

Conformation control of the histidine kinase BceS of *Bacillus subtilis* by its cognate ABC-transporter facilitates need-based activation of antibiotic resistance

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- Supplementary Information -

Figure S1. Cysteine scanning and site-directed mutagenesis of the BceS DHP domain.

Figure S2. Cysteine cross-linking following activation of signaling.

Figure S3. Equilibration and principle component analysis of Gaussian accelerated molecular dynamics simulations of BceS DHP domain conformation.

Figure S4. Cysteine scanning mutagenesis of the BceS HAMP-like domain.

Figure S5. Cysteine scanning mutagenesis of the second transmembrane helix of BceS.

Figure S6. Arginine substitutions in transmembrane helix 2 of BceS.

Figure S7. Signaling phenotypes of BceAB variants.

Table S1. Strains, primers and plasmids used in this study.

Table S2. Minimal inhibitory concentration of bacitracin in strains carrying BceAB variants.

Table S3. Parameters used for GaMD simulations.

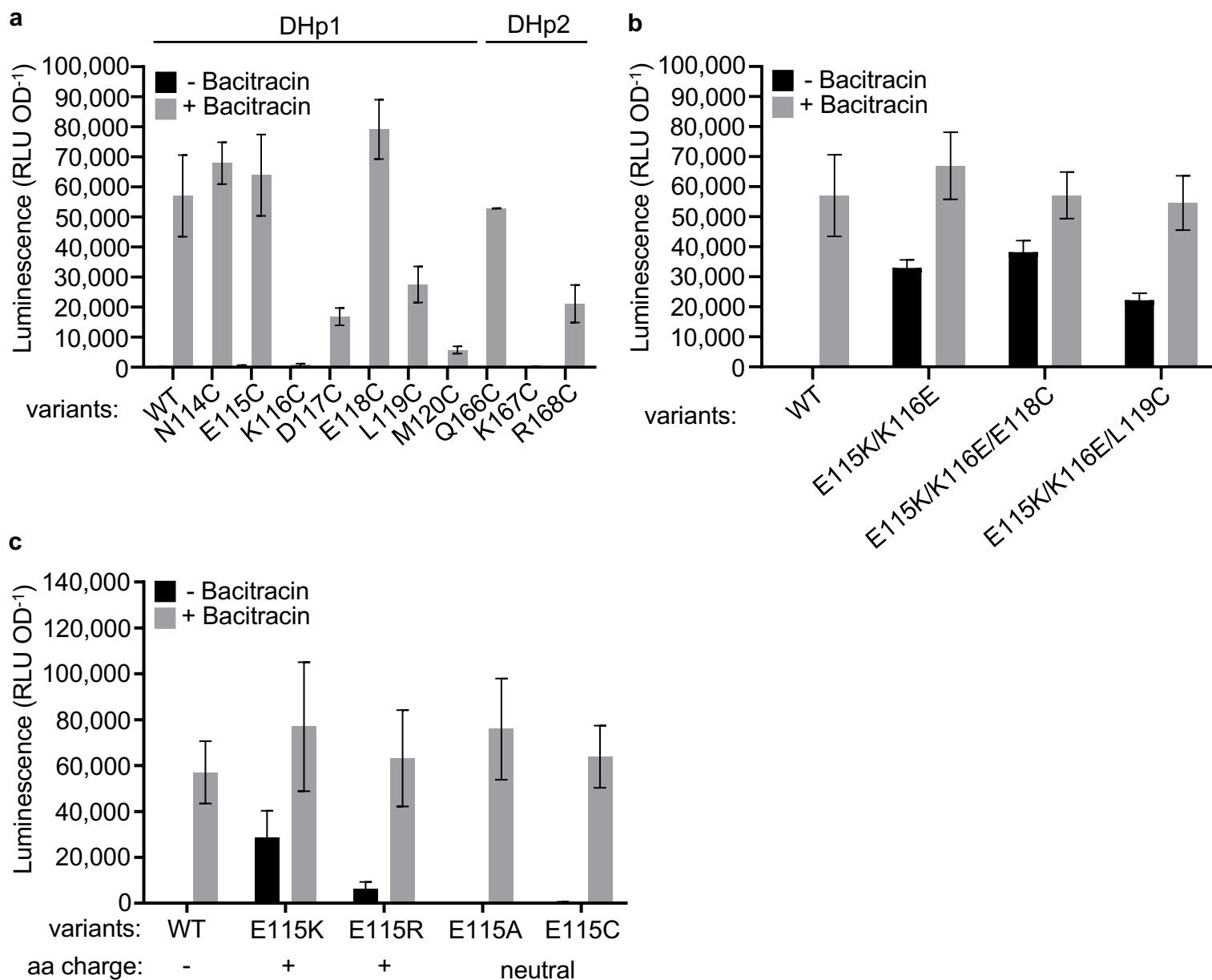


Figure S1. Cysteine scanning and site-directed mutagenesis of the BceS DHp domain. BceS signalling activity measured in strains harbouring P_{bceA} -*luxABCDE* and P_{xyf} -*bceS* wild-type (WT) or variants as indicated. Amino acid substitutions were introduced in the parent construct *bceS*^{WT}-His₈ (SGB369). **a**, Single Cys-substitution variants. Residues belonging to helices DHp1 or DHp2 are indicated above. **b**, Combination of single Cys substitutions with the constitutive ON substitutions E115K/K116E. **c**, Series of substitutions at position 115, with the resulting side-chain charge indicated below. Signalling was measured in cells challenged with 1 $\mu\text{g ml}^{-1}$ of bacitracin (grey) or left unchallenged (black). Data are shown as mean \pm SD from 4-22 biological repeats.

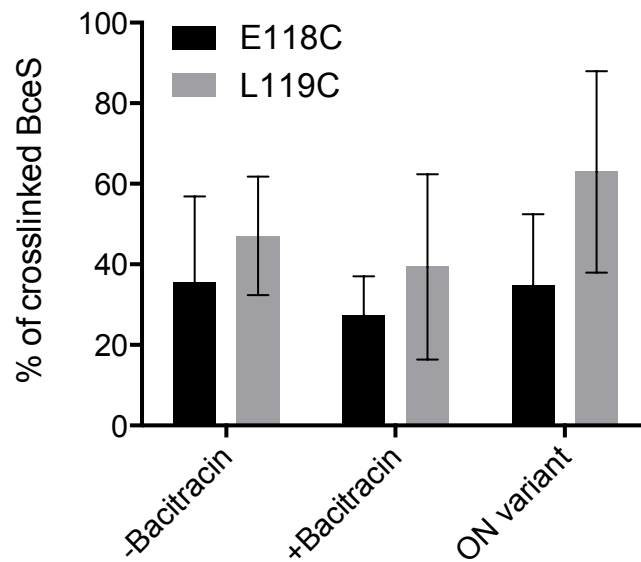


Figure S2. Cysteine cross-linking following activation of signalling. The percentage of crosslinking for BceS Cys variants E118C (black) and L119C (grey) was calculated from relative band intensities between monomer and dimer within each lane, following Western blot detection of BceS-His₈ as described in Methods. ‘+/- Bacitracin’ shows results for cells producing otherwise wild-type BceS following activation with the native stimulus. ‘ON variant’ shows results in cells carrying the additional substitutions E115K/K116E causing constitutive activity of BceS.

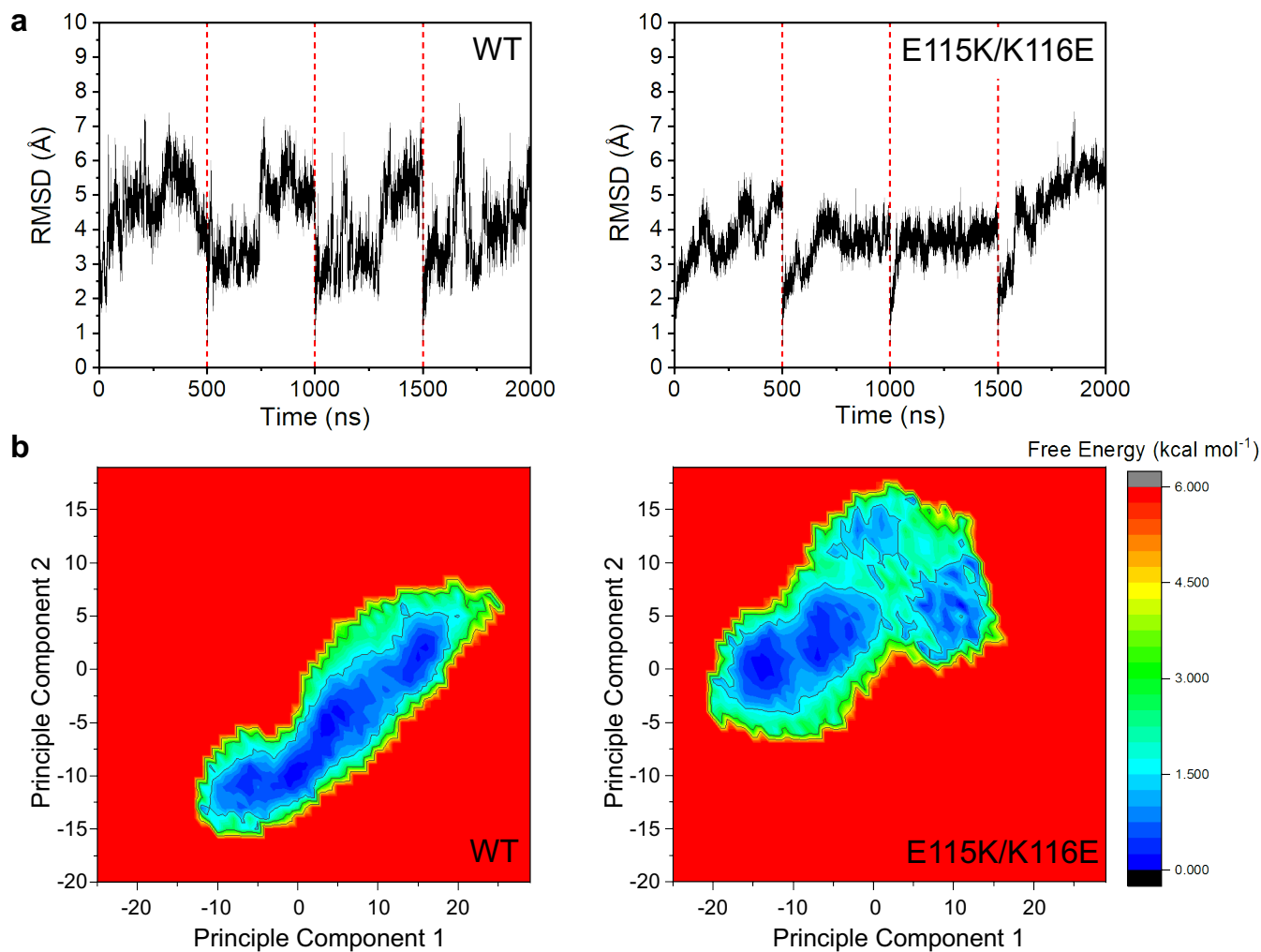


Figure S3. Equilibration and principle component analysis of Gaussian accelerated molecular dynamics (GAMD) simulations of BceS DHP domain conformation. **a**, $C\alpha$ RMSD over the course of GAMD simulations for simulations of wild type (WT, left) and the E115K/K116E variant (right) protein. The red dotted lines divides up the four different 500 ns long replicas used. RMSD was measured against the homology model starting structure. **b**, Projection of the first two principal components (PCs) from PC analysis (PCA) of the GAMD simulations for the WT (left) and E115K/K116E variant (right) protein. PCA was performed on the $C\alpha$ carbon of the DHP domain residues.

