How to make a dial not a switch: Control of histidine kinase conformation by an ABC-transporter facilitates need-based activation of antibiotic resistance

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ABSTRACT

Bacteria exert close control over gene expression to ensure optimal physiological responses to their environment, balancing metabolic costs against physiological gain. Classically we consider such regulatory responses as being switch-like, with gene expression on or off. Is it possible for such responses to be more analog and act as graded dials rather than digital switches? If it is, how might this work? We previously described a novel regulatory logic that produces an analog response, where the signaling output is gradually dialed up over an input dynamic range of several orders of magnitude. This extraordinarily fine-tuned response is achieved via a two-component regulatory system that forms a sensory complex with an ABC-transporter, where signaling is proportional to transport activity. The resulting gradual tuning of gene expression leads to a very cost-effective activation of antibiotic resistance in response to the cell's need for detoxification capacity. Here, we show how such analog signaling behavior can be achieved mechanistically. Using experimentation and computation we show that the histidine kinase within the sensory complex is activated by piston-like displacements in the membrane, which are converted to helical rotations in the catalytic core via an intervening HAMP-like domain. Intriguingly, the transporter was found not only to be required for kinase activation, but also to actively maintain the kinase in its inactive state in the absence of the drug. Such slaving of kinase activity to that of the transporter will allow the level of complete control required to achieve transport flux-dependent analog signaling. Moreover, we show that the transporter has evolved for sub-maximal signaling sensitivity, highlighting yet another layer of fine-tuning and energy-conservation in signaling. These results provide the first mechanistic overview of the design principles of an analog dial, which has importance in energy-efficient decision making in bacteria more broadly, and antibiotic resistance and synthetic biology more specifically.

41 **INTRODUCTION**

42 Bacteria have exquisite abilities to physiologically respond to environmental conditions to
43 ensure their survival and proliferation. Decision-making has to balance the benefits of a
44 particular response against the metabolic cost of the associated changes in gene expression.
45 To achieve this, bacteria employ regulatory systems that can detect extracellular or
46 intracellular information and modulate the expression of a defined set of target genes. In the
47 simplest case, regulation is dependent on the presence or absence of a ligand, and this is often
48 thought of as a digital response, where gene expression is either switched ON or OFF.
49 However, some regulatory systems display a much more analog behavior, where the output is
50 gradually dialed up in response to the strength of the input information. While it is
51 straightforward to explain digital switch behavior, e.g. DNA-binding of a regulator in the
52 presence of a ligand, it is much harder to envision what molecular mechanisms may realize a
53 gradated, analog response.

54 We previously described a new sensory strategy that produces an unprecedented degree of
55 fine-tuning, with three orders of magnitude of output modulation over an input dynamic
56 range of three orders of magnitude [1]. This regulatory logic is achieved by a two-component
57 system whose histidine kinase works in a sensory complex with an ABC-transporter [1,2] (Fig.
58 1). Such systems are wide-spread among low G+C content Gram-positive bacteria, such as
59 bacilli, lactobacilli, staphylococci, streptococci, enterococci and clostridia [3-8]. They are
60 collectively referred to as Bce-like systems and impart resistance against a broad range of cell
61 wall-active peptide antibiotics [9,10]. Based on computational modelling of the response
62 dynamics, we have established that signaling is proportional to transport activity [1]. Because
63 the transporter mediates the actual resistance, presumably by removal of the antibiotic from
64 its target in the cell envelope [1,11-13], increased transporter production acts a negative feedback

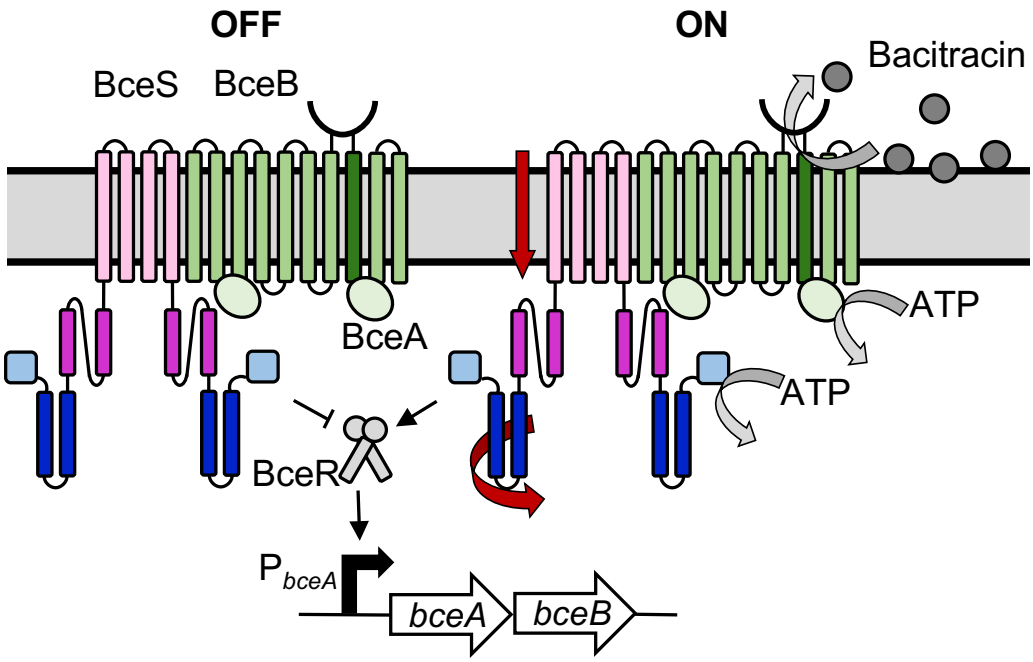

Figure 1. Function of the BceRS-BceAB resistance system of *B. subtilis.* BceS and its domains are shown in shades of pink and blue, with the HAMP-like domain in dark pink, and DHp domain in dark blue; BceAB is in green with the 8th transmembrane helix marked in dark green. The left section of the schematic shows the system in its inactive (OFF) state; the right shows the active (ON) state in the presence of antibiotics, exemplified by bacitracin. ATP hydrolysis reactions and antibiotic removal are indicated by grey arrows; for simplicity ATP hydrolysis is only shown for one ATPase each. The motions of kinase domains upon activation are shown as red block arrows.

65 on signaling, which creates the characteristic gradual, fine-tuned response [1]. This flux-
66 sensing mechanism effectively allows the bacterium to adjust its response according to its
67 need for additional detoxification capacity, implementing a cost-effective 'produce-to-
68 demand' strategy. However, the molecular mechanism of this new signaling strategy, i.e. how
69 the histidine kinase is activated within the sensory complex and how this creates the observed
70 analog response behavior, remains unknown.

71 Histidine kinases typically relay extracellular information to the cytoplasm via
72 autophosphorylation at a conserved histidine residue. The phosphoryl group is then
73 transferred to a cytoplasmic response regulator, usually a DNA-binding protein that elicits
74 changes in gene expression [14]. Despite their importance in bacterial signaling, we still have
75 limited understanding of the molecular mechanisms by which histidine kinases transmit
76 information through the cytoplasmic membrane. This is in part due to the modular
77 architecture of the kinases, which allows a plethora of different input domains to be coupled
78 to a conserved catalytic core. The core consists of a DHp (also known as HisKA) domain that
79 facilitates dimerization and histidine phosphotransfer, and a C-terminal ATPase domain [14].
80 The extracellular input and cytoplasmic core domains are separated by transmembrane
81 segments and usually at least one cytoplasmic signal transfer domain (e.g. HAMP or PAS
82 domains) [14,15]. While we still have no knowledge of the complete set of conformational
83 changes during transmembrane signaling by histidine kinases, recent studies of domains in
84 isolation or in context with an adjacent domain suggest a combination of rotating and
85 scissoring motions and piston-like helical displacements [15-18].

