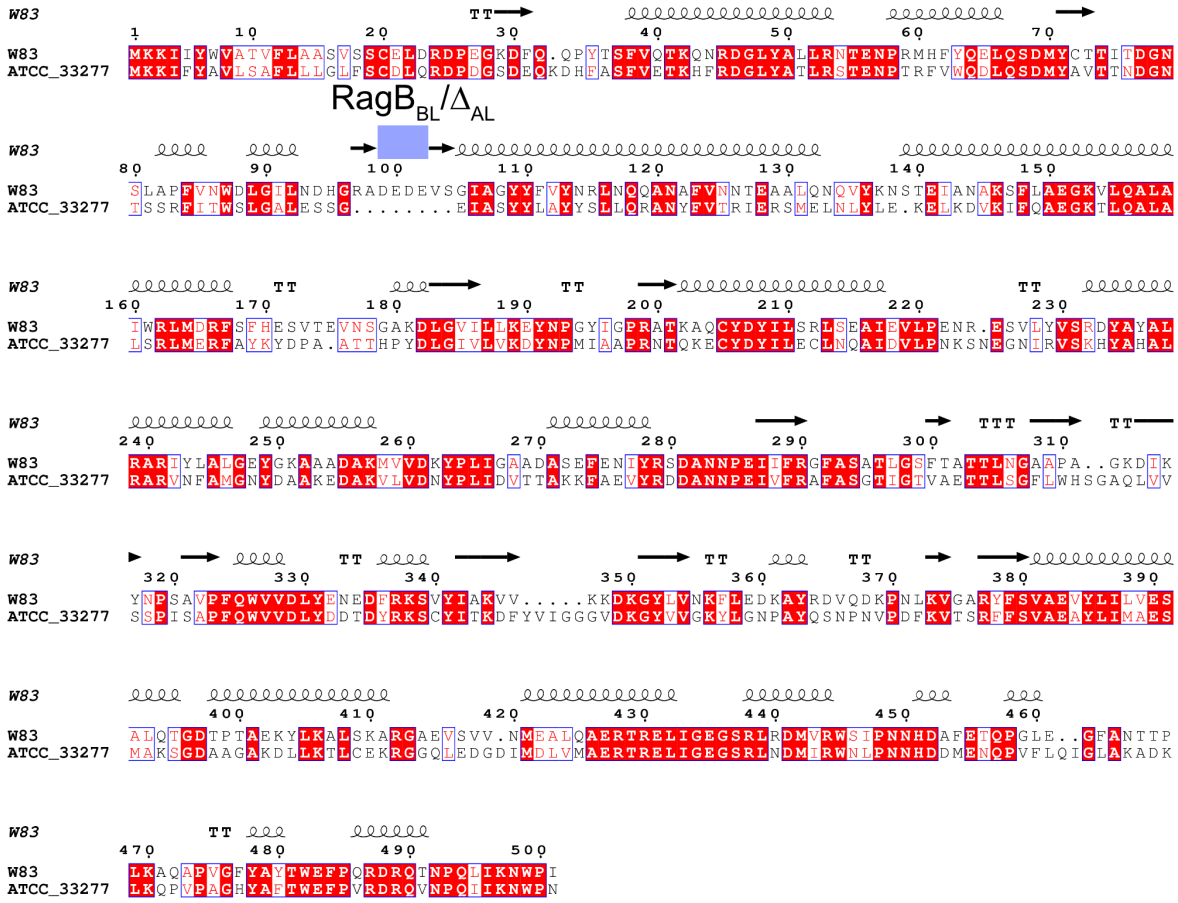
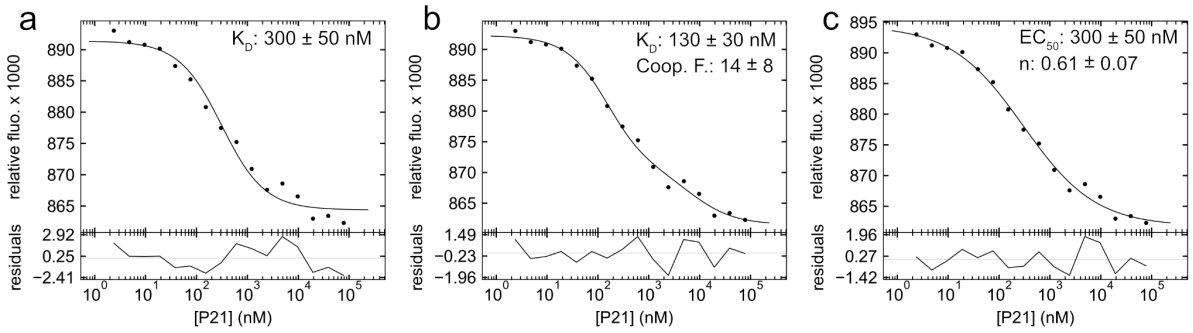
Supplementary Information