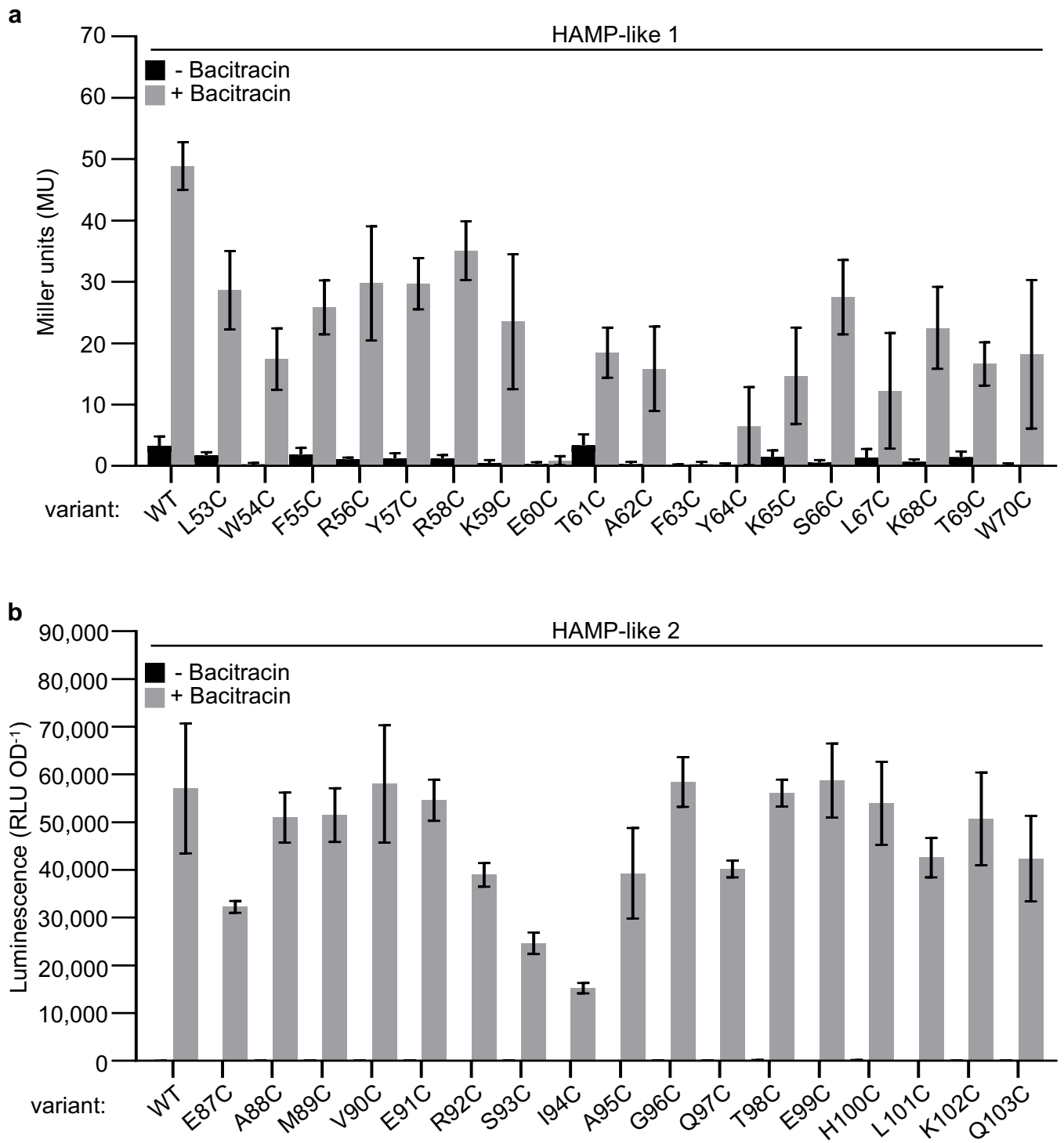


Figure S4: Cysteine scanning mutagenesis of the BceS HAMP-like domain. Amino acid substitutions were introduced into the P_{xyI} -*bceS*-His₈ parent construct, pSD2E01. Cells were challenged with 1 $\mu\text{g ml}^{-1}$ of bacitracin (grey) or left unchallenged (black). **a**, Cys scanning of the helix HAMP-like 1. Signalling was assessed by β -galactosidase activity (Miller Units) in cells carrying the P_{bceA} -*lacZ* reporter (SGB401 and derivatives). **b**, Cys scanning of helix HAMP-like 2. Signalling was assessed by luminescence (RLU OD⁻¹) in cells carrying the P_{bceA} -*luxABCDE* reporter (SGB369 and derivatives). Data are shown as mean \pm SD from 3-22 independent repeats.

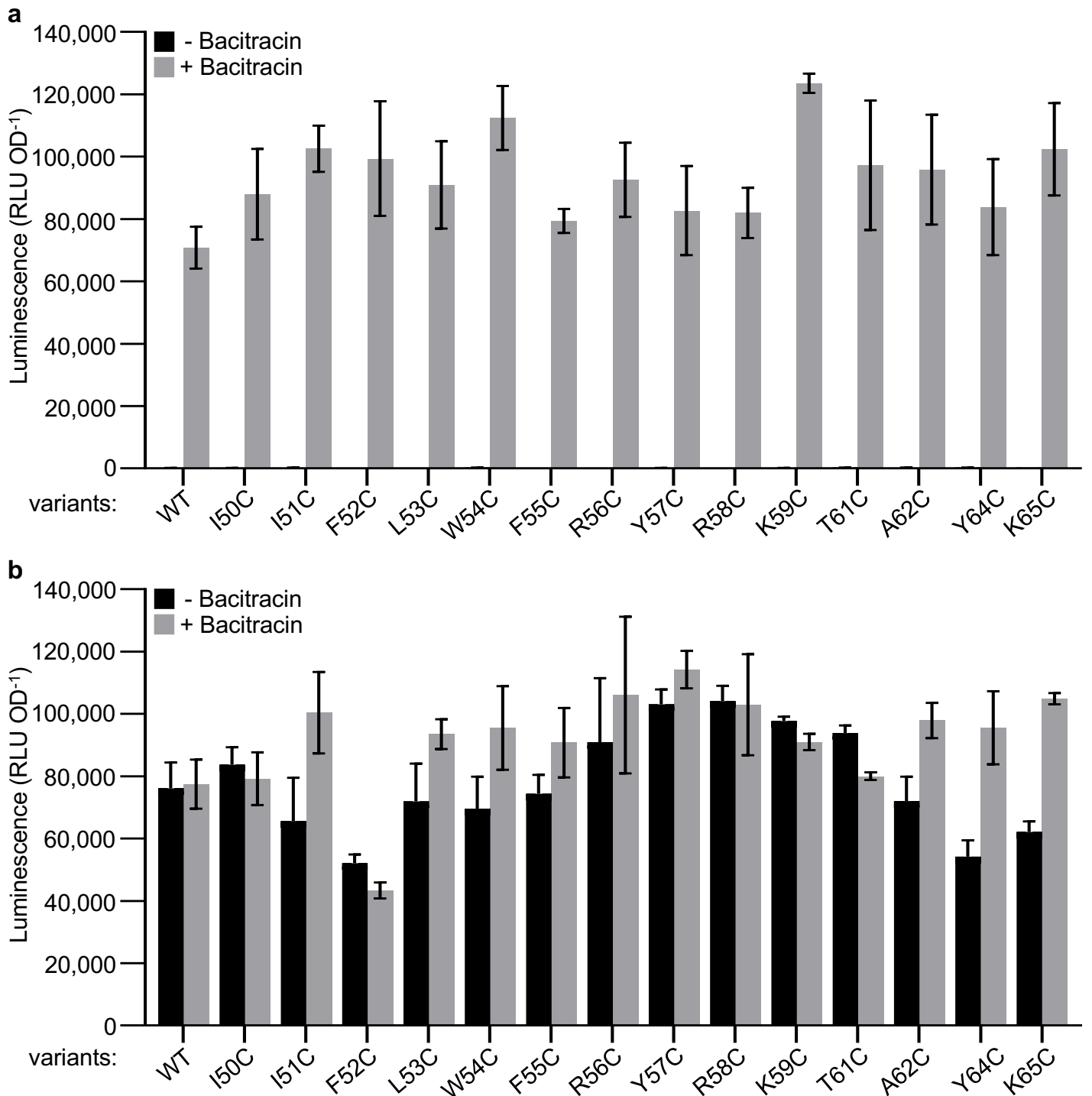


Figure S5. Cysteine scanning mutagenesis of the second transmembrane helix of BceS. BceS signaling activity was assessed in cells harbouring the P_{bceA} -*luxABCDE* reporter and P_{xyI} -*bceS*-His₈. Cells were challenged with 1 $\mu\text{g ml}^{-1}$ of bacitracin (grey) or left unchallenged (black), and signalling measured by luminescence (RLU OD⁻¹). **a**, Signalling activities of the otherwise wild-type, cysteine-free variant BceS^{C45S/C198S/C259S} (WT; SGB936) and its single-cysteine derivatives as indicated. **b**, Signalling activities of the constitutively active, cysteine-free variant BceS^{E115K/K116E/C45S/C198S/C259S} (WT; SGB951) and its single-cysteine derivatives as indicated. Data are shown as mean \pm SD from 3 independent repeats.