86 Most histidine kinases respond directly to an environmental cue via their input domains, but
87 some are controlled by accessory proteins, which are often transporters [19,20]. The majority of
88 transporters with a signaling role act as repressors of kinase activity, and deletion of the

89 transporter leads to constitutive activation of signaling [21-23]. While the molecular mechanisms
90 are unknown, it is conceivable that such transporters act as a physical block on their
91 respective kinases, and that this block is disrupted during active transport, allowing signaling
92 to proceed. In the flux-sensing Bce-like systems, however, the histidine kinases fully rely on
93 their cognate transporters for activation, and remain inactive when the transporter is deleted
94 [9,24]. Moreover, implementing the reported flux-sensing strategy requires kinase activity to be
95 directly proportional to transport activity [1]. To our knowledge, no information is available on
96 how accessory proteins may achieve such positive and precise control over a histidine kinase.

97 In the present study, we sought to understand the mechanistic basis of histidine kinase
98 activation in the Bce system of *Bacillus subtilis* and how this links to the system's analog
99 signaling behavior. Through computational predictions, detailed mutagenesis and cysteine
100 cross-linking and accessibility analyses, we provide evidence that the kinase BceS is
101 activated via helical rotations in the DHp domain, accompanied by a piston movement in the
102 second transmembrane domain. Physical motions are most likely relayed via a poorly-
103 conserved intervening HAMP-like domain. To our knowledge this is the first report of a
104 complete set of conformational changes *in vivo* during activation of a membrane-bound
105 histidine kinase. Artificial activation of the kinase through charge substitutions in the DHp
106 domain revealed that the transporter not only acts as the activator of the kinase, but also
107 controls its inactive state. We propose it is this complete control over kinase conformation
108 that slaves its activity to that of the transporter. Surprisingly, mutagenesis of the putative
109 signaling domain of the transporter, BceAB, highlighted that the system has not evolved for
110 maximal signaling sensitivity, which likely represents an additional layer of fine-tuning and
111 minimizing energy expenditure in control of antibiotic resistance.

112

113 **RESULTS**

114 **Generation and validation of a BceS homology model.** Analysis of the domain architecture
115 of BceS using SMART [25] showed two N-terminal transmembrane helices, predicted to end at
116 residue F55, and a prototypical cytoplasmic kinase core with a DHp domain from residues
117 E115 to I172 and ATPase domain from S216 to N326. The 60 amino acid linker between the
118 transmembrane and DHp domains contained no predicted domains. A recent NMR structure
119 of the homologous kinase NsaS from *Staphylococcus aureus* reported the existence of a
120 coiled-coil conformation in this region of the protein [17]. However, with 23 amino acids the
121 linker in NsaS is much shorter than that in BceS. To investigate the cytoplasmic composition
122 of BceS in more detail, we used I-TASSER [26] to predict the tertiary structure of the linker
123 region and DHp domain (residues R56 to N174; Fig. 2a). The closest structural analog was
124 the hybrid kinase Af1503-EnvZ from *Archaeoglobus fulgidus* and *E. coli*, respectively (PDB
125 3ZRW). BceS showed the typical DHp domain fold of two antiparallel helices (DHp1 and
126 DHp2), joined by a short loop. In the functional kinase dimer, this will form the four-helix
127 bundle characteristic for most histidine kinases [14] (Fig.2c). Interestingly, the linker between
128 the membrane interface and DHp domain adopted a HAMP-like conformation with two short
129 parallel helices (HAMP1 and HAMP2), where HAMP2 continues into DHp1 as a single
130 continuous helix (Fig. 2a). The relevance of this domain, which had not been predicted in
131 previous analyses, is discussed in more detail below.

132 To test the accuracy of the homology model, we probed the relative orientation of helices
133 within the DHp domain four-helix bundle, based on proximity of individual residues, using
134 oxidative cysteine crosslinking. These experiments were done *in vivo* using cells deleted for
135 the native copy of *bceS* and carrying a single-copy ectopic construct for expression of *bceS*
136 with a C-terminal His_8-tag, allowing slight over-production of the kinase to facilitate