Supplementary Figure 1 Sequence alignments for RagA from W83 and ATCC 33277. The secondary structure assignment based on the RagAB W83 crystal structure is indicated. The positions of the site-directed mutants made are indicated with blue bars. Only the transmembrane β-strands are numbered in RagA. The image was produced by ESPript 3.0[1]. Sequence alignment was performed using Clustal Omega[2].



Supplementary Figure 2 Sequence alignments for RagB from W83 and ATCC 33277. The secondary structure assignment based on the RagAB W83 crystal structure is indicated. The positions of the site-directed mutants made are indicated with blue bars. The image was produced by ESPript 3.0[1]. Sequence alignment was performed using Clustal Omega[2].



Supplementary Figure 3 Additional analysis of MST data. MST profiles for unlabelled P21 binding to His-tag labelled RagAB W83 with fitted line from a 1:1 model (**a**), a 1:2 Macro model[3] (**b**) and the Hill model (**c**). The residuals between the data and the fit line are indicated in the bottom panel. Experiments and listed K_D and EC50 values represent the mean of three independent experiments ± SD. Cooperativity factor (Coop. F.) and n factor were used to determine the mode of cooperativity. Data were analysed in PALMIST version 1.4.04[3]. MST figures were rendered using GUSSI[4]. Statistical analysis was performed using the F-test, with p-values < 0.05 considered significant. The fits for the 1:2 Macro model and the Hill model are both better than the 1:1 model, with p-values of 0.0009 and 0.0002, respectively. The goodness-of-fits of the 1:2 Macro model and Hill model are statistically indistinguishable (p-value = 0.57).

Supplementary Movie Dynamics of the RagAB transporter. Cryo-EM map of open-closed (OC) RagAB, with cartoon models shown for RagA (blue) and RagB (yellow or grey) subunits.

Supplementary Table 1 Crystallographic data collection and refinement statistics

	W83-KRAB	W83-wild type	W83-wild type + P21
Data collection[#]			
Space group	$C222_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions			
a, *b*, *c* (Å)	191, 377, 369	131, 142, 242	130, 143, 250
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	83.9-3.38 (3.44-3.38)*	80.5-3.04 (3.09-3.04)	142.7-2.61 (2.65-2.61)
R_{pim}	8.4 (44.9)	15.7 (55.8)	10.2 (75.2)
$I / \sigma I$	8.5 (2.0)	3.7 (1.3)	5.6 (1.0)
Completeness (%)	99.6 (100)	100 (99.5)	99.9 (96.8)
Redundancy	7.6 (8.1)	7.4 (7.6)	7.3 (7.6)
Refinement			
Resolution (Å)	83.9-3.38	71.0-3.04	124.0-2.61
No. reflections	184,138	86,882	140,898
R_{work} / R_{free} (%)	20.5/25.5	22.3/29.3	20.7/26.4
No. atoms			
Protein (RagA/RagB)	7048/3839	7069/3838	7064/3834
Peptide	95	72	72
Water	-	-	
B-factors			
Protein (RagA/RagB)	89/66	44/37	57/50
Peptide	95	72	72
Water	-	-	
R.m.s. deviations			
Bond lengths (Å)	0.011	0.011	0.008
Bond angles (°)	1.37	1.33	1.03

[#] One crystal was used for each data collection.

* Values in parentheses are for highest-resolution shell.