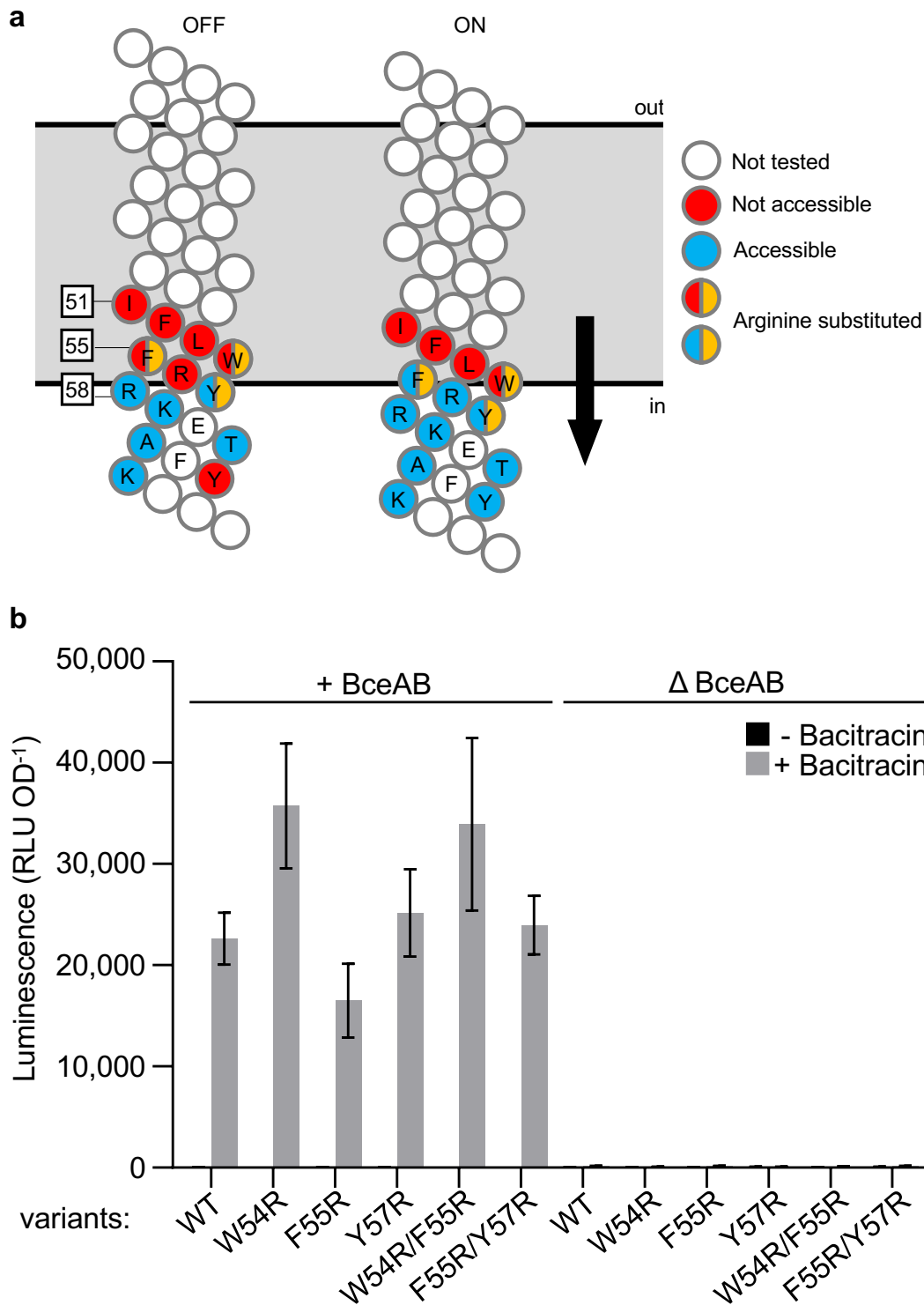


Figure S6. Arginine substitutions in transmembrane helix 2 of BceS. **a**, Schematic representation of Arg substitutions within transmembrane helix 2, shown in the OFF (left) and ON (right) states. Solvent-accessible Cys residues are indicated in blue, inaccessible Cys residues in red. The introduced Arg substitution are shown as yellow half-circles. **b**, Signalling activity of BceS Arg variants. Amino acid substitutions were introduced into the parent construct P_{xyI} -*bceS*-His₈, in a strain background carrying a $\Delta bceS$ deletion (+BceAB; SGB792), or a $\Delta bceSAB$ deletion (Δ BceAB; SGB818). Cells were challenged with $10 \mu\text{g ml}^{-1}$ of bacitracin (grey) or left unchallenged (black), and signalling was assessed using the P_{bceA} -*luxABCDE* reporter, expressed as luminescence (RLU OD⁻¹). Data are shown as mean \pm SD from 7-12 independent repeats.

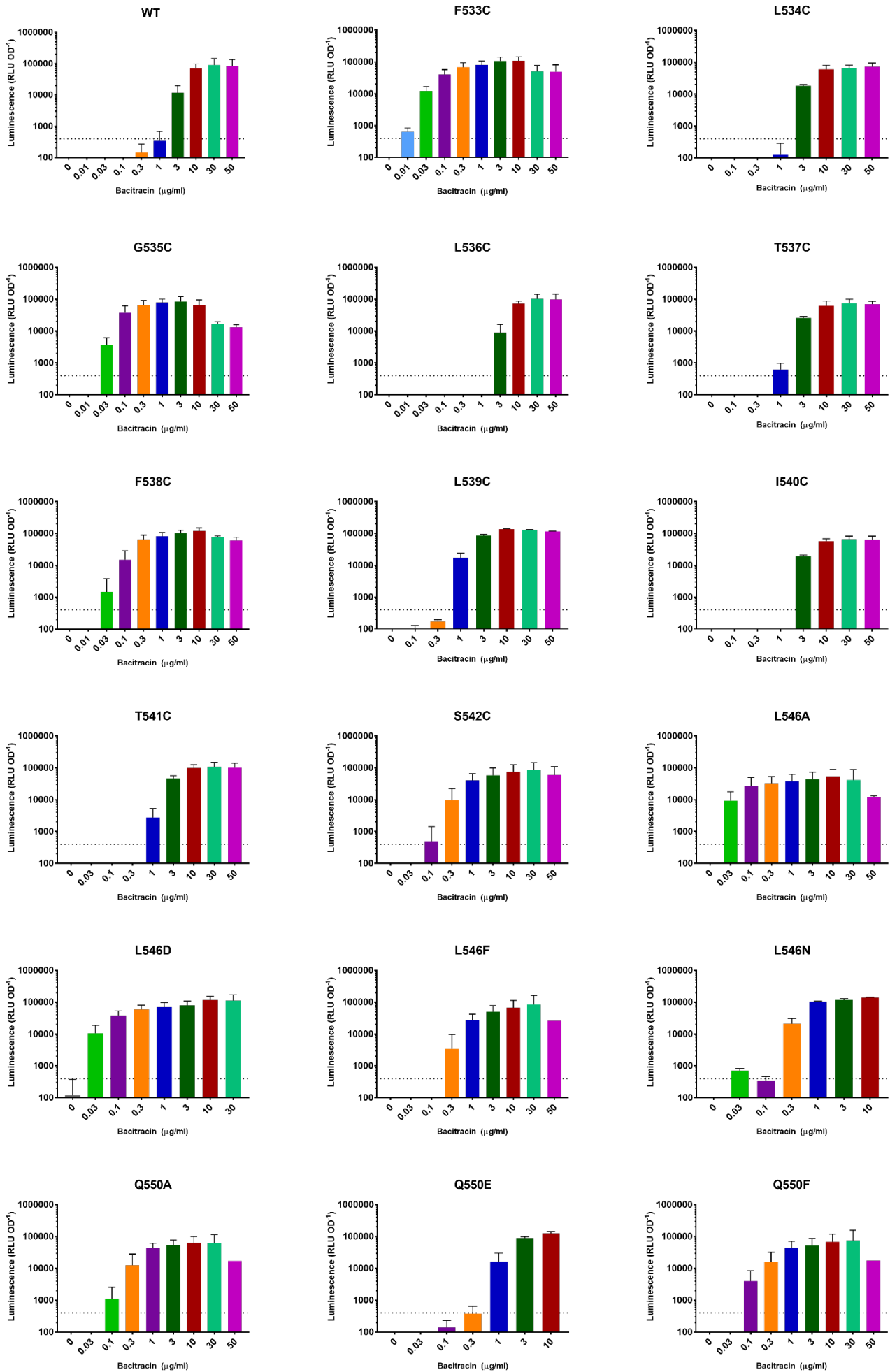
a

Figure S7. Signalling phenotypes of BceAB variants. complete legend after panel b.

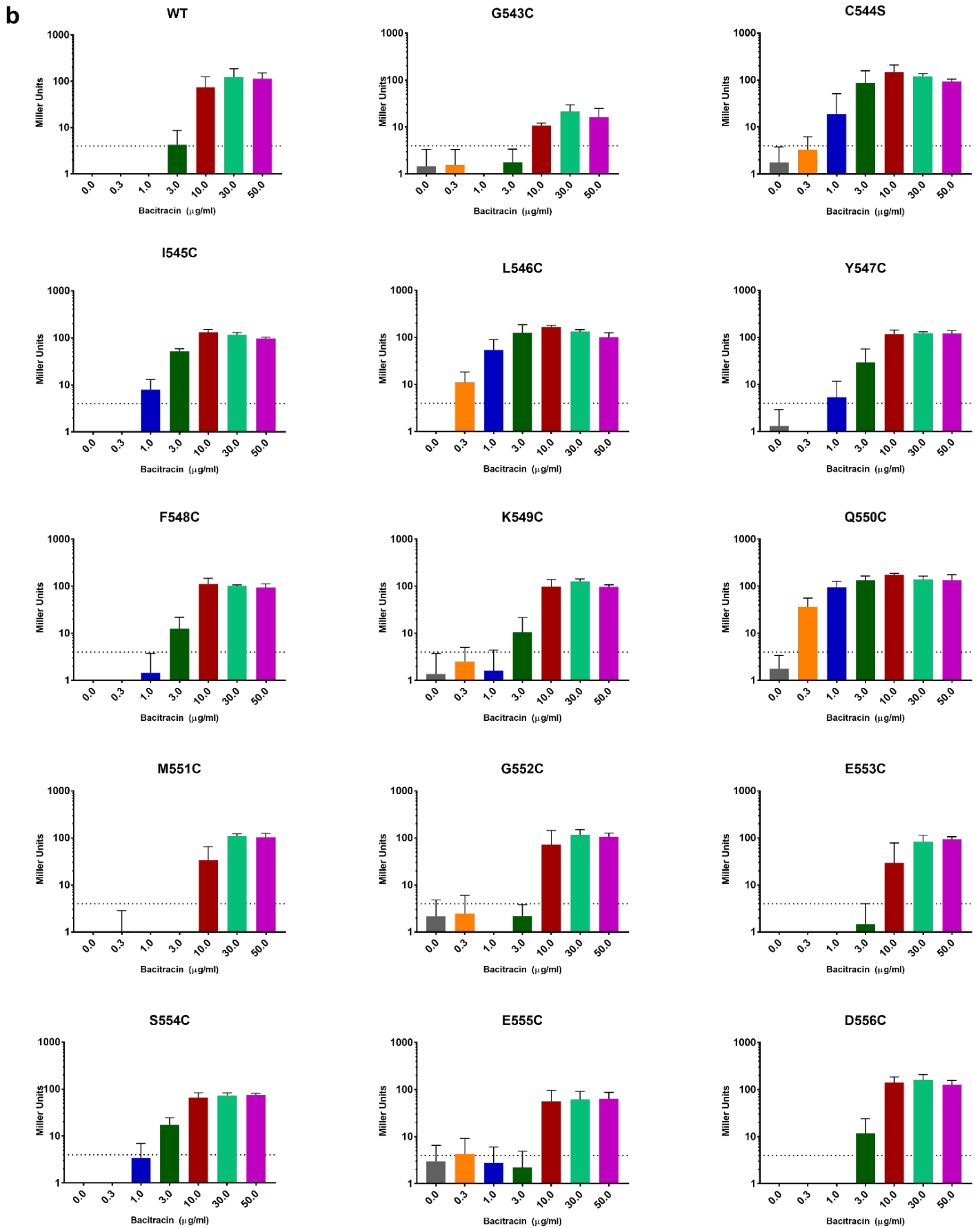


Figure S7. Signalling phenotypes of BceAB variants. Amino acid substitutions were introduced into the $P_{xyI}bceAB\text{-FLAG}_3$ parent construct, pFK727. Cells were challenged with a series of bacitracin concentrations as indicated. **a**, Signalling was assessed by luminescence (RLU OD^{-1}) in cells carrying the $P_{bceA}\text{-luxABCDE}$ reporter (SGB731 and derivatives). **b**, Signalling was assessed by b-galactosidase activity (Miller Units) in cells carrying the $P_{bceA}\text{-lacZ}$ reporter (SGB370 and derivatives). Data are shown as mean \pm SD of a minimum of three independent repeats. The threshold activity applied to determine signalling above background is indicated by dotted lines and was used to generate the data displayed in Fig. 6b of the main manuscript.