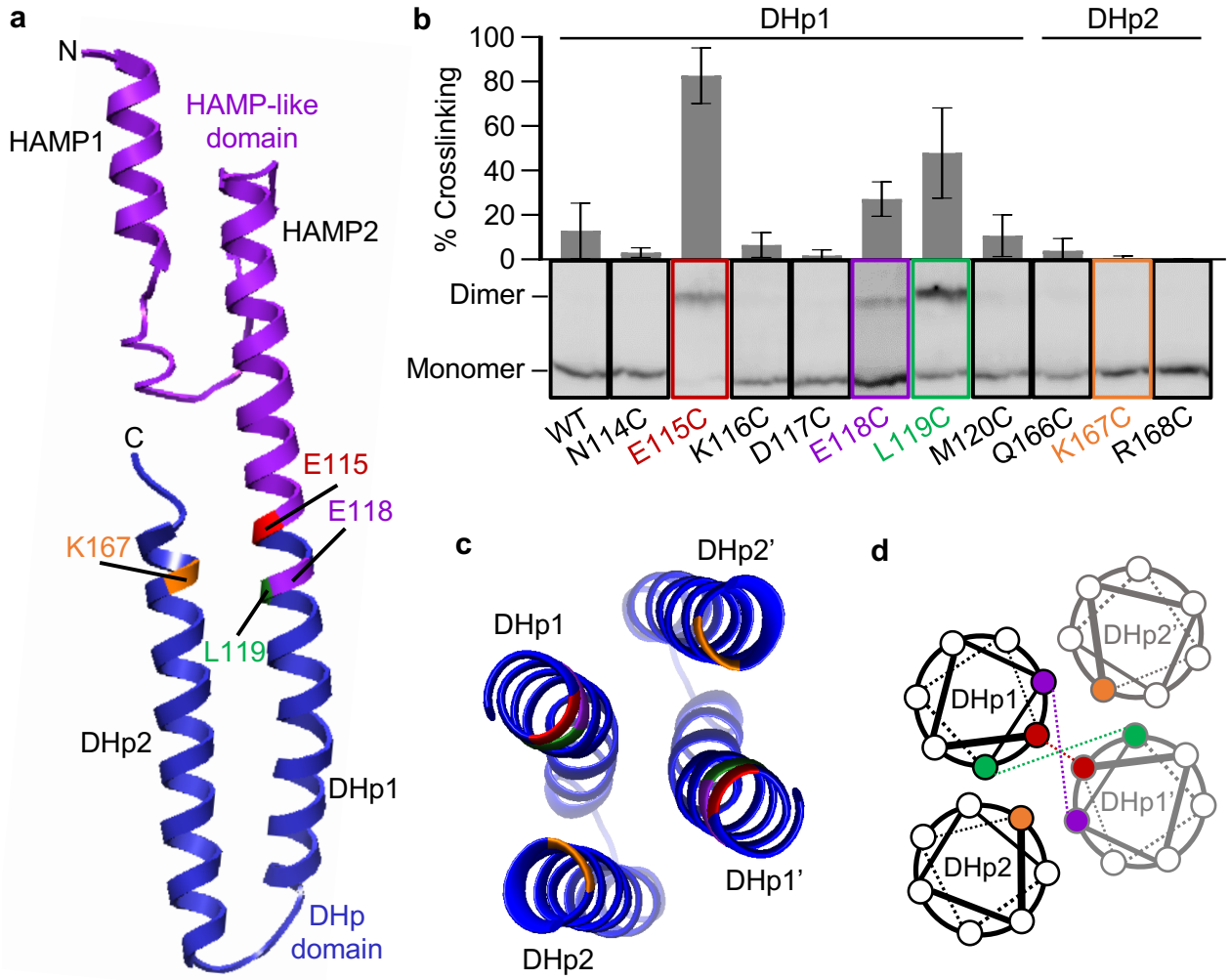

Figure 2: BceS DHp domain forms a four-helix bundle. a, Homology model of the BceS HAMP-like and DHp domains generated by I-TASSER (C-score = -0.76). The HAMP-like domain is shown in purple and the DHp domain in blue. The residues that form Cys-crosslinks in panel b are highlighted, E115 (red), E118 (purple) and L119 (green). Residue K167, which sits opposite E115, is shown in orange. **b**, Percentage of crosslinking for each BceS Cys variant was calculated from relative band intensities between monomer and dimer within each lane using Fiji [42]. Amino acid substitutions were introduced in parent construct *bceS*^{WT}-His$_8$ (SGB369) and BceS production was controlled by addition of 0.02 % (w/v) xylose. Data are shown as mean ± SD from 2-5 independent repeats. **c, d**, Schematic diagram of the BceS DHp dimer configuration. Helices labelled DHp1' and DHp2' show the second protomer. Left, top down view of the helices in the structural model; right, helical wheel diagram of the top two helical turns in the DHp dom ain; residues that form crosslinks are highlighted as before.

137 detection by Western blot analysis. BceS contains three native Cys residues, C45 in the
138 second transmembrane helix and C198 and C259 in the ATPase domain. Based on their
139 physical distance from each other and the DHp domain, we did not expect these residues to
140 interfere with our analysis, and indeed no crosslinking product was observed upon oxidation
141 with iodine in wild-type BceS (Fig. 2b). Next, single cysteine substitutions were introduced
142 in the first two helical turns of the helix DHp1 (N114 to M120), and in equivalent positions
143 near the end of the helix DHp2 (Q166 to R168). Disulfide bond formation in these variants
144 should only occur if the substituted residues come into close proximity in the kinase dimer [27].
145 The only residues that consistently formed detectable crosslinks were at positions 115, 118
146 and 119, with the highest degree of crosslinking at position 115 (Fig. 2b). This is in good
147 agreement with their positioning at the core of the four-helix bundle and shows that the
148 structural model closely reflects the conformation of BceS in the cell (Fig. 2c).

149 To ensure that the introduction of cysteines had not affected the integrity of the protein, we
150 tested the activity of each BceS variant *in vivo*. This was based on their ability to induce
151 expression from the system's target promoter fused to a reporter gene (P_{bceA}-*luxABCDE* or
152 P_{bceA}-*lacZ*), following exposure to the antibiotic bacitracin [1]. Only the K116C and K167C
153 substitutions led to a complete loss of activity (Fig. S1a). It is therefore possible that the lack
154 of crosslinking observed for the latter, which also faces the core of the DHp domain (Fig. 2c),
155 could be explained by defects in the protein rather than the distance between DHp2 helices in
156 the kinase dimer. However, the majority of BceS variants retained at least partial signaling
157 activity, confirming structural integrity of the kinase during the crosslinking analyses.

158 **Charge substitutions in the DHp core suggest a rotation mechanism of kinase**
159 **activation.** Closer inspection of the DHp domain revealed a striking concentration of charged
160 residues at the membrane-proximal end of the four-helix bundle (Fig. 3). In the wild-type

161 kinase, E115 and K167 create a net neutral charge in the bundle core. As described above and
162 previously [1], cells harbouring wild-type BceS strongly activate expression of the P_{bceA}-*lux*
163 reporter in the presence of bacitracin, while in the absence of the antibiotic the activity of the
164 reporter is below the detection limit (Fig. 3a, WT). Interestingly, creation of an 'all negative'
165 (K167D substitution) or 'all positive' (E115K substitution) DHp core resulted in a massive
166 increase in the basal level of the kinase, while the activity in the presence of the inducer
167 remained unaffected (Fig. 3a). To test whether this effect was indeed due to the changes in
168 core charge and not to substitutions at this position *per se*, we introduced a series of other
169 amino acids into position 115. Exchanging E115 for a different positively charged amino
170 acid, Arg, again resulted in an increase in basal activity, although less pronounced (Fig. S1c).
171 Substitution for Ala or Cys had no detectable effect on activity, showing that the presence of
172 charged amino acids was not in itself important for kinase function. Moreover, the
173 constitutive kinase activity could be returned to near wild-type levels by restoring the net
174 neutral charge through combining the E115K and K167D substitutions (Fig. 3a). We
175 therefore concluded that the high constitutive activity in the absence of the inducer was
176 indeed due to the presence of four residues with the same charge in the DHp domain core.

177 In the current model for histidine kinase activation, the DHp domain undergoes a helical
178 rotation [15]. The strong activating effect we observed upon introduction of four residues of the
179 same charge at the core of the domain made it tempting to speculate that charge repulsion
180 may force BceS to undergo such a rotating motion, placing it in its activated state. According
181 to our homology model, rotation of the helix DHp1 in a counter-clockwise direction and
182 DHp2 in clockwise should create two new points of contact between charged residues: point I
183 between E118 and K167, and point II between K116 and R168 (Fig. 3b). The opposing
184 charges in point I should have a stabilizing effect on the rotated state, and removing this in
185 the K167D variant may explain its lower basal activity compared to E115K (Fig. 3a). In