Supplementary Table 2 RagAB peptidomics and *in vitro* binding of peptides to RagAB and RagB. Both analyses contain two separate Excel spreadsheets: all information retrieved from Mascot (Mascot) and a reduced spreadsheet with summed spectra (duplicates) and one charge variant of each peptide (Spectral C.) For spectral C., additional statistics were calculated: Spectral count peptide - summed number of spectra of particular peptide; Spectral count protein - summed number of spectra per particular protein; Spectral count sample - summed number of spectra per particular sample; Ratio peptide/protein - ratio of total number of particular peptide spectra to total number of spectra per protein; Ratio peptide/sample - ratio of total number of particular peptide spectra to total number of spectra per protein.

Supplementary Table 3 Cryo-EM data collection, refinement and validation statistics

	RagAB (CC) (EMD-10241) (PDB 6SM3)	RagAB (OC) (EMD-10245) (PDB 6SMQ)	RagAB (OO) (EMD-10243) (PDB 6SML)
Data collection and processing			
Magnification	130,000 x	130,000 x	130,000 x
Voltage (kV)	300	300	300
Electron exposure (e–/Å^2)	77.88	77.88	77.88
Defocus range (μm)	-1.2 to -3.0	-1.2 to -3.0	-1.2 to -3.0
Pixel size (Å)	1.07	1.07	1.07
Symmetry imposed	C2	C1	C2
Initial particle images pre-classification (no.)		491,870	
Initial particle images post-classification (no.)	87,897	218,052	52,754
Final particle images (no.)	86,877	213,143	51,849
Map resolution (Å)	3.3	3.3	3.4
FSC threshold 0.143			
Map resolution range (Å)	4.26-3.14	4.10-3.11	4.49-3.21
Refinement			
Initial model used (PDB code)	6SLI	6SLI	6SLI
Model resolution (Å)	3.2	3.2	3.4
FSC threshold	0.5	0.5	0.5
Model resolution range (Å)	∞ - 3.2	∞ - 3.2	∞ - 3.4
Map sharpening *B* factor (Å^2)	-120	-114.1	-119.9
Model composition			
Non-hydrogen atoms protein	11,020	22,136	11,076
Non-hydrogen atoms peptide ligand	79	82	65
Protein residues	1,398	2,791	1,405
B factors (Å^2)			
Protein	30.85	36.50	40.29
Peptide	30.37	26.53	46.22
R.m.s. deviations			
Bond lengths (Å)	0.009	0.011	0.009
Bond angles (°)	0.938	0.998	0.996
Validation			
MolProbity score	1.50	1.59	1.59
Clashscore	2.45	3.32	3.67
Poor rotamers (%)	0.26	0.34	0.09
Ramachandran plot			
Favored (%)	92.39	92.44	93.27
Allowed (%)	7.61	7.38	6.66
Disallowed (%)	0	0.18	0.07

Supplementary Table 4 Primers, plasmids and strains used in this study.