Table S1. Strains, primers and plasmids used in this study

Section A - Strains

Strain	Genotype	Parent strain ^a	Plasmid/Strain used ^b	Source
SGB16	<i>W168 bceAB::kan amyE::bceA-lacZ</i>	TMB035	pER603	this work
SGB79	<i>W168 bceAB::kan sacA::PbceA-luxABCDE</i>			(Kallenberg <i>et al.</i> , 2013)
SGB176	<i>W168 bceAB::kan thrC::Pxyl-bceAB-FLAG</i>			(Kallenberg <i>et al.</i> , 2013)
SGB276	<i>W168 ΔbceS sacA::PbceA-luxABCDE lacA::Pxyl-bceS-His8</i>			(Dintner <i>et al.</i> , 2014)
SGB334	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{E553C}-FLAG</i>	SGB16	pMG707	this work
SGB337	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{L546C}-FLAG</i>	SGB16	pAM703	this work
SGB338	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{D556C}-FLAG</i>	SGB16	pJL705	this work
SGB340	<i>W168 sacA::PbceA-luxABCDE</i>	W168	SGB276	this work
SGB341	<i>W168 sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG</i>	SGB340	SGB176	this work
SGB345	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{Y547C}-FLAG</i>	SGB16	pMG704	this work
SGB352	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{K549C}-FLAG</i>	SGB16	pMG713	this work
SGB357	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG</i>	SGB341	pAK102	this work
SGB359	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K}-His8</i>	SGB357	pAK2E02	this work
SGB369	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{WT}-His8</i>	SGB357	pSD2E01	this work
SGB370	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB-FLAG</i>	SGB16	pFK727	this work
SGB371	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{G543C}-FLAG</i>	SGB16	pMG718	this work
SGB372	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{L545C}-FLAG</i>	SGB16	pMG719	this work
SGB373	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{F548C}-FLAG</i>	SGB16	pMG720	this work
SGB374	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{Q550C}-FLAG</i>	SGB16	pMG721	this work
SGB375	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{M551C}-FLAG</i>	SGB16	pMG722	this work
SGB376	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{G552C}-FLAG</i>	SGB16	pMG723	this work
SGB377	<i>W168 ΔbceSAB</i>	W168	pAK102	this work
SGB378	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE</i>	SGB340	pAK102	this work
SGB379	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{C544S}-FLAG</i>	SGB16	pMG724	this work
SGB380	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115A}-His8</i>	SGB357	pAK2E12	this work
SGB381	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115R}-His8</i>	SGB357	pAK2E13	this work
SGB382	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115C}-His8</i>	SGB357	pAK2E14	this work

SGB383	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{Q166C}-His8</i>	SGB357	pAK2E15	this work
SGB384	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{R168C}-His8</i>	SGB357	pAK2E16	this work
SGB385	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{K116C}-His8</i>	SGB357	pAK2E17	this work
SGB392	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE lacA::Pxyl-bceS^{WT}-His8</i>	SGB378	pSD2E01	this work
SGB395	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{N114C}-His8</i>	SGB357	pAK2E21	this work
SGB396	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{K167C}-His8</i>	SGB357	pAK2E22	this work
SGB400	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG</i>	SGB377	TMB279 and SGB176	this work
SGB401	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{WT}-His8</i>	SGB400	pSD2E01	this work
SGB402	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{L53C}-His8</i>	SGB400	pMG2E01	this work
SGB403	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{W54C}-His8</i>	SGB400	pMG2E02	this work
SGB404	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{F55C}-His8</i>	SGB400	pMG2E03	this work
SGB405	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{R56C}-His8</i>	SGB400	pMG2E04	this work
SGB406	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{Y57C}-His8</i>	SGB400	pMG2E05	this work
SGB407	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{R58C}-His8</i>	SGB400	pMG2E06	this work
SGB408	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{K59C}-His8</i>	SGB400	pMG2E07	this work
SGB409	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{E60C}-His8</i>	SGB400	pMG2E08	this work
SGB410	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{T61C}-His8</i>	SGB400	pMG2E09	this work
SGB411	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{A62C}-His8</i>	SGB400	pMG2E10	this work
SGB413	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{E60C/E115K}-His8</i>	SGB400	pMG2E12	this work
SGB414	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{F63C}-His8</i>	SGB400	pAK2E54	this work
SGB415	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{Y64C}-His8</i>	SGB400	pAK2E55	this work
SGB416	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{K65C}-His8</i>	SGB400	pAK2E56	this work
SGB417	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{S66C}-His8</i>	SGB400	pAK2E57	this work
SGB418	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{L67C}-His8</i>	SGB400	pAK2E58	this work
SGB419	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{K68C}-His8</i>	SGB400	pAK2E59	this work
SGB420	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{T69C}-His8</i>	SGB400	pAK2E60	this work
SGB421	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{W70C}-His8</i>	SGB400	pAK2E61	this work
SGB424	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{F63C/E115K}-His8</i>	SGB400	pMG2E15	this work
SGB430	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{S554C}-FLAG</i>	SGB16	pMG738	this work

SGB431	<i>W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB^{E555C}-FLAG</i>	SGB16	pMG739	this work
SGB433	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{K116E}-His8</i>	SGB357	pAK2E26	this work
SGB436	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{K167D}-His8</i>	SGB357	pAK2E29	this work
SGB440	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E118C}-His8</i>	SGB357	pAK2E33	this work
SGB441	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E}-His8</i>	SGB357	pAK2E34	this work
SGB444	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{Q94C}-His8</i>	SGB357	pAK2E37	this work
SGB445	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{A95C}-His8</i>	SGB357	pAK2E38	this work
SGB446	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{G96C}-His8</i>	SGB357	pAK2E39	this work
SGB447	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{Q97C}-His8</i>	SGB357	pAK2E40	this work
SGB448	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{T98C}-His8</i>	SGB357	pAK2E41	this work
SGB449	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E99C}-His8</i>	SGB357	pAK2E42	this work
SGB450	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{H100C}-His8</i>	SGB357	pAK2E43	this work
SGB451	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{L101C}-His8</i>	SGB357	pAK2E44	this work
SGB452	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{K102C}-His8</i>	SGB357	pAK2E45	this work
SGB453	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{Q103C}-His8</i>	SGB357	pAK2E46	this work
SGB454	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E87C}-His8</i>	SGB357	pAK2E47	this work
SGB455	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{A88C}-His8</i>	SGB357	pAK2E48	this work
SGB456	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{M89C}-His8</i>	SGB357	pAK2E49	this work
SGB457	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{V90C}-His8</i>	SGB357	pAK2E50	this work
SGB458	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E91C}-His8</i>	SGB357	pAK2E51	this work
SGB459	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{R92C}-His8</i>	SGB357	pAK2E52	this work
SGB460	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{S93C}-His8</i>	SGB357	pAK2E53	this work
SGB465	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{L67C}-His8</i>	SGB357	pAK2E58	this work
SGB470	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{D117C}-His8</i>	SGB357	pAK2E63	this work
SGB473	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{K167D/K116E}-His8</i>	SGB357	pAK2E66	this work
SGB513	<i>W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB^{WT}-FLAG lacA::Pxyl-bceS^{E115K}-His8</i>	SGB400	pAK2E02	this work
SGB532	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K167D}-His8</i>	SGB357	pAK2E118	this work
SGB562	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{L119C}-His8</i>	SGB357	pAK2E134	this work
SGB565	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{M120C}-His8</i>	SGB357	pAK2E137	this work

SGB693	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{L67F}-His8</i>	SGB357	pAK2E154	this work
SGB696	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{L67G}-His8</i>	SGB357	pAK2E157	this work
SGB707	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{L94G}-His8</i>	SGB357	pAK2E168	this work
SGB709	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{L94F}-His8</i>	SGB357	pAK2E170	this work
SGB718	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{T541C}-FLAG</i>	SGB79	pMG759	this work
SGB719	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{S542C}-FLAG</i>	SGB79	pMG760	this work
SGB721	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{L546A}-FLAG</i>	SGB79	pMG762	this work
SGB722	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{L546F}-FLAG</i>	SGB79	pMG763	this work
SGB723	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{Q550A}-FLAG</i>	SGB79	pMG764	this work
SGB724	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{Q550F}-FLAG</i>	SGB79	pMG765	this work
SGB731	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB-FLAG</i>	SGB79	pFK727	this work
SGB732	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{F538C}-FLAG</i>	SGB79	pMG712	this work
SGB765	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{L546N}-FLAG</i>	SGB79	pMG770	this work
SGB766	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{Q550E}-FLAG</i>	SGB79	pMG771	this work
SGB771	<i>W168 ΔbceS sacA::PbceA-luxABCDE</i>	TMB1036	SGB378	this work
SGB790	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/E118C}-His8</i>	SGB357	pAK2E176	this work
SGB791	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/L119C}-His8</i>	SGB357	pAK2E177	this work
SGB792	<i>W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{WT}</i>	SGB771	pAKXT03	this work
SGB795	<i>W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{E115K}</i>	SGB771	pAKXT06	this work
SGB807	<i>W168 bceAB::kan sacA::PbceA-lux thrC::pXT-BceAB^{L546D}-FLAG</i>	SGB79	pMG772	this work
SGB818	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{WT}</i>	SGB378	pAKXT03	this work
SGB820	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{E115K}</i>	SGB378	pAKXT06	this work
SGB868	<i>W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{E115K/K116E}</i>	SGB771	pAKXT13	this work
SGB869	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{E115K/K116E}</i>	SGB378	pAKXT13	this work
SGB886	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE lacA::Pxyl-bceS^{L67F}-His8</i>	SGB378	pAK2E154	this work
SGB895	<i>W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{W54R}</i>	SGB771	pAKXT15	this work
SGB896	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{W54R}</i>	SGB378	pAKXT15	this work
SGB897	<i>W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{F55R}</i>	SGB771	pAKXT22	this work
SGB898	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{F55R}</i>	SGB378	pAKXT22	this work
SGB899	<i>W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-BceS^{Y57R}</i>	SGB771	pAKXT20	this work
SGB900	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{Y57R}</i>	SGB378	pAKXT20	this work
SGB901	<i>W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-BceS^{W54R/F55R}</i>	SGB771	pAKXT16	this work
SGB902	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{W55R/F55R}</i>	SGB378	pAKXT16	this work
SGB903	<i>W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-BceS^{F55R/Y57R}</i>	SGB771	pAKXT21	this work