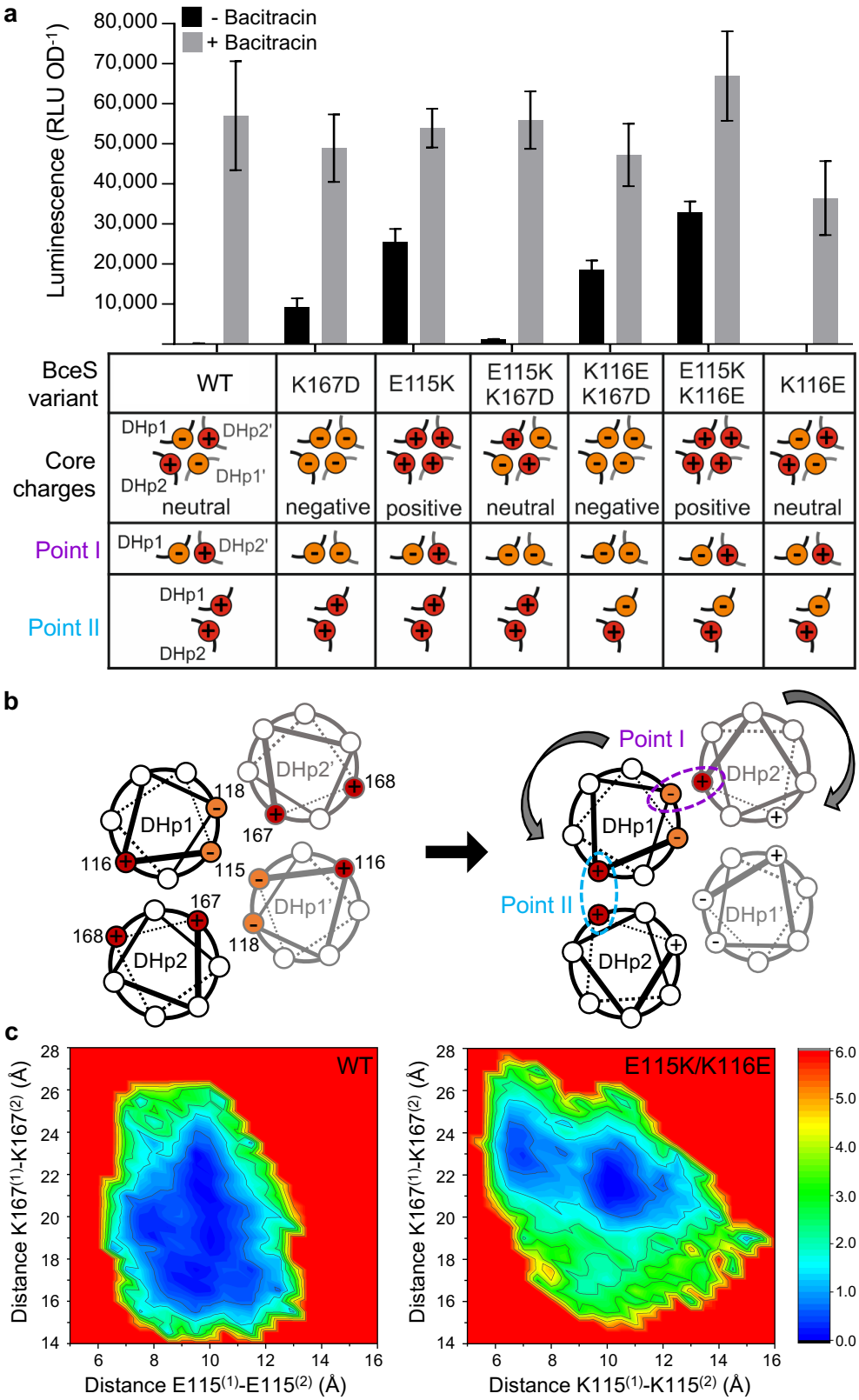

Figure 3: Charges within the BceS DHp domain core affect kinase activity. a, BceS signaling activity in strains harbouring P_{bceA}-*luxABCDE* and P_{xyl}-*bceS*. Amino acid substitutions were introduced in parent construct *bceS*^{WT}-His$_8$ (SGB369). Signaling was induced with 1µg ml^{-1} of bacitracin. Data are shown as mean ± SD from 4-22 biological repeats. The schematic below shows the location of individual and net charges in the core and stabilisation points within the four-helix bundle, as illustrated in panel b. Red, positive charge; orange, negative charge. **b**, Helical wheel diagram of the top two helical turns in the DHp dimer configuration showing location of charges and stabilisation points (pink and cyan). Helices labelled DHp1' and DHp2' show the second protomer. Black curve arrows denote the direction of helical rotation. **c**, 2D plots of potential of mean force (PMF) obtained in GaMD simulations of the wild type (WT, left) and E115K/K116E variant (right) protein. Inter-monomer distances shown are between the Cα carbon atoms of residues at positions 115 (x-axes) and 167 (y-axes). The PMFs are projected onto the surface in kcal mol^{-1} indicated by the heat scale.

186 contrast, the proximity of two positive charges in point II should have a de-stabilizing effect.
187 Replacing one of these with a negative charge (K116E) by itself had no effect on kinase
188 activity. However, combining K116E with the activating effect of the E115K substitution led
189 to a further increase of basal kinase activity compared to E115K alone (Fig. 3a). This effect is
190 entirely consistent with kinase activation through helical rotations in the DHp domain, where
191 we could achieve the highest artificial activation of the protein through creating charge
192 repulsion in the kinase core, while stabilizing the rotated state through opposing charges in
193 the two new points of contact.

194 Attempts were made to detect this DHp domain rotation *in vivo* using cysteine crosslinking,
195 either following induction with bacitracin or using the constitutively active E115K/K116E
196 variant. No quantitative conclusions could be drawn, however, due to the very large
197 experimental errors (Fig. S2). To provide further evidence of conformational change in the
198 DHp domain, we turned to molecular dynamics (MD) simulations. As discussed above, the
199 homology model (Fig. 2) shows excellent agreement with experimental validation, indicating
200 that it is a reasonable starting point for MD simulations. Given the relatively large putative
201 rotation, we applied Gaussian accelerated MD (GaMD) to more completely sample
202 conformational space. This approach applies a harmonic boost potential to effectively
203 'flatten' the free energy landscape, thereby enhancing the conformational sampling achieved
204 during MD simulation [28].

205 To assess conformational differences in the DHp domain between the wild-type kinase and
206 the constitutively active E115K/K116E variant, GaMD simulations were performed on both
207 for a total of 2 μs each (four replicas of 500 ns; Figure S3a). From our simulations, we
208 calculated the potential of mean force (PMF) for a series of internuclear distances for both
209 wild-type and variant residues. These values can serve as a proxy for the energetic barrier

210 between conformational sub-states. To best illustrate the relative orientation of helices in the
211 DHp domain bundle, we focused on positions 115 and 167, facing the bundle core (Fig. 3).
212 The PMF plots for the internuclear pairs of these residues between monomeric units of the
213 DHp domain are shown in Figure 3c. The lower the PMF value, the more likely the helices
214 will occupy the conformational state defined by the internuclear distances shown. In the wild-
215 type DHp domain, the PMF plot for distances between monomers at amino acids 115 and 167
216 shows a broad minimum across most of conformational space (Fig. 3c, left). This suggest that
217 there is a relatively high propensity for the domain to explore a diversity of conformational
218 space. Figure 3c (right) shows the same data for the variant DHp. Instead of the broad
219 minimum that characterizes the wild-type domain, the variant now shows two distinct, more
220 restricted minima. More specifically, the tendency towards a longer K167 distance in the
221 variant DHp would seem consistent with our model for helix rotation (Figure 3b). To more
222 clearly explore the differences between conformational space accessed by the wild-type and
223 variant DHp domain, we performed principal components analysis (PCA) of the data from
224 Figure 3c. This indicates that there is a significant difference in the principal component
225 space sampled by the wild-type and variant DHp (Fig. S3b). That is, the variant DHp
226 occupies a conformational space that is not accessible to the wild-type domain. Based on the
227 high constitutive activity of the variant, it is plausible to assume that this new conformational
228 space represents the active state of the kinase. Importantly, our simulations show that the
229 wild-type kinase is unable to adopt this conformation on the simulated time scales. This is
230 consistent with our *in vivo* experimental data that shows no detectable kinase activity without
231 activation through the sensory transporter BceAB.

232 **BceS contains a HAMP-like signal transfer domain.** Contrary to previous predictions, the
233 BceS homology model showed a domain with structural similarity to a HAMP domain
234 between the second transmembrane helix and the DHp domain. HAMP domains adopt a

235 parallel four-helix bundle arrangement with two alternative states of helix packing that
236 transmit a conformational change from an upstream input to a downstream output domain
237 [15,29,30]. While there is some argument over the exact motions within HAMP domains, there
238 seems to be agreement that they turn multiple input movements consistently into a helical
239 rotation [15,30]). The four-helix bundle is stabilized through multiple packing layers, forming
240 both inter- and intramolecular interactions, predominantly mediated by hydrophobic residues
241 [30,31]. A protein sequence alignment of known HAMP domains and the HAMP-like domain of
242 BceS is shown in Fig. 4a, with the positions of the critical packing points highlighted. The
243 hydrophobicity at these positions is largely conserved in BceS.