Primers

Name	Sequence (5'→3')
RagBaII plasmid	
RagB_A_KpnI_F	ATTGGTACCATGAAAAAAATAATTTATTGGGTTG
RagB_A_BamHI_R	ATAGGATCCTTATATCGGCCAGTTCTTTATTAAC
RagB_tet_BamHI_F	ATAGGATCCACAACGAATTATCTCCTTAACGTACG
RagB_tet_XbaI_R	TCGTCTAGATTTTATTGCCAAGTTCTAATGCTTCT
RagB_B_XbaI_F	TGCTCTAGATTTAGTTGTAGATCTTACTATGAAA
RagB_B_SphI_R	CATGCATGCACAAAGATAAGATATCTGCC
RagAaII plasmid	
RagA_A_SmaI_F	GTACCCGGGTGAAAAAAAGGATAATAGGATTAGTCT
RagA_A_NdeI_R	TATCATATGTTAGAAAGACAACTGAATACCCGC
RagA_erm_NdeI_F	TAACATATGATAGCTTCCGCTATTGCTTTTTTG
RagA_erm_XhoI_R	ATCCTCGAGTCTAGAGGATCCCCGAAGCTG
RagA_B_XhoI_F	AGACTCGAGGATTTACTTATTCTTAAGAAACATTTGATATGAA
RagA_B_SalI_R	CAGGTCGACGAAAGGGACTGCGCTCGG
RagB-8His plasmid	
RagB_8H_Fl	CACCATCACCATCACCATCACCATTAAGGATCCACAACGAATTATCTC
RagB_8H_Fs	TAAGGATCCACAACGAATTATCTC
RagB_8H_Rl	ATGGTGATGGTGATGGTGATGGTGTATCGGCCAGTTCTTTATTAACTG
RagB_8H_Rs	TATCGGCCAGTTCTTTATTAACTG
Δ*ragB* plasmid	
delRagB_KpnI_F	CAGTGGTACCCCAATTCGTTCTATATGGCT
delRagB_BamHI_R	GTTGTGGATCCATCAAATGTTTCTTAAGAATAAGTAAATC
Δ*ragA* plasmid	
delRagA_A_F	TGAATTCGAGCTCGGTACCCGACACGAAGGAGTTTATTGCG
delRagA_A_R	AAAGCAATAGCGGAAGCTATTCTAAGCAATTTGCTCACCATAC
delRagA_erm_F	TGGTGAGCAAATTGCTTAGAATAGCTTCCGCTATTGCTTTTTT
delRagA_erm_R	TTCTTAAGAATAAGTAAATCTCTAGAGGATCCCCGAAGCT
delRagA_B_F	GCTTCGGGGATCCTCTAGAGATTTACTTATTCTTAAGAAACATTTGAT
delRagA_B_R	GTCGACTCTAGAGGATCCCCTTTGTCGATCTCGCTGTG
Δ*ragAB* plasmid	
delRagAB_KpnI_F	CAGTGGTACCGACACGAAGGAGTTTATTGCG
delRagAB_BamHI_R	GTTGTGGATCCTCTAAGCAATTTGCTCACCATAC
$RagB_{BL}$ plasmid	
RagB_DEDEloop_Fl	GGACGTGCTCGTAAGCGTAAGGTCTCCGGTATAGCTGGCTACTATT
RagB_DEDEloop_Rl	GGAGACCTTACGCTTACGAGCACGTCCATGGTCGTTA
RagB_DEDEloop_Fs	GTCTCCGGTATAGCTGGCTACTATT
RagB_DEDEloop_Rs	AGCACGTCCATGGTCGTTA
Δ_{AL} plasmid	
RagB_delAcLoop_Fl	GGCATACTTAACGACCATGGAGGTATAGCTGGCTACTATTTCGTAT
RagB_delAcLoop_Rl	GAAATAGTAGCCAGCTATACCTCCATGGTCGTTAAGTATGC
RagB_delAcLoop_Fs	GGTATAGCTGGCTACTATTTCGTAT
RagB_delAcLoop_Rs	TCCATGGTCGTTAAGTATGC
$RagAB_{mono}$ plasmid	
RagAmono6H_Fl	GCCAGCATCATCATCATCATCATGGAAAAACCGGAAATAGTTTG
RagAmono6H_Rl	TTCCATGATGATGATGATGATGCTGGCTCAGTAACATCAACTTATC
RagAmono6H_Fs	GGAAAAACCGGAAATAGTTTG
RagAmono6H_Rs	CTGGCTCAGTAACATCAACTTATC
Δhinge1 plasmid	
RagBdelHinge1_Fl	GAGATTGGTAATTACAACCACAATCCCGACCTCTCGTGG
RagBdelHinge1_Rl	TCCCACGAGAGGTCGGGATTGTGGTTGTAATTACCAATCTCCGAG
RagBdelHinge1_Fs	AATCCCGACCTCTCGTGG
RagBdelHinge1_Rs	GTGGTTGTAATTACCAATCTCCGAG
Δhinge2 plasmid	
RagBdelHinge2_Fl	CGCACTACGAATGATATGGGCGTAGGCTCTATGAAAAATACGGG
RagBdelHinge2_Rl	ATTTTTCATAGAGCCTACGCCCATATCATTCGTAGTGCGGAC
RagBdelHinge2_Fs	GTAGGCTCTATGAAAAATACGGG
RagBdelHinge2_Rs	CATATCATTCGTAGTGCGGAC
ΔTonB plasmid	
RagAdelTonB_Fl	GTACTGGATCCGGACTCTAAGGGTACGGGACAGAAACTCAG
RagAdelTonB_Rl	GCTGAGTTTCTGTCCCGTACCCTTAGAGTCCGGATCCAGTACG
RagAdelTonB_Fs	GGTACGGGACAGAAACTCAG
RagAdelTonB_Rs	CCTTAGAGTCCGGATCCAGTACG