SGB904	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{EF55R/Y57R}</i>	SGB378	pAKXT21	this work
SGB936	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S}-His8</i>	SGB357	pAK2E185	this work
SGB937	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I50C}-His8</i>	SGB367	pAK2E186	this work
SGB938	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I51C}-His8</i>	SGB357	pAK2E187	this work
SGB939	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I52C}-His8</i>	SGB357	pAK2E188	this work
SGB940	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I53C}-His8</i>	SGB357	pAK2E189	this work
SGB941	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I54C}-His8</i>	SGB357	pAK2E190	this work
SGB942	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I55C}-His8</i>	SGB357	pAK2E191	this work
SGB943	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I56C}-His8</i>	SGB357	pAK2E192	this work
SGB944	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I57C}-His8</i>	SGB357	pAK2E193	this work
SGB945	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I58C}-His8</i>	SGB357	pAK2E194	this work
SGB946	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I59C}-His8</i>	SGB357	pAK2E195	this work
SGB947	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I61C}-His8</i>	SGB357	pAK2E196	this work
SGB948	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I62C}-His8</i>	SGB357	pAK2E197	this work
SGB949	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I64C}-His8</i>	SGB357	pAK2E198	this work
SGB950	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{C45S/C198S/C259S/I65C}-His8</i>	SGB357	pAK2E199	this work
SGB951	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S}-His8</i>	SGB357	pAK2E202	this work
SGB952	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I50C}-His8</i>	SGB357	pAK2E203	this work
SGB953	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I51C}-His8</i>	SGB357	pAK2E204	this work
SGB954	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I52C}-His8</i>	SGB357	pAK2E205	this work
SGB955	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I53C}-His8</i>	SGB357	pAK2E206	this work
SGB956	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I54C}-His8</i>	SGB357	pAK2E207	this work
SGB957	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I55C}-His8</i>	SGB357	pAK2E208	this work
SGB958	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I56C}-His8</i>	SGB357	pAK2E209	this work
SGB959	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I57C}-His8</i>	SGB357	pAK2E210	this work
SGB960	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I58C}-His8</i>	SGB357	pAK2E211	this work
SGB961	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I59C}-His8</i>	SGB357	pAK2E212	this work
SGB962	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I61C}-His8</i>	SGB357	pAK2E213	this work
SGB963	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I62C}-His8</i>	SGB357	pAK2E214	this work
SGB964	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/I64C}-His8</i>	SGB357	pAK2E215	this work

SGB965	<i>W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{E115K/K116E/C45S/C198S/C259S/K65C}-His8</i>	SGB357	pAK2E216	this work
SGB976	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{F533C}-FLAG</i>	SGB79	pMG775	this work
SGB977	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{L534C}-FLAG</i>	SGB79	pMG776	this work
SGB978	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{G535C}-FLAG</i>	SGB79	pMG777	this work
SGB979	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{L536C}-FLAG</i>	SGB79	pMG778	this work
SGB980	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{T537C}-FLAG</i>	SGB79	pMG779	this work
SGB981	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{L539C}-FLAG</i>	SGB79	pMG780	this work
SGB982	<i>W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{I540C}-FLAG</i>	SGB79	pMG781	this work
TMB035	<i>W168 bceAB::kan</i>			(Rietkötter <i>et al.</i> , 2008)
TMB1036	<i>trpC2 ΔbceS</i>			gift from Mascher lab
W168	Laboratory (wild-type) strain of <i>Bacillus subtilis</i> ; <i>trpC2</i>			laboratory stock

^a Parent strain indicates the previous step in the genetic construction of the strain.

^b Plasmids or genomic DNA of the indicated strain used in the last transformation step of genetic construction of the strain.

Section B - Primers^a

Primer	Sequence (5'→3')
SG0068	CCTTTATTTTTGTCAAATGGGTGAAAGTGAAGATGAAAAACCGAG
SG0069	CACCCATTTGACAAAAATAAAGGATACAACCTGATGTAATCAGG
SG0109	TGAAAGTGAATGTGAAAAACCGAGCTATACAATTTTAAGAAAACCTCGG
SG0110	TCGGTTTTTCACATTCACCTTCACCCATTTGTTTAAAAATAAAGGATAC
SG0117	AGGTTGTATCTGTTATTTTAAACAAATGGGTGAAAGTGAAGATGAAAAACC
SG0118	GTTTAAAAATAACAGATACAACCTGATGTAATCAGGAACGTTAACCC
SG0119	ACAAATGGGTGTAGTGAAGATGAAAAACCGAGCTATACAATTTTAAG
SG0120	CATCTTCACTACAACCCATTTGTTTAAAAATAAAGGATACAACCTGATG
SG0127	CATCGCTTAGCATTGGAAAATAAAAAAGATG
SG0128	CCATGCCATCAGCTCATCTTTTTTATTTTC
SG0175	GTATCCTTTGTTTTAAACAAATGGGTGAAAGTGAAGATGAAAAACCGAGC
SG0176	CATTTGTTTTAAACAAAGGATACAACCTGATGTAATCAGGAACGTTAACCC
SG0179	AATTTAATTTCCATGGCTTGTCAACCTGGCCGTTCCGATG
SG0180	AATTTAATTTGAATTCCTGCTGAAACAAAAACGCGGCAAT
SG0181	AATTTAATTTGAATTCCTGTCAGTTCTTTATTATAAGAAG
SG0182	AATTTAATTTGGATCCGAAATGGTATTAGCATTCTTAGGT
SG0197	GGGTAAACGTGCCTGATTACATCAGGTTGTATCCTTTATTTTAAAC
SG0198	GTAATCAGGCACGTTAACCCTAGAAGCCGACGATG
SG0215	CGGCATCGCTTAGCATTGGAAAATGCTAAAGATGAG
SG0216	GATCCATGCCATCAGCTCATCTTTAGCATTTTCC
SG0217	CGGCATCGCTTAGCATTGGAAAATCGTAAAGATGAG
SG0218	GATCCATGCCATCAGCTCATCTTTACGATTTTCC
SG0219	GGCATCGCTTAGCATTGGAATGCGAAAAAG
SG0220	GCCATCAGCTCATCTTTTTTCGCATTCCAATG
SG0221	CATCGCTTAGCATTGGAAAATTGCAAAGATG
SG0222	CCATGCCATCAGCTCATCTTTGCAATTTTC
SG0223	GCTTAGCATTGGAAAATGAATGCGATGAG
SG0224	GATCCATGCCATCAGCTCATCGCATTTCAT
SG0225	CTTCTTGATCAGCAGCTTCATTGCAAACG
SG0226	GTTTTCAATAAATGAAATGCGTTTGCAATGAAG
SG0227	CTTGATCAGCAGCTTCATCAATGCCGCATTTTC
SG0228	GTCGTTTTCAATAAATGAAATGCGGCATTGATG
SG0229	GATCAGCAGCTTCATCAAAAAATGCATTTTC
SG0230	GGTCGTTTTCAATAAATGAAATGCATTTTTG
SG0231	CATCATGTTGTATCCTTTATTTTAAACAAATGGGTG
SG0232	GATACAACATGATGTAATCAGGAACGTTAAC
SG0233	CAGGTTGTTGCCTTTATTTTAAACAAATGGGTG
SG0234	AAATAAAGGCAACAACCTGATGTAATCAGG
SG0235	CTTTATTGTAAACAAATGGGTGAAAGTGAAGATG
SG0236	GTTTACAATAAAGGATACAACCTGATGTAATCAG
SG0237	TTGTATCCTTTATTTTAAATGCATGGGTG
SG0238	CATCTTCACTTTCACCCATGCATTTAA

SG0239	GGTTGTATCCTTTATTTTAAACAATGCGGTGAAAG
SG0240	CGGTTTTTCATCTTCACTTTCACCGCATTGTT
SG0241	CAAATGTGTGAAAGTGAAGATGAAAAACCGAGC
SG0242	CTTTCACACATTTGTTTAAAATAAAGGATACAACC
SG0243	CAGGTAGTATCCTTTATTTTAAACAAATGGG
SG0244	GGATACTACCTGATGTAATCAGGAACG
SG0245	GGCAACCGAGCGTTCTG
SG0246	CTGACAGCGTTTCGATCC
SG0247	CATATTCTGTTGGTTCGCTATCGGAAAGAAAC
SG0248	CGGAACCAACAGAATATGATAAAAAACAAGATGCAC
SG0249	GTGCATCTTGTTTTTTATCATATTCCTTTGCTTCCGC
SG0250	GCTGTTTCCTTCCGATAGCGGAAGCAAAG
SG0251	TTTGGTGCCGCTATCGGAAAGAAACAGCGTTTTATAAAAGC
SG0252	CCGATAGCGGCACCAAAGGAATATGATAAAAAACAAGATGC
SG0253	TGGTTC TGCTATCGGAAAGAAACAGC
SG0254	CCGATAGCAGAACCAAAGGAATATGATAAA
SG0255	CGCTGTCGGAAAGAAACAGCGTTTTATAAAAGC
SG0256	CTTTCGACAGCGGAACCAAAGGAATATGATAAA
SG0257	CCGCTATTGTAAAGAAACAGCGTTTTATAAAAGC
SG0258	GTTTCTTTACAATAGCGGAACCAAAGGAATATG
SG0259	TATCGGTGCGAAACAGCGTTTTATAAAAGCTTGAAAACATGG
SG0260	CGCTGTTTCACAGCGATAGCAGAACCAAAGG
SG0261	GGAAATGCACAGCGTTTTATAAAAGCTTGAAAACATGGG
SG0262	GCTGTGCATTTCCGATAGCGGAACCAAAGG
SG0263	CGGAAAGAATGCGCGTTTTATAAAAGCTTGAAAACATGG
SG0264	AACGCGCATTCTTTGCGATAGCAGAACCAAAGG
SG0265	GAAACATGCTTTTTATAAAAGCTTGAAAACATGGGAGAAC
SG0266	TAAAAGCATGTTTCTTTCGATAGCGGAACCAAAG
SG0267	GCTTAGCATTGGAAAATGAAGAAGATGAG
SG0268	GATCCATGCCATCAGCTCATCTTCTTTCAT
SG0275	CTTGATCAGCAGCTTCATCAAGACCGCATTTTC
SG0276	GTCGTTTTCAATAAATGAAATGCGGTCTTGATG
SG0283	CTTAGCATTGGAAAATGAAAATGCGGAGCTG
SG0284	GGATCCATGCCATCAGCTCGCATTTTTTC
SG0285	GCATTGGAAAATGAAAAGATTGCCTGATG
SG0286	CTCATGGATCCATGCCATCAGGCAATCTTT
SG0289	CGCTTAGCATTGGAAAATAAAGAAGATGAG
SG0290	GATCCATGCCATCAGCTCATCTTCTTTATTTTC
SG0295	GAAGCAATGGTTGAAAGAAGCTGCGCCGGG
SG0296	CAAGTGTTCTGTTTGCCCGGCGCAGCTTC
SG0297	GCAATGGTTGAAAGAAGCATTGCGGGCAA
SG0298	CTTCAAGTGTTCTGTTTGCCCGCAAATGC
SG0299	CAATGGTTGAAAGAAGCATTGCCTGCCAAAC
SG0300	CTGCTTCAAGTGTTCTGTTTGCCAGGCAATG