244 Previous investigations of HAMP domains have highlighted one particular residue with key
245 influence on the signaling state. This residue corresponds to L67 in BceS, a position most
246 commonly occupied by small hydrophobic residues that favour bundle packing (A or V,
247 occasionally I, L; Fig. 4a, black arrow) [30]. Based on extensive structural work on the HAMP
248 domain of the *A. fulgidus* protein Af1503, this residue (A291 in Af1503) has a crucial role in
249 controlling the conformational state of the HAMP domain. Specifically, it is thought that
250 increasing residue size at this position decreases the threshold for conversion between
251 packing states of the HAMP domain [30,32]. In Af1503, larger residues at this position increased
252 the signal responsiveness of a hybrid Tsr-HAMP-adenylate cyclase protein or of the HAMP-
253 EnvZ chimera our BceS homology model is most similar to [32,33]. Similarly, increasing the
254 size of the corresponding amino acid (V228I) in the HAMP domain of the histidine kinase
255 PhoQ of *Salmonella enterica* serovar Typhimurium led to elevated activity of the kinase,
256 while decreasing residue size had the opposite effect and reduced the PhoQ response to signal
257 [34].

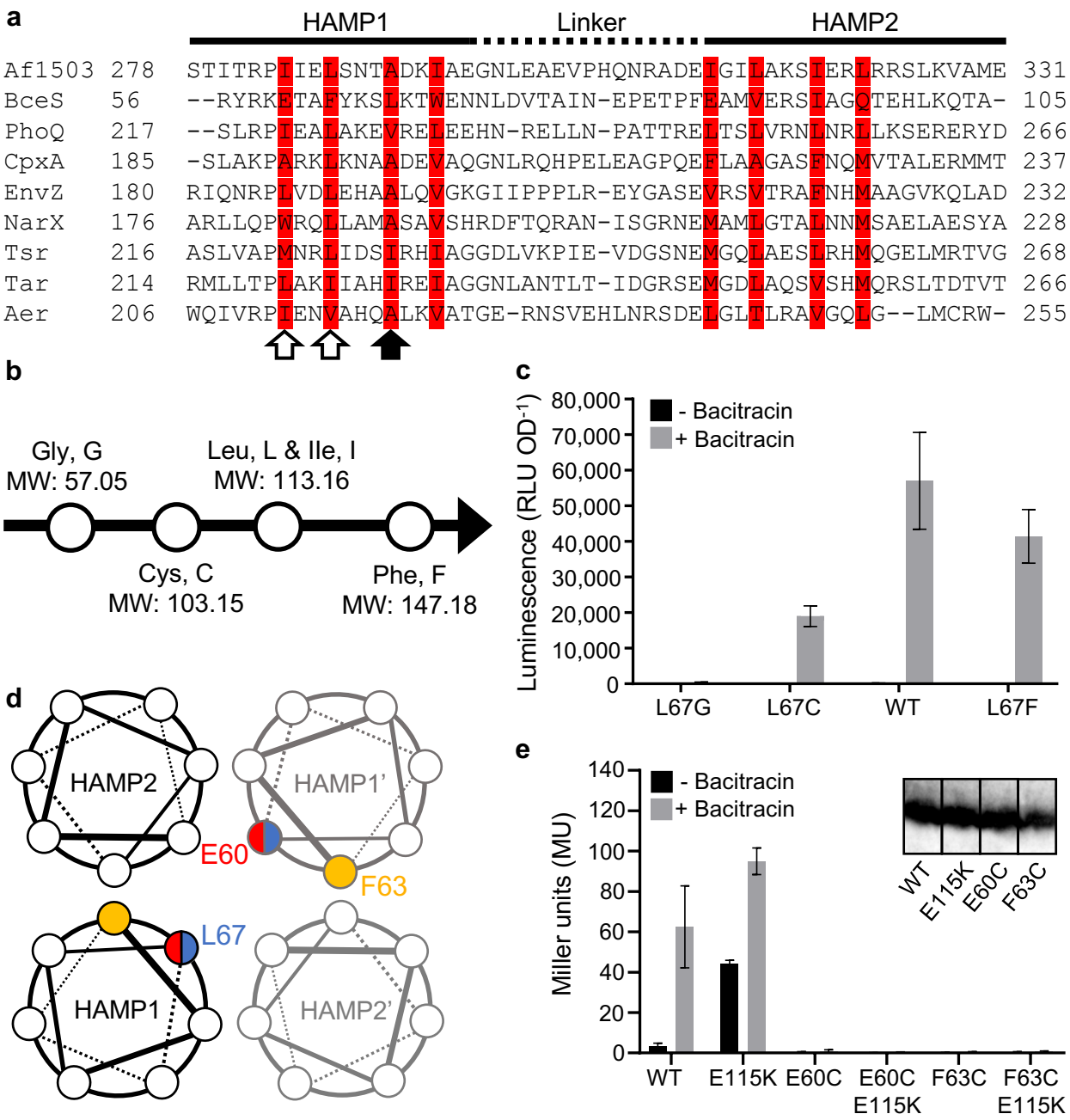

Figure 4: BceS contains a HAMP-like signal transfer. a, Sequence alignment of known HAMP domains and the BceS HAMP-like domain generated with ClustalO. Residues in red form the packing layers in the Af1503 HAMP domain. The black solid arrow indicates the packing residue that influences signaling state in some HAMP domains. White open arrows indicate E60 and F63 critical for BceS function. Proteins included and their GenBank identifiers are Af1503 (O28769), BceS (BSU30390), PhoQ (P23837), CpxA (P0AE82), EnvZ (P0AEJ4), NarX (P0AFA2), Tsr (P02942), Tar (P07017), Aer (P50466). **b**, Schematic showing amino acids and their sizes in MW used in size substitutions. **c**, BceS signaling activity following L67 amino acid size substitution harbouring P_{bceA}-*luxABCDE* and P_{xyl}-*bceS*. Amino acid substitutions were introduced in the parent construct *bceS*^{WT}-His$_8$ (SGB369). Signaling was induced with 1µg ml^{-1} of bacitracin. Data are shown as mean ± SD from 5-18 biological repeats. **d**, Helical wheel diagram of the HAMP-like domain. Helices labelled HAMP1' and HAMP2' show the second protomer . Residues essential for BceS activity are E60 (red) and F63 (yellow); the key packing residue L67 is shown in blue; red/blue semicircles are used to indicate positioning of residues in subsequent helical layers. **e**, BceS signaling activity of the constitutive ON variant (BceS^{E115K}) combined with loss-of-function substitutions at positions 60 and 63. Amino acid substitutions were introduced in parent construct *bceS*^{WT}-His$_8$ (SGB401). Signaling in cells harbouring P_{bceA}-*lacZ* and P_{xyl}-*bceS* was induced with 1µg ml^{-1} of bacitracin, BceS production was controlled by addition of 0.02 % (w/v) xylose. Data are shown as mean ± SD from 3-7 biological repeats. The inset shows an anti-His Western blot analysis to confirm membrane localisation and protein levels of wild-type (WT) BceS and its variants.

258 To test if the HAMP-like domain discovered in BceS fulfils an equivalent function to other
259 HAMP domains, we therefore investigated the role of amino acid size at this position by
260 changing L67 to the smaller Gly and Cys, as well as the larger Phe side chain (Fig. 4b).
261 Indeed, smaller size substitutions reduced BceS activity, up to a near-complete loss of
262 induction in the Gly variant (Fig. 4c). Increasing the size to Phe, however, did not lead to an
263 increase of signaling nor changed the basal activity of BceS. Our data are thus consistent with
264 the notion that small residues at this position may increase the threshold for conversion
265 between HAMP-domain conformations [30,32], but we could not find a stimulating effect
266 through a larger residue. The latter was also found in *E. coli* NarX, where an A189V
267 substitution at the equivalent position to L67 in BceS led to a decrease of signal output [35].
268 The precise effect of changing this residue may therefore depend on the specific context in
269 different protein. We nevertheless conclude from our data that the HAMP-like domain in
270 BceS appears to be a functional equivalent to conventional HAMP domains in other proteins
271 and likely contributes to signal transfer within the histidine kinase.