RagB W83 in ATCC plasmid	
ATCC_RagB_vect_F	ACAACGAATTATCTCCTTAACG
ATCC_RagB_vect_R	AATGATTTACTGTTAATCGTTAGTAC
RagBW83inATCC_F	ACGATTAACAGTAAATCATTATGAAAAAAATAATTTATTGGGTTGC
RagBW83inATCC_R	TAAGGAGATAATTCGTTGTTTATATCGGCCAGTTCTTTATTAAC
Δ*ragB*-ATCC plasmid	
delRagB_ATCC_A_F	TGAATTCGAGCTCGGTACCCTTTTTGGAACAATCGTTTG
delRagB_ATCC_A_R	TTAAGGAGATAATTCGTTGTAATGATTTACTGTTAATCGTTAGTACTC
delRagB_ATCC_tet_F	ACGATTAACAGTAAATCATTACAACGAATTATCTCCTTAACGTACG
delRagB_ATCC_tet_R	CGTCGAAACATCTAATTGAATTTTATTGCCAAGTTCTAATGCTTC
delRagB_ATCC_B_F	ATTAGAACTTGGCAATAAAATTCAATTAGATGTTTCGACG
delRagB_ATCC_B_R	GTCGACTCTAGAGGATCCCCTACGGAGAACATATGTTCC
Δ*ragAB*-ATCC plasmid	
delRagAB_ATCC_A_F	TGAATTCGAGCTCGGTACCCTTTCCGTCTCTCTTATGACGAAGAG
delRagAB_A_R	TTAAGGAGATAATTCGTTGTTCTAAGCAATTTGCTCACCATAC
delRagAB_ATCC_tet_F	TGGTGAGCAAATTGCTTAGA ACAACGAATTATCTCCTTAACGTAC
delRagAB_ATCC_tet_	CGTCGAAACATCTAATTGAATTTTATTGCCAAGTTCTAATGCTTC
delRagAB_ATCC_B_F	ATTAGAACTTGGCAATAAAATTCAATTAGATGTTTCGACG
delRagAB_ATCC_B_R	GTCGACTCTAGAGGATCCCCTACGGAGAACATATGTTCC
RagAB-W83-pTIO plasmid	
RagAB_W_pTIO_F	GCAGCCCGGGTTGGCGCGAGAAGTAAAAAAATC
RagAB_W_pTIO_R	CCGCTCTAGATTATATCGGCCAGTTCTTTATTAACTG
ΔNTE plasmid	
RagAdelNTE_Fl	GCTATGGCCCAGAATAGAACCGTTCTGGAGCAGGTAGTTGTAT
RagAdelNTE_Rl	TACAACTACCTGCTCCAGAACGGTTCTATTCTGGGCCATAG
RagAdelNTE_Fs	GTTCTGGAGCAGGTAGTTGTAT
RagAdelNTE_Rs	ACGGTTCTATTCTGGGCCATAG