SG0301	GTTGAAAGAAGCATTGCCGGGTGCACAGAAC
SG0302	GTCTGCTTCAAGTGTCTGTGCACCCGGC
SG0303	GAAAGAAGCATTGCCGGGCAATGCGAACAC
SG0304	GCGGTCTGCTTCAAGTGTTCGCATTGCC
SG0305	GAAGCATTGCCGGGCAAACATGCCACTTG
SG0306	GCTGCGGTCTGCTTCAAGTGGCATGTTTG
SG0307	GCATTGCCGGGCAAACAGAATGCTTGAAG
SG0308	CGTGCTGCGGTCTGCTTCAAGCATTCTG
SG0309	CATTGCCGGGCAAACAGAACACTGCAAGCAG
SG0310	GCCGTGCTGCGGTCTGCTTGCAGTGTTG
SG0311	CCGGGCAAACAGAACACTTGTGCCAGACC
SG0312	GATGCCGTGCTGCGGTCTGGCACAAGTG
SG0313	GGCAAACAGAACACTTGAAGTGCACCGC
SG0314	TAAGCGATGCCGTGCTGCGGTGCACTTCAA
SG0315	GAACCGGAAACGCCGTTTTGCGCAATG
SG0316	CAATGCTTCTTTCAACCATTGCGCAAAACGG
SG0317	GAACCGGAAACGCCGTTTGAATGCATGGTTG
SG0318	CGGCAATGCTTCTTTCAACCATGCATTCAAAC
SG0319	CCGGAAACGCCGTTTGAAGCATGCGTTGAA
SG0320	CCCGGCAATGCTTCTTTCAACGCATGCTTC
SG0321	GAAACGCCGTTTGAAGCAATGTGCGAAAG
SG0322	GCCCGGCAATGCTTCTTTGCGACATTGC
SG0323	ACGCCGTTTGAAGCAATGGTTTGCAGAAGC
SG0324	CTGTTTGCCCGGCAATGCTTCTGCAAACCAT
SG0325	CCGTTTGAAGCAATGGTTGAATGCAGCATTG
SG0326	GTTCTGTTTGCCCGGCAATGCTGCATTCAAC
SG0327	GTTTGAAGCAATGGTTGAAAGATGCATTGCCG
SG0328	GTGTTCTGTTTGCCCGGCAATGCATCTTTG
SG0329	CGCTATCGGAAAGAAACAGCGTGCTATAAAAG
SG0330	CTCCCATGTTTTCAAGCTTTTATAGCAGCTGT
SG0331	GCTATCGGAAAGAAACAGCGTTTTGCAAACGC
SG0332	CTCCCATGTTTTCAAGCTTTTGCAAAACGC
SG0333	CGGAAAGAAACAGCGTTTTATTGCAGCTTG
SG0334	GTTCTCCCATGTTTTCAAGCTGCAATAAAAC
SG0335	TCGGAAAGAAACAGCGTTTTATAAATGCTTGAAAAC
SG0336	GAGATTGTTCTCCCATGTTTTCAAGCATTTATA
SG0337	GAAACAGCGTTTTATAAAAGCTGCAAACATG
SG0338	GAGATTGTTCTCCCATGTTTTGCAGCTTTTA
SG0339	GAAACAGCGTTTTATAAAAGCTTGTGCACATGG
SG0340	CATCGAGATTGTTCTCCCATGTGCACAAGCT
SG0341	GCGTTTTATAAAAGCTTGAATGCTGGGAGA
SG0342	CACATCGAGATTGTTCTCCAGCATTTCAAG
SG0343	GCGTTTTATAAAAGCTTGAACATGCGAGAAC
SG0344	GTCACATCGAGATTGTTCTCGCATGTTTTG

SG0356	GGTGAATGTGAAGATGAAAAACCGAGCTATAC
SG0357	CATCTTCACATTCACCCATTTGTTTTAAAATAAAGGATAC
SG0358	GAAAGTTGCGATGAAAAACCGAGCTATACAATTTTAAG
SG0359	TTCATCGCAACTTTCACCCATTTGTTTTAAAATAAAGGATAC
SG0400	CAGCGTGTATAAAAAGCTTGAAAACATGGGAGAACAATCTCG
SG0401	CTTTTATAACACGCTGTTTTCTTTCCGATAGCGGAACCAAAG
SG0402	GCGTTTTGTAAAAGCTTGAAAACATGGGAGAACAATCTCGATGTGAC
SG0403	GCTTTTACAAAACGCTGTTTTCTTTCCGATAGCGGAACCAAAGG
SG0404	GTTTTATTGTAGCTTGAAAACATGGGAGAACAATCTCGATGTGACAGC
SG0405	CAAGCTACAATAAAACGCTGTTTTCTTTCCGATAGCGGAACC
SG0480	TGAGTGCATGGCATGGATCCATGAGGTC
SG0481	GCCATGCACTCATCTTTTTTCATTTTCC
SG0486	GCTGTGCGCATGGATCCATGAGGTCAA
SG0487	ATGCGCACAGCTCATCTTTTTTCATTTTCC
SG0534	AAGCTTCAAACATGGGAGAACAATCTCGATG
SG0535	GTTTTGAAGCTTTTATAAAACGCTGTTTTCTTTC
SG0542	AAGCGTAAAACATGGGAGAACAATCTCGATG
SG0543	GTTTTACCGCTTTTATAAAACGCTGTTTTCTTTC
SG0579	GAAGCGGTGCCGGGCAAACAGAACAACCTTGAAG
SG0580	CCGGCACCGCTTCTTTCAACCATTGCTTCAAAC
SG0583	GAAGCTTCGCCGGGCAAACAGAACAACCTTGAAG
SG0584	CCGGCGAAGCTTCTTTCAACCATTGCTTCAAAC
SG0614	GTATCGCTTATTTTAAACAAATGGGTG
SG0615	AATAAGCGATACAACCTGATGTAATCAG
SG0616	GTATCTTTTATTTTAAACAAATGGGTG
SG0617	AATAAAAGATACAACCTGATGTAATCAG
SG0618	TTGTATCCTTTATTTTAAAGCAATGGGTG
SG0619	CATCTTCACTTTCACCCATTGCTTTAA
SG0620	TTGTATCCTTTATTTTAAATTTATGGGTG
SG0621	CATCTTCACTTTCACCCATAAATTTAA
SG0622	GATTACATGCGGTTGTATCCTTTATTTTAA
SG0623	CAACCGCATGTAATCAGGAACGTTA
SG0624	CTGATTTGTTTCAGGTTGTATCCTTTA
SG0625	ACCTGAACAAATCAGGAACGTTAAC
SG0659	GAAAATAAAGAAGATTGCCTGATGGCATGGATCCATGAG
SG0660	CATCAGGCAATCTTCTTTATTTTCCAATGCTAAGCGATG
SG0661	GAAAATAAAGAAGATGAGTGCATGGCATGGATCCATGAGGTC
SG0662	TGCCATGCACTCATCTTCTTTATTTTCCAATGCTAAGCGATG
SG0671	AGTCGGTCTCGGATCCTACATCGGAAGGAAGAGG
SG0672	AGTCGGTCTCGAATTCGGGCTTTTCTTCGATACGG
SG0675	GTATCAATTATTTTAAACAAATGGGTG
SG0676	AATAATTGATACAACCTGATGTAATCAG
SG0677	TTGTATCCTTTATTTTAAAGAAATGGGTG
SG0678	CATCTTCACTTTCACCCATTTCTTTAA

SG0715	GTATCGATTATTTTAAACAAATGGGTG
SG0716	AATAATCGATAACAACCTGATGTAATCAG
SG0727	TTTTTGCATATTCCTTTGGTTCCGCTAT
SG0729	TATCTGCTTCCTTTGGTTCCGCTATCGG
SG0731	CATATGCCTTTGGTTCCGCTATCGGAAA
SG0733	ATTCTGCTGGTTCCGCTATCGGAAAGAA
SG0734	ACCAGCAGAATATGATAAAAAACAAGAT
SG0735	CCTTTGCTTCCGCTATCGGAAAGAAACA
SG0736	GGAAGCAAAGGAATATGATAAAAAACAA
SG0737	TTGGTGCCGCTATCGGAAAGAAACAGCG
SG0738	AGCGGCACCAAAGGAATATGATAAAAAA
SG0739	GTTCTGCTATCGGAAAGAAACAGCGTTT
SG0740	GATAGCAGAACCAAAGGAATATGATAAAA
SG0741	CCGCTGCCGGAAGAAACAGCGTTTTTAT
SG0742	TCCGGCAGCGGAACCAAAGGAATATGAT
SG0743	CTATTGCAAAGAAACAGCGTTTTATAAAA
SG0744	CTTTGCAATAGCGGAACCAAAGGAATAT
SG0745	TCGGTGCGAAACAGCGTTTTATAAAAAGC
SG0746	TTTCGCACCGATAGCGGAACCAAAGGAA
SG0779	GTGGTCTATCCAAAAGGGATCGGTTTT
SG0780	GGATAGACCACGATTGTAAATCTTTGAT
SG0781	AGATTCTGGCAGGGGCATTGATCCAAAA
SG0782	TGCCAGAATCTTTCACCTGGAGCTGCGT
SG0783	ATATGCAAAAAACAAGATAGACAAATA
SG0784	GGAAGCAGATAAAAAACAAGATAGACAA
SG0785	AAAGGCATATGATAAAAAACAAGATAGA
SG0806	AAATAAAGAAGATGAGCTGATGGCATGGATCCATGA
SG0807	CATCTTCTTTATTTTCCAATGCTAAGCGATGCCGTG
SG0812	CCTTCGTTTCCGCTATCGGAAAGAAACAGCGTT
SG0813	GGAAACGAAGGAATATGATAAAAAACAAGATGC
SG0814	TTGGCGTCGCTATCGGAAAGAAACAGCGTTTTA
SG0815	AGCGACGCCAAAGGAATATGATAAAAAACAAGA
SG0816	CCTTCGTCGTCGCTATCGGAAAGAAACAGCGTTTTA
SG0817	AGCGACGACGAAGGAATATGATAAAAAACAAGATGC
SG0828	TTTGTCTATCTTGTTTTTTATCATATTCCTTTG
SG0829	AGATAGACAAATACACCATATAAAGTACATTCC
SG0897	TCCGCCGTCCGAAAGAAACAGCGTTTTAT
SG0898	TCCGACGGCGGAACCAAAGGAATATGAT
SG899	TTGGCGTCGCCGTCCGAAAGAAACAGCGTTTTAT
SG0900	TCCGACGGCGACGCCAAAGGAATATGATAAAAAAC
SG0932	CGTCGGCTGCTTAGGGTTAACGTTTCTG
SG0933	CCCTAAGCAGCCGACGATGAACATCACC
SG0934	CGGCTTCTGTGGGTTAACGTTTCTGATTAC
SG0935	GTTAACCCACAGAAGCCGACGATGAACATCACC

SG0936	GCTTCTTATGTTTAAAGTTTCCTGATTACATC
SG0937	GAACGTTAAACATAAGAAGCCGACGATGAAC
SG0938	CTTAGGGTGCACGTTTCCTGATTACATCAGG
SG0939	GGAACGTGCACCCTAAGAAGCCGACG
SG0940	GGGTTATGCTTCCTGATTACATCAGGTTG
SG0941	CAGGAAGCATAACCCTAAGAAGCCGACG
SG0942	TAACGTTCTGCATTACATCAGGTTGTATCCTT
SG0943	GATGTAATGCAGAACGTTAACCCTAAGAAGC
SG0944	CGTTCCTGTGTACATCAGGTTGTATCCTTTAT
SG0945	CCTGATGTACACAGGAACGTTAACCCTAAG