272 For further functional investigation, we carried out Cys scanning mutagenesis of both helices
273 within the HAMP-like domain. The majority of variants maintained at least partial signaling
274 activity (Fig. S4), and in contrast to other studies [34,35], we did not find any substitutions that
275 led to a constitutively active state of BceS. The only two substitutions that caused a complete
276 loss of function were E60C and F63C (Fig. 4 and S4). Interestingly, these fall on the first two
277 positions of the packing motif (Fig. 4a, white arrows and 4d), with E60 located one packing
278 layer above the critical L67 discussed earlier, and are therefore likely of structural
279 importance. Both variant proteins are present in the cytoplasmic membrane at the same levels
280 as the wild type protein according to Western blot analysis (Fig. 4e inset), showing that the
281 lack of signaling is not caused by general protein misfolding. To test if the defect of the two
282 variants was due to a loss of signal transfer from either the TM region of the protein or the

283 transporter, we combined each of the LOF mutations with the E115K substitution that
284 activates BceS in the absence of a stimulus. All resulting variants remained completely
285 inactive (Fig. 4e), which showed that the substitutions in the HAMP-like domain had a
286 dominant effect and thus likely caused substantial structural disruptions in the protein, rather
287 than specifically affecting signal transfer.

288 **Activation of BceS involves a piston-like displacement in transmembrane helix 2.** We
289 next investigated the conformation of the second transmembrane helix in BceS. In many
290 histidine kinases, conformational changes in an extracellular sensory domain upon ligand
291 binding or signal perception are transmitted to the cytoplasmic region of the protein through
292 physical movement of the intervening transmembrane helix [14]. In the case of BceS, however,
293 signaling is entirely reliant on the BceAB transport system and therefore does not involve
294 canonical transmembrane information transfer. To investigate if BceS activation nevertheless
295 involved transmembrane helix movements, we carried out a cysteine accessibility scan (Scan-
296 SCAM, [18]) of its second transmembrane helix. This analysis requires a cysteine-free
297 background, and we therefore replaced the three native Cys residues mentioned earlier by
298 serine. The C45S/C198S/C259S BceS variant showed comparable signaling activity to the
299 wild-type kinase and was therefore deemed fully functional (Fig. S5). Transmembrane
300 topology predictions of BceS place the membrane interface approximately at residues W54 or
301 F55. We therefore individually replaced each residue from positions 51 to 65 with Cys, with
302 the exception of E60 and F63, which were essential for BceS activity (see above). Again,
303 none of the resulting variants showed any defects in signaling (Fig. S5). The position relative
304 to the membrane was then tested for each Cys residue based on its solvent accessibility, using
305 a modified version of the established *E. coli* protocol [18]. In brief, treatment of whole cells
306 with N-ethyl-maleimide (NEM) should block any solvent-exposed Cys residue through
307 reaction with the sulfhydryl group, whereas in untreated cells these groups should remain

308 available for later reactions. Following isolation of membranes, any unblocked Cys residues
309 are then reacted with methoxypolyethylene glycol maleimide (PEG-mal), resulting in a 10
310 kDa increase in size, detectable by Western Blot analysis. For cytoplasmic Cys residues in
311 BceS, we therefore expected to see a shift in size only in samples that had not been treated
312 with NEM (Fig. 5, blue labels). In the established methodology used for *E. coli*, membrane-
313 embedded Cys residues are protected by their membrane environment from blocking by
314 NEM, but become available for PEGylation upon solubilisation in SDS [18]. However, in *B.*
315 *subtilis* membranes we could not observe PEGylation of membrane-embedded Cys residues
316 regardless of the presence of SDS, possibly due to differences in membrane composition. We
317 therefore interpreted those residues as being membrane embedded that did not show a size
318 shift in either presence or absence of NEM treatment (Fig. 5, red labels). The results from the
319 Scan-SCAM analysis showed that residues up to position 56 resided in the solvent-
320 inaccessible membrane environment, while residues from Y57 onwards were accessible and
321 therefore cytoplasmic (Fig. 5, 'OFF state'). The apparent inaccessibility of Y64 was
322 disregarded based on its distance from the likely membrane interface. We then repeated the
323 same analysis in the background of the E115K/K116E substitutions that place the kinase in a
324 constitutively active conformation. Here, residues from F55 onwards became consistently
325 solvent-accessible (Fig. 5, 'ON state'). Comparison of the two states therefore shows that
326 BceS activation involved an inward piston-like displacement by two amino acids of the
327 second transmembrane helix.

328 We next sought to test whether BceS could be activated by artificially inducing a shift
329 through introduction of arginine residues near the membrane interface. Due to their positive
330 charge, Arg residues prefer to move towards a hydrophilic environment. When introduced in
331 a transmembrane segment, this can induce a shift towards the nearest solvent interface, which
332 has been shown to cause signal-independent activation of the *E. coli* histidine kinase DcuS [18].