Plasmids

Plasmid	Relevant features	Source
pUC19	*E. coli* cloning vector, Ap^R	Thermo Scientific
pTIO-1	*E. coli-Bacteroides* shuttle vector, Ap^R	[5]
RagBaII	Master plasmid for RagB modifications, derivative of pUC19	This study
RagB-8His	Plasmid for insertion of 8xHis at C-terminal of RagB, used for purification of RagAB complex, derivative of RagBaII,	This study
Δ*ragB*	Plasmid for deletion of *ragB* gene, derivative of RagBaII	This study
Δ*ragA*	Plasmid for deletion of *ragA* gene, derivative of RagAaII	This study
Δ*ragAB*	Plasmid for deletion of *ragA* and *ragB* genes, derivative of delRagB	This study
RagAaII	Master plasmid for RagA modifications, derivative of pUC19	This study
$RagB_{BL}$	Plasmid for substitution of D^{99}-E^{102} with RKRK in *ragB* gene, derivative of RagBaII	This study
$Δ_{AL}$	Plasmid for deletion of acidic loop R^{97}-S^{104} in *ragB* gene, derivative of RagBaII	This study
$RagAB_{mono}$	Plasmid for insertion of 6xHis after Q^{570} in *ragA* gene, derivative of RagAaII	This study
Δhinge1	Plasmid for deletion of Q^{670}- G^{691} in *ragA* gene, derivative of RagAaII	This study
Δhinge2	Plasmid for deletion of L^{731} -N^{748} and insertion of glycine in postion 731 in *ragA* gene, derivative of RagAaII	This study
ΔTonB	Plasmid for deletion of V^{100}-Y^{109} in *ragA* gene, derivative of RagAaII	This study
ΔNTE	Plasmid for deletion of V^{25}-K^{99} in *ragA* gene, derivative of RagAaII	This study
RagB W83 in ATCC	Plasmid for substitution of *ragB* from ATCC33277 with *ragB* from W83 strain	This study
Δ*ragB*-ATCC	Plasmid for deletion of *ragB* gene in ATCC33277 strain	This study
Δ*ragAB*-ATCC	Plasmid for deletion of *ragA* and *ragB* genes in ATCC33277 strain	This study
RagAB-W83-pTIO	Shuttle plasmid for expression of *ragAB* genes from W83 strain	This study

Strains

Strain	Relevant genotype	Source
	E. coli	
DH5α	*fhuA2* Δ(*arg*F-*lac*Z)U169 *pho*A *gln*V44 Φ80 Δ(*lac*Z)M15 *gyr*A96 *rec*A1 *rel*A1 *end*A1 *thi*-1 *hsd*R17	New England Biolabs
BL21 (DE3)	*fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*	Invitrogen
S-17 λpir	*creC510 hsdR17 thiE1 endA1 recA1 LAMpir pro-82 RP4-2(Km::Tn7,Tc::Mu-1)*	[6]
	P. gingivalis	
W83	Wild type	[7]
ATCC33277	Wild type	[8]
HG66	Wild type	[9]
A7436	Wild type	[10]
381	Wild type	[11]
KRAB	*rgpA rgpB kgp* (Cm^R)(Em^R)(Tet^R)	[12]
ΔragA	*ragA* (NCBI:PG_0185)(Em^R)	This study
Δ*ragB*	*ragB* (NCBI:PG_0186)(Tet^R)	This study
Δ*ragAB*	*ragA ragB* (Tet^R)	This study
ΔTonB	*ragA* $\Delta^{101-108}$ (Em^R)	This study
$RagAB_{mono}$	*ragA* p.Q^{570}_ins$HHHHHH$_G^{571} (Em^R)	This study
Δhinge1	*ragA* $\Delta^{670-691}$(Em^R)	This study
Δ_{AL}	*ragB* Δ^{97-104}(Tet^R)	This study
$RagB_{BL}$	*ragB*p.D99R;E100K;D101R;E102K(Tet^R)	This study
ΔNTE	*ragA* Δ^{25-99} (Em^R)	This study
Δhinge2	*ragA* $\Delta^{670-691}$(Em^R)	This study
RagB-8His	*ragA*p.L731G;$\Delta^{731-748}$;(Em^R)	This study
RagB W83 in ATCC 33277	*ragB*(NCBI:PGN_0294):*ragB*(NCBI:PG_0186)(Tet^R)	This study
∆*ragAB* ATCC 33277	*ragA*(NCBI: PGN_0293); *ragB*(NCBI: PGN_0294)(Tet^R)	This study
RagAB W83 in ATCC 33277	*ragA*(NCBI: PGN_0293); *ragB*(NCBI: PGN_0294)(Tet^R)/*ragA* (NCBI:PG_0185) *ragB* (NCBI:PG_0186) (Em^R)	This study

Supplementary references

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