^a See section C for use of each primer

Section C - Plasmids

Plasmid	Description ^a	Parent plasmid	Plasmid construction ^{b,c}	Reference
pAK101	<i>bla</i> 'ytrF' <i>bgaB erm</i>	pMAD	SG0179/SG0180 (W168 gDNA)	this work
pAK102	<i>bla</i> 'ytrF <i>bgaB erm yttA</i> '	pMAD	SG0181/SG0182 (W168 gDNA)	this work
pAK2E02	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{E115K} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0127/SG0128 (QC)	this work
pAK2E12	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{E115A} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0215/SG0216 (QC)	this work
pAK2E13	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{E115R} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0217/SG0218 (QC)	this work
pAK2E14	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{E115C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0221/SG0222 (QC)	this work
pAK2E15	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{Q166C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0225/SG0226 (QC)	this work
pAK2E16	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{R168C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0229/SG0230 (QC)	this work
pAK2E17	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{K116C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0223/SG0224 (QC)	this work
pAK2E21	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{N114C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0220 & SG0246/SG0219	this work
pAK2E22	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{K167C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0228 & SG0246/SG0227	this work
pAK2E26	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{K116E} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0268 & SG0246/SG0267	this work
pAK2E29	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{K167D} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0276 & SG0246/SG0275	this work
pAK2E33	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{E118C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0286 & SG0246/SG0285	this work
pAK2E34	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{E115K/K116E} - <i>His8</i>) <i>mls</i>	pSD2E02	SG0245/SG0290 & SG0246/SG0289	this work
pAK2E37	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{I94C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0296 & SG0246/SG0295	this work
pAK2E38	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{A95C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0298 & SG0246/SG0297	this work
pAK2E39	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{G96C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0300 & SG0246/SG0299	this work
pAK2E40	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{Q97C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0302 & SG0246/SG0301	this work
pAK2E41	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{T98C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0304 & SG0246/SG0303	this work
pAK2E42	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{E99C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0306 & SG0246/SG0305	this work
pAK2E43	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{H100C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0308 & SG0246/SG0307	this work
pAK2E44	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{L101C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0310 & SG0246/SG0309	this work
pAK2E45	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{K102C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0312 & SG0246/SG0311	this work
pAK2E46	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{Q103C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0314 & SG0246/SG0313	this work
pAK2E47	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{E87C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0316 & SG0246/SG0315	this work
pAK2E48	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{A88C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0318 & SG0246/SG0317	this work
pAK2E49	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{M89C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0320 & SG0246/SG0319	this work
pAK2E50	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{V90C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0322 & SG0246/SG0321	this work
pAK2E51	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{E91C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0324 & SG0246/SG0323	this work
pAK2E52	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{R92C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0326 & SG0246/SG0325	this work
pAK2E53	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{S93C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0328 & SG0246/SG0327	this work
pAK2E54	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{F63C} - <i>His8</i>) <i>mls</i>	pBS2E01	SG0245/SG0330 & SG0246/SG0329	this work
pAK2E55	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{Y64C} - <i>His8</i>) <i>mls</i>	pBS2E01	SG0245/SG0332 & SG0246/SG0331	this work
pAK2E56	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{K65C} - <i>His8</i>) <i>mls</i>	pBS2E01	SG0245/SG0334 & SG0246/SG0333	this work
pAK2E57	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{S66C} - <i>His8</i>) <i>mls</i>	pBS2E01	SG0245/SG0336 & SG0246/SG0335	this work
pAK2E58	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{L67C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0338 & SG0246/SG0337	this work
pAK2E59	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{K68C} - <i>His8</i>) <i>mls</i>	pBS2E01	SG0245/SG0340 & SG0246/SG0339	this work
pAK2E60	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{T69C} - <i>His8</i>) <i>mls</i>	pBS2E01	SG0245/SG0342 & SG0246/SG0341	this work
pAK2E61	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{W70C} - <i>His8</i>) <i>mls</i>	pBS2E01	SG0245/SG0344 & SG0246/SG0343	this work
pAK2E63	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{D117C} - <i>His8</i>) <i>mls</i>	pSD2E01	SG0245/SG0284 & SG0246/SG0283	this work
pAK2E66	<i>bla lacA</i> ::(P _{xyI} - <i>bceS</i> ^{K167D/K116E} - <i>His8</i>) <i>mls</i>	pAK2E26	SG0245/SG0276 & SG0246/SG0275	this work

pAK2E118	<i>bla lacA::(P_{xyI}-bceS^{E115K/K167D}-His8) mls</i>	pAK2E29	SG0245/SG0128 & SG0246/SG0127	this work
pAK2E134	<i>bla lacA::(P_{xyI}-bceS^{D117C}-His8) mls</i>	pSD2E01	SG0480/SG0481(QC)	this work
pAK2E137	<i>bla lacA::(P_{xyI}-bceS^{M120C}-His8) mls</i>	pSD2E01	SG0486/SG0487 (QC)	this work
pAK2E154	<i>bla lacA::(P_{xyI}-bceS^{L67F}-His8) mls</i>	pSD2E01	SG0534/SG0535 (QC)	this work
pAK2E157	<i>bla lacA::(P_{xyI}-bceS^{L67G}-His8) mls</i>	pSD2E01	SG0542/SG0543 (QC)	this work
pAK2E168	<i>bla lacA::(P_{xyI}-bceS^{I94G}-His8) mls</i>	pSD2E01	SG0579/SG0580 (QC)	this work
pAK2E170	<i>bla lacA::(P_{xyI}-bceS^{I94F}-His8) mls</i>	pSD2E01	SG0583/SG0584 (QC)	this work
pAK2E176	<i>bla lacA::(P_{xyI}-bceS^{E115K/K116E/E118C}-His8) mls</i>	pSD2E01	SG0659/SG0660 (QC)	this work
pAK2E177	<i>bla lacA::(P_{xyI}-bceS^{E115K/K116E/L119C}-His8) mls</i>	pSD2E01	SG0661/SG0662 (QC)	this work
pAK2E183	<i>bla lacA::(P_{xyI}-bceS^{C45S}-His8) mls</i>	pSD2E01	SG0245/SG0829 & SG0246/SG0828	this work
pAK2E184	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S}-His8) mls</i>	pAK2E183	SG0245/SG0780 & SG0246/SG0779	this work
pAK2E185	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S}-His8) mls</i>	pAK2E184	SG0245/SG0782 & SG0246/SG0781	this work
pAK2E186	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/I50C}-His8) mls</i>	pAK2E185	SG0245/SG0783 & SG0246/SG0727	this work
pAK2E187	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/I51C}-His8) mls</i>	pAK2E185	SG0245/SG0784 & SG0246/SG0729	this work
pAK2E188	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/F52C}-His8) mls</i>	pAK2E185	SG0245/SG0785 & SG0246/SG0731	this work
pAK2E189	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/L53C}-His8) mls</i>	pAK2E185	SG0245/SG0734 & SG0246/SG0733	this work
pAK2E190	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/W54C}-His8) mls</i>	pAK2E185	SG0245/SG0736 & SG0246/SG0735	this work
pAK2E191	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/F55C}-His8) mls</i>	pAK2E185	SG0245/SG0738 & SG0246/SG0737	this work
pAK2E192	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/R56C}-His8) mls</i>	pAK2E185	SG0245/SG0740 & SG0246/SG0739	this work
pAK2E193	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/Y57C}-His8) mls</i>	pAK2E185	SG0245/SG0742 & SG0246/SG0741	this work
pAK2E194	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/R58C}-His8) mls</i>	pAK2E185	SG0245/SG0744 & SG0246/SG0743	this work
pAK2E195	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/K59C}-His8) mls</i>	pAK2E185	SG0245/SG0746 & SG0246/SG0745	this work
pAK2E196	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/T61C}-His8) mls</i>	pAK2E185	SG0245/SG0264 & SG0246/SG0263	this work
pAK2E197	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/A62C}-His8) mls</i>	pAK2E185	SG0245/SG0266 & SG0246/SG0265	this work
pAK2E198	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/Y64C}-His8) mls</i>	pAK2E185	SG0245/SG0403 & SG0246/SG0402	this work
pAK2E199	<i>bla lacA::(P_{xyI}-bceS^{C45S/C198S/C259S/K65C}-His8) mls</i>	pAK2E185	SG0245/SG0405 & SG0246/SG0404	this work
pAK2E200	<i>bla lacA::(P_{xyI}-bceS^{E115K/K116E/C45S}-His8) mls</i>	pAK2E34	SG0245/SG0829 & SG0246/SG0828	this work
pAK2E201	<i>bla lacA::(P_{xyI}-bceS^{E115K/K116E/C45S/C198S}-His8) mls</i>	pAK2E200	SG0245/SG0780 & SG0246/SG0779	this work
pAK2E202	<i>bla lacA::(P_{xyI}-bceS^{E115K/K116E/C45S/C198S/C259S}-His8) mls</i>	pAK2E201	SG0245/SG0782 & SG0246/SG0781	this work
pAK2E203	<i>bla lacA::(P_{xyI}-bceS^{E115K/K116E/C45S/C198S/C259S/I50C}-His8) mls</i>	pAK2E202	SG0245/SG0783 & SG0246/SG727	this work
pAK2E204	<i>bla lacA::(P_{xyI}-bceS^{E115K/K116E/C45S/C198S/C259S/I51C}-His8) mls</i>	pAK2E202	SG0245/SG0784 & SG0246/SG0729	this work
pAK2E205	<i>bla lacA::(P_{xyI}-bceS^{E115K/K116E/C45S/C198S/C259S/F52C}-His8) mls</i>	pAK2E202	SG0245/SG0785 & SG0246/SG0731	this work