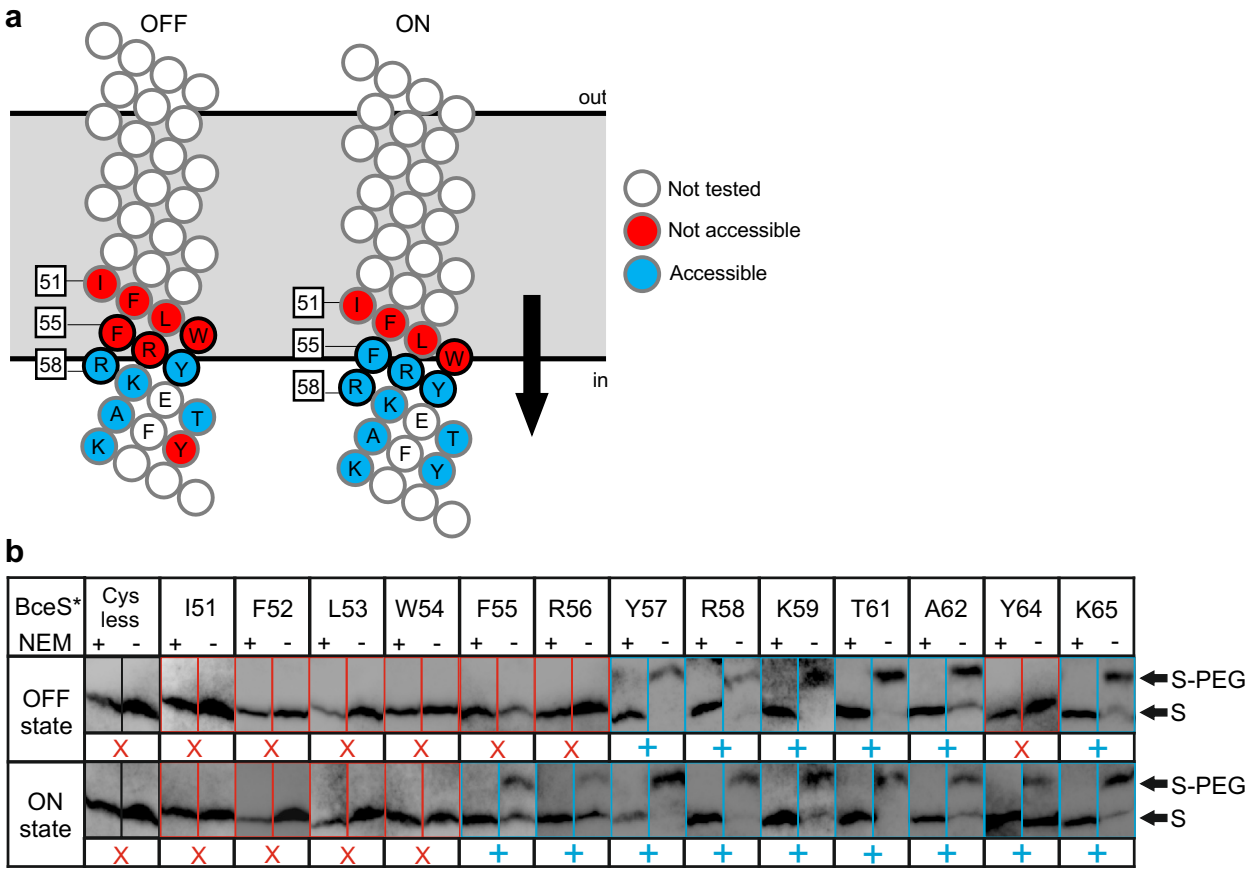

Figure 5: Accessibility of Cys residues at the membrane interface shows a piston-like displacement upon BceS activation. a, Schematic representation of transmembrane helix 2 in the OFF and ON states, respectively. Relevant native amino acids are indicated in one-letter code. Solvent-accessible (PEGylated) Cys replacements are shown in blue, inaccessible (unPEGylated) Cys replacements in red and residues not tested in white. Residues circled in black were tested in triplicate, those circled in grey in duplicate. **b**, Anti-His Western blot analysis of solvent-accessibility of Cys replacements (Scan-SCAM). Bands corresponding to PEGylated (S-PEG) and unmodified (S) BceS are indicated; individual lanes are outlined in the same colour code as used in panel a. 'Cys-less' indicates the parent variant of BceS lacking its native Cys residues. OFF state: amino acids substituted in parent construct *bceS*$^{C45S/C198S/C259S}$-His$_8$ (SGB936). ON state: amino acids substituted in parent construct *bceS*$^{E115K/K116E/C45S/C198S/C259S}$-His$_8$ (SGB951).

333 We therefore substituted three of the residues near the cytoplasmic end of transmembrane
334 helix 2 of BceS (W54, F55 and Y57) with Arg, individually and in pairs. (Fig. S6). Variants
335 containing the W54R substitution had approximately 60% higher activities upon stimulation
336 with bacitracin (Fig. S6), which may indicate a stabilisation of or lowered transition threshold
337 to the activated state. However, none of the variants showed a detectable change of basal
338 activity in the presence or absence of the input transporter BceAB, leading us to conclude
339 that Arg substitution alone was not sufficient to activate the kinase, which still relied on
340 BceAB for signaling.

341 **The transporter BceAB has complete control over kinase conformation**. It was
342 previously shown for Bce of *B. subtilis* and several other examples of the same type of
343 resistance system that activation of signaling is completely reliant on the ABC transporter
344 [3,4,7,8,24]. We further showed through computational modelling that the signaling output via
345 BceS is directly proportional to the activity of BceAB and does not follow the switch-like
346 on/off behaviour seen in most other systems using transporters as accessory sensing proteins
347 [1,20]. This led us to hypothesise that BceAB completely controls the conformation of BceS and
348 thus slaves kinase activity directly to its own transport activity. Our discovery of the DHp
349 domain variants that cause artificial activation of BceS now for the first time enabled us to
350 test this hypothesis experimentally by investigating the role of BceAB in controlling the OFF
351 state of the kinase.

352 In cells harbouring the wild-type sequence of BceS, the OFF-state activity in un-stimulated
353 cells is below the detection limit of our assays, and deletion of the transporter therefore has
354 no measurable effect (Fig. 6a). However, in the E115K or the E115K/K116E variants, basal
355 kinase activity was high, allowing us to test the effect of *bceAB* deletion in the absence of
356 bacitracin. Removing the transporter in this background caused a striking further increase in

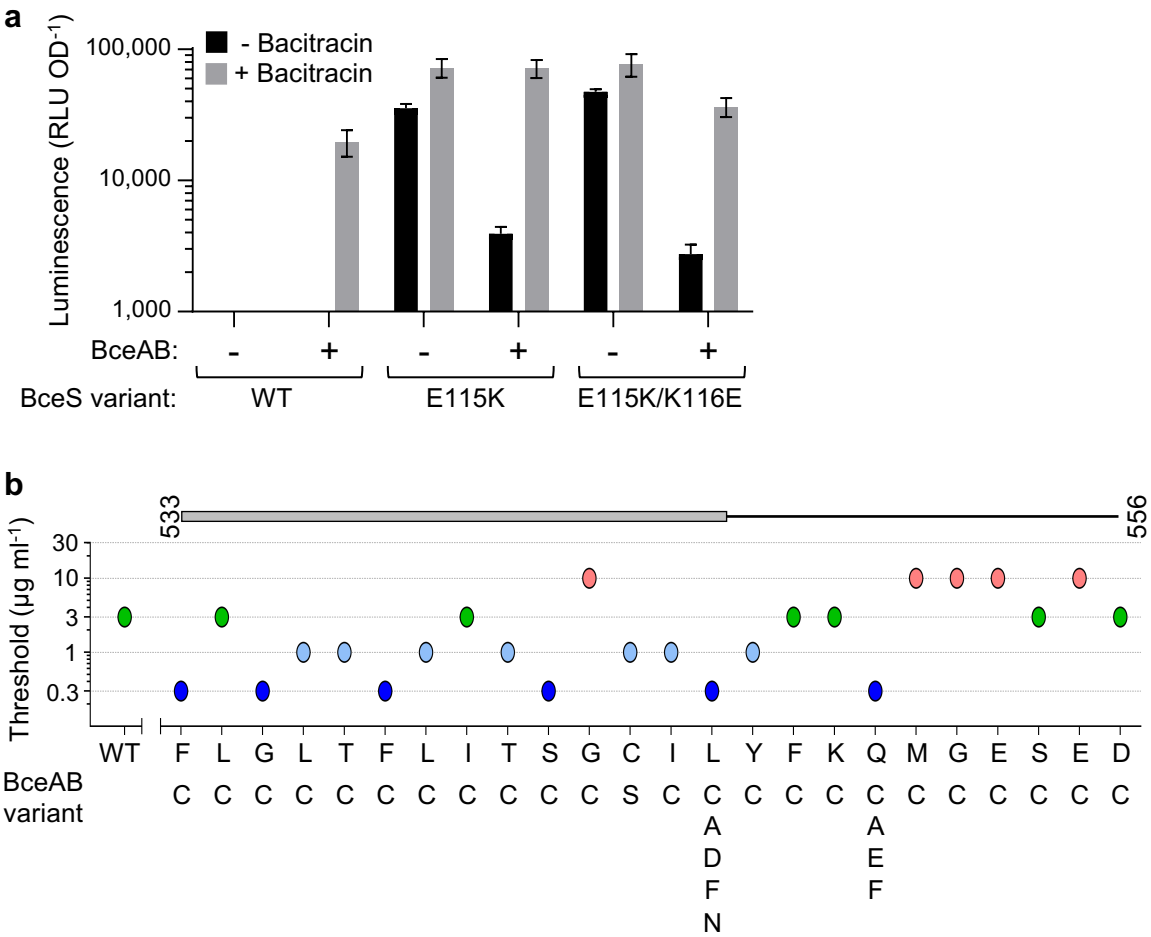

Figure 6: Control of BceS activity by the transporter BceAB. a, Signaling activity of BceS measured in strains harbouring P_{bceA}-*luxABCDE* and challenged with 10µg ml^{-1} of bacitracin (grey) or left untreated (black). Amino acid substitutions were introduced into parent construct P_{xyl}-*bceS*-His_8, in a strain background carrying a Δ*bceS* deletion (BceAB +; SGB792), or a Δ*bceSAB* deletion (BceAB -; SGB818). Data are shown as mean ± SD from 5-12 biological repeats. **b,** Signaling phenotype of BceAB variants. Amino acid substitutions were introduced into the P_{xyl}-*bceAB*-$FLAG_3$ parent construct, and signaling assessed using either a P_{bceA}-*lacZ* (SGB370 and derivatives) or P_{bceA}-*luxABCDE* (SGB731 and derivatives) reporter. Cells were challenged with a series of bacitracin concentrations, and the lowest concentration giving signaling above background is shown by circles in the plot (see Fig. S7 for complete datasets). Analyses were based on mean values from at least three independent repeats. The first line of x-axis labels gives native amino acids, the lines below indicate substitutions tested. The bar above the graph shows positions in the BceB sequence and areas within the membrane (grey bar) or in the cytoplasm (black line). Circle colours are assigned green, wild-type (WT) signaling threshold; red, increased signaling threshold; blue, reduced signaling threshold.