pAK2E206	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/L53C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0734 & SG0246/SG0733	this work
pAK2E207	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/W54C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0736 & SG0246/SG0735	this work
pAK2E208	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/F55C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0738 & SG0246/SG0737	this work
pAK2E209	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/R56C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0740 & SG0246/SG0739	this work
pAK2E210	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/Y57C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0742 & SG0246/SG0741	this work
pAK2E211	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/R58C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0744 & SG0246/SG0743	this work
pAK2E212	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/K59C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0746 & SG0246/SG0745	this work
pAK2E213	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/T61C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0264 & SG0246/SG0263	this work
pAK2E214	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/A62C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0266 & SG0246/SG0265	this work
pAK2E215	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/Y64C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0403 & SG0246/SG0402	this work
pAK2E216	<i>bla lacA::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E/C45S/C198S/C259S/K65C} -His8) <i>mls</i>	pAK2E202	SG0245/SG0405 & SG0246/SG0404	this work
pAKXT03	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{WT}) <i>spec</i>	pXT	SG0671/SG0672 (pAS719)	this work
pAKXT06	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{E115K}) <i>spec</i>	pXT	SG0671/SG0672 (PCF705)	this work
pAKXT13	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{E115K/K116E}) <i>spec</i>	pXT	SG0671/SG0807 & SG0672/SG0806 (pAS719)	this work
pAKXT15	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{W54R}) <i>spec</i>	pAKXT03	SG0671/SG0813 & SG0672/SG0812 (pAKXT03)	this work
pAKXT16	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{W54R/F55R}) <i>spec</i>	pAKXT03	SG0671/SG0817 & SG0672/SG0816 (pAKXT03)	this work
pAKXT20	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{Y57R}) <i>spec</i>	pAKXT03	SG0671/SG0898 & SG0672/SG0897 (pAKXT03)	this work
pAKXT21	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{F55R/Y57R}) <i>spec</i>	pAKXT03	SG0671/SG0900 & SG0672/SG0899 (pAKXT03)	this work
pAKXT22	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{F55R}) <i>spec</i>	pAKXT03	SG0671/SG0815 & SG0672/SG0814 (pAKXT03)	this work
pAM703	<i>bla thrC::</i> (P _{xyl} - <i>bceAB</i> ^{L546C} -Flag ₃) <i>spec</i>	pFK727	SG0117/SG0118 (QC)	this work
pAS719	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{WT}) <i>spec</i>	pXT		gift from Mascher lab
pCF705	<i>bla thrC::</i> (P _{xyl} - <i>bceS</i> ^{E115K}) <i>spec</i>	pXT		Laboratory stock
pER603	<i>bla amyE::P</i> _{bceA} - <i>lacZ cm</i>			(Rietkötter <i>et al.</i> , 2008)
pFK727	<i>bla thrC::</i> (P _{xyl} - <i>bceAB</i> ^{WT} -Flag ₃) <i>spec</i>	pXT		(Kallenberg <i>et al.</i> , 2013)
pJL705	<i>bla thrC::</i> (P _{xyl} - <i>bceAB</i> ^{D556C} -Flag ₃) <i>spec</i>	pFK727	SG0109/SG0110 (QC)	this work

pMAD	<i>bla bgaB erm</i>			(Arnaud <i>et al.</i> , 2004)
pMG2E01	<i>bla lacA::(P_{xyI}-bceS^{L53C}-His8) mls</i>	pBS2E01	SG0247/SG0248 (QC)	this work
pMG2E02	<i>bla lacA::(P_{xyI}-bceS^{W54C}-His8) mls</i>	pBS2E01	SG0245/SG0250 & SG0246/SG0249	this work
pMG2E03	<i>bla lacA::(P_{xyI}-bceS^{F55C}-His8) mls</i>	pBS2E01	SG0251/ SG0252 (QC)	this work
pMG2E04	<i>bla lacA::(P_{xyI}-bceS^{R56C}-His8) mls</i>	pBS2E01	SG0253/ SG0254 (QC)	this work
pMG2E05	<i>bla lacA::(P_{xyI}-bceS^{Y57C}-His8) mls</i>	pBS2E01	SG0255/ SG0256 (QC)	this work
pMG2E06	<i>bla lacA::(P_{xyI}-bceS^{R58C}-His8) mls</i>	pBS2E01	SG0257/ SG0258 (QC)	this work
pMG2E07	<i>bla lacA::(P_{xyI}-bceS^{K59C}-His8) mls</i>	pBS2E01	SG0259/ SG0260 (QC)	this work
pMG2E08	<i>bla lacA::(P_{xyI}-bceS^{E60C}-His8) mls</i>	pBS2E01	SG0261/SG0262 (QC)	this work
pMG2E09	<i>bla lacA::(P_{xyI}-bceS^{T61C}-His8) mls</i>	pBS2E01	SG0263/SG0264 (QC)	this work
pMG2E10	<i>bla lacA::(P_{xyI}-bceS^{A62C}-His8) mls</i>	pBS2E01	SG0265/SG0266 (QC)	this work
pMG2E12	<i>bla lacA::(P_{xyI}-bceS^{E60C/E115K}-His8) mls</i>	pBS2E01	SG0261/SG0262 (QC)	this work
pMG2E15	<i>bla lacA::(P_{xyI}-bceS^{F63C/E115K}-His8) mls</i>	pBS2E01	SG0400/SG0401 (QC)	this work
pMG704	<i>bla thrC::(P_{xyI}-bceAB^{Y547C}-Flag₃) spec</i>	pFK727	SG0175/SG0176 (QC)	this work
pMG707	<i>bla thrC::(P_{xyI}-bceAB^{E553C}-Flag₃) spec</i>	pFK727	SG0119/SG0120 (QC)	this work
pMG712	<i>bla thrC::(P_{xyI}-bceAB^{F538C}-Flag₃) spec</i>	pFK727	SG0197/SG0198 (QC)	this work
pMG713	<i>bla thrC::(P_{xyI}-bceAB^{K549C}-Flag₃) spec</i>	pFK727	SG0068/SG0069 (QC)	this work
pMG718	<i>bla thrC::(P_{xyI}-bceAB^{G543C}-Flag₃) spec</i>	pFK727	SG0231/SG0232 (QC)	this work
pMG724	<i>bla thrC::(P_{xyI}-bceAB^{C544S}-Flag₃) spec</i>	pFK727	SG0243/SG0244 (QC)	this work
pMG719	<i>bla thrC::(P_{xyI}-bceAB^{I545C}-Flag₃) spec</i>	pFK727	SG0233/SG0234 (QC)	this work
pMG720	<i>bla thrC::(P_{xyI}-bceAB^{F548C}-Flag₃) spec</i>	pFK727	SG0235/SG0236 (QC)	this work
pMG721	<i>bla thrC::(P_{xyI}-bceAB^{Q550C}-Flag₃) spec</i>	pFK727	SG0237/SG0238 (QC)	this work
pMG722	<i>bla thrC::(P_{xyI}-bceAB^{M551C}-Flag₃) spec</i>	pFK727	SG0239/SG0240 (QC)	this work
pMG723	<i>bla thrC::(P_{xyI}-bceAB^{G552C}-Flag₃) spec</i>	pFK727	SG0241/SG0242 (QC)	this work
pMG738	<i>bla thrC::(P_{xyI}-bceAB^{S554C}-Flag₃) spec</i>	pFK727	SG0356/SG0357 (QC)	this work
pMG739	<i>bla thrC::(P_{xyI}-bceAB^{E555C}-Flag₃) spec</i>	pFK727	SG0358/SG0359 (QC)	this work
pMG759	<i>bla thrC::(P_{xyI}-bceAB^{T541C}-Flag₃) spec</i>	pFK727	SG0624/SG0625 (QC)	this work
pMG760	<i>bla thrC::(P_{xyI}-bceAB^{S542C}-Flag₃) spec</i>	pFK727	SG0622/SG0623 (QC)	this work
pMG762	<i>bla thrC::(P_{xyI}-bceAB^{L546A}-Flag₃) spec</i>	pFK727	SG0614/SG0615 (QC)	this work
pMG763	<i>bla thrC::(P_{xyI}-bceAB^{L546F}-Flag₃) spec</i>	pFK727	SG0616/SG0617 (QC)	this work
pMG764	<i>bla thrC::(P_{xyI}-bceAB^{Q550A}-Flag₃) spec</i>	pFK727	SG0618/SG0619 (QC)	this work
pMG765	<i>bla thrC::(P_{xyI}-bceAB^{Q550F}-Flag₃) spec</i>	pFK727	SG0620/SG0621 (QC)	this work
pMG770	<i>bla thrC::(P_{xyI}-bceAB^{L546N}-Flag₃) spec</i>	pFK727	SG0675/SG0676 (QC)	this work
pMG771	<i>bla thrC::(P_{xyI}-bceAB^{Q550E}-Flag₃) spec</i>	pFK727	SG0677/SG0678 (QC)	this work
pMG772	<i>bla thrC::(P_{xyI}-bceAB^{L546D}-Flag₃) spec</i>	pFK727	SG0715/SG0716 (QC)	this work
pMG775	<i>bla thrC::(P_{xyI}-bceAB^{F533C}-Flag₃) spec</i>	pFK727	SG0932/SG0933 (QC)	this work
pMG776	<i>bla thrC::(P_{xyI}-bceAB^{L534C}-Flag₃) spec</i>	pFK727	SG0934/SG0935 (QC)	this work
pMG777	<i>bla thrC::(P_{xyI}-bceAB^{G535C}-Flag₃) spec</i>	pFK727	SG0936/SG0937 (QC)	this work
pMG778	<i>bla thrC::(P_{xyI}-bceAB^{L536C}-Flag₃) spec</i>	pFK727	SG0938/SG0939 (QC)	this work
pMG779	<i>bla thrC::(P_{xyI}-bceAB^{T537C}-Flag₃) spec</i>	pFK727	SG0940/SG0941 (QC)	this work
pMG780	<i>bla thrC::(P_{xyI}-bceAB^{L539C}-Flag₃) spec</i>	pFK727	SG0942/SG0943 (QC)	this work
pMG781	<i>bla thrC::(P_{xyI}-bceAB^{I540C}-Flag₃) spec</i>	pFK727	SG0944/SG0945 (QC)	this work
pSD2E01	<i>bla lacA::(P_{xyI}-bceS^{WT}-His8) mls</i>			(Dintner <i>et al.</i> , 2014)

pSDlux101	pAH328-P _{bceA} -luxABCDE			(Kallenberg et al., 2013)
pXT	bla thrC::(P _{xyI}) spec			(Derré et al., 2000)

^a *E. coli* and *B. subtilis* antibiotic resistance markers are listed first and second respectively. *bla*, ampicillin resistance; *cm*, chloramphenicol resistance. *m/s*, macrolide-lincosamide-streptogramin resistance; *spec*, spectinomycin resistance.

^b Plasmid mutagenesis were performed by the PCR overlap extension method (Ho et al., 1989) where two primer pairs are given, or by QuickChange (QC) with a single primer pair where indicated.

^c DNA template used for mutagenesis was plasmid pSD2E01 unless stated otherwise in parentheses.

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Table S2. Minimal inhibitory concentration (MIC) of bacitracin in strains carrying BceAB variants.

Amino acid exchange in BceB	MIC of bacitracin ($\mu\text{g ml}^{-1}$) ^a
F533C	16
L534C	32
G535C	4
L536C	32
T537C	64
F538C	32
L539C	32
I540C	32
T541C	32
S542C	32-64
G543C	16
C544S	16-32
I545C	16-32
L546C	32-64
L546A	32-64
L546F	64
L546D	16-32
L546N	32
Y547C	16
F548C	16
K549C	16-32
Q550C	32-64
Q550A	32-64
Q550F	64
Q550E	32-64

^a MICs given as a range of values showed variable results between biological triplicates; the MIC for strains carrying the wild-type construct was 32-64 $\mu\text{g ml}^{-1}$; the MIC of strains lacking *bceAB* was 4 $\mu\text{g ml}^{-1}$.

Table S3. Parameters used for GaMD simulations (timestep = 2 fs).

Parameter	Assignment
igamd	3 (Boost on both dihedral and total potential energy).
ntcmdprep	3000000 (Number of preparation MD steps).
ntcmd	6000000 (Number of initial conventional MD steps to calculate system potential energies).
ntebprep	3000000 (Number of preparation biasing MD steps).
nteb	33000000 (Total number of biasing MD steps).
ntave	300000 (Time step to calculate standard deviation of potential energies).
sigma0P	6 (Upper limit of standard deviation of total potential boost).
sigma0D	6 (Upper limit of standard deviation of dihedral potential boost).