357 activity, rendering the kinase fully activated irrespective of the presence of the inducing
358 antibiotic (Fig. 6a). This shows that in the absence of its substrate bacitracin, the inactive
359 transporter can inhibit the activity of the constitutively active DHp domain variants, which
360 manifests as an approximately ten-fold reduction in basal activity compared to the
361 transporter-free state. Our data are therefore consistent with a model where inactive BceAB
362 exerts close control over BceS keeping the kinase in its inactive state. This reveals a further
363 layer of kinase control in addition to the transporter's known activating effect in the presence
364 of the antibiotic [1].

365 **BceAB has not evolved for maximal signaling sensitivity.** Recent structural biology work
366 has provided new insights into the functional mechanism of the Type VII superfamily of
367 ABC transporters, of which BceAB is a member. This group of proteins, exemplified by
368 MacB of *E. coli*, is thought to act as mechanotransmitters, using cytoplasmic ATP hydrolysis
369 to provide energy for periplasmic processes, such as removal of antibiotics [36]. In MacB,
370 mechanotransmission is achieved by the transmembrane helix linking the cytoplasmic
371 ATPase interaction site to the periplasmic domain [36]. In BceAB, the equivalent region is its
372 eighth transmembrane helix, which connects the transporter's extracellular domain to a
373 cytoplasmic loop containing one of the ATPase contact interfaces (Fig. 1) [11]. Strikingly, in a
374 previous random mutagenesis analysis we had discovered that this region of BceB contained
375 a particularly high density of mutations leading to a loss of signaling [37]. It was therefore
376 tempting to speculate that this region was crucial for the communication between transporter
377 and kinase. To investigate this further, we carried out another Cys scan, individually
378 replacing each residue from positions 533 to 556 in BceB, encompassing approximately half
379 of transmembrane helix 8 and the first alpha-helical segment of the adjacent cytoplasmic
380 region. The native Cys at position 544 was replaced by Ser. The resulting variants were tested
381 for their ability to complement a *bceAB* deletion strain for signaling and resistance activities.

382 With the exception of the strain harbouring the G535C variant, all displayed comparable
383 bacitracin resistance to the control containing the wild-type sequence of *bceAB* on the same
384 genetic construct (Table S4), showing that the resulting transporters were fully functional.
385 Signaling was tested using our standard reporter gene assays (P_{bceA}-*luxABCDE* or P_{bceA}-*lacZ*).
386 All variants except G543C reached a similar amplitude of reporter induction as strains
387 containing the wild-type construct (Fig. S7 a&b). However, we observed some clear
388 difference in the sensitivity of signaling, i.e. the minimal concentration of antibiotic required
389 to elicit a response. In cells with the wild-type construct, the lowest bacitracin concentration
390 to trigger signaling was 3 μg ml^{-1}, while a number of variants had a higher threshold of 10 μg
391 ml^{-1} (Fig. 6b, green vs red dots; Fig. S7 a&b). The majority of these fell into the C-terminal
392 part of the scanned region, indicating potential defects in coupling to the ATPase. To our
393 surprise, we also found several variants that showed a marked increase in signaling
394 sensitivity and responded already at 0.3 μg ml^{-1} or even 0.03 μg ml^{-1} bacitracin (Fig. 6b, dark
395 blue dots; Fig. S7). We had previously seen that a reduction of BceAB transport activity can
396 cause such a shift in signaling threshold, because the negative feedback created by removal of
397 the antibiotic is missing [1]. However, as none of these variants showed any change in
398 bacitracin resistance (Table S4), loss of antibiotic removal cannot explain the increased
399 signaling sensitivity. Mapping of these residues showed that they occurred with a regular
400 periodicity of every four amino acids (Fig. 6b), suggesting they are positioned along the same
401 face of the transmembrane helix. Figure S7c shows a homology model from I-TASSER of
402 the final three transmembrane helices of BceB, for which MacB was the closest structural
403 analog, to illustrate the potential arrangement of these residues. Substitutions with the
404 relatively small amino acid Cys may have enabled tighter helix packing, potentially
405 contributing to more effective signaling. To test this, we replaced two of the positions, L546
406 and Q550, with a series of other amino acids, including small Ala; charged Glu or Asp; and

407 large Phe. Regardless of the substitution made, deviation from the wild-type sequence at
408 these positions consistently led to an increase in signaling sensitivity (Fig. 6b). It is difficult
409 to envisage that all these substitutions caused tighter interactions with the kinase. An
410 alternative explanation may be that they are involved in tightly coupling ligand binding by
411 the extracellular domain to ATP hydrolysis, and that substitutions weaken this coupling,
412 allowing ATP hydrolysis and therefore signaling to occur at lower substrate concentrations. It
413 is beyond the scope of this study to investigate the mechanistic details of the transporter
414 further to answer this question. However, our findings clearly show that the transporter
415 contains a series of residues that likely form a continuous face on transmembrane helix 8,
416 which prevent signaling from occurring at lower bacitracin concentrations. Given that any
417 replacement we tested led to more sensitive signaling, we therefore conclude that the
418 transporter has evolved to avoid hyper-sensitive signaling, and that this likely constitutes a
419 further layer in minimizing the fitness cost of resistance.

420 **DISCUSSION**

421 In this study, we set out to investigate the molecular mechanisms of the flux-sensing strategy
422 employed by Bce-like antibiotic resistance systems. Their hallmark feature is that histidine
423 kinase activity not only fully depends on the presence of its cognate transporter, but is
424 directly proportional to transport activity across a large input dynamic range of antibiotic
425 concentrations [1]. This careful adaptation of signaling activity ensures the resistance
426 transporter is produced precisely to the cell's demand for detoxification capacity, a very cost-
427 efficient strategy to control gene expression. The results presented here now provide
428 molecular level understanding of how such close control over kinase activity, implementing
429 regulation akin to an analog dial, can be achieved.

430 Analyses of BceS conformation in the inactive and active state showed a piston-like inward
431 displacement of the second transmembrane helix and helical rotation within the DHp domain
432 upon kinase activation. The intervening linker region is not sufficiently conserved in
433 sequence for *in silico* identification of any specific domains. However, tertiary structure
434 predictions and experimental validation indicate that BceS possesses a HAMP-like domain
435 that likely fulfils the signal transfer function. Based on crystallographic evidence from a
436 truncated kinase, it has been proposed that in HAMP-containing histidine kinases ligand
437 binding typically induces piston-displacements in the membrane, and these are converted by
438 the HAMP domain into rotational motion of the DHp domain [15]. Kinases without a HAMP
439 domain instead directly experience rotational motions in their transmembrane segments upon
440 ligand binding [15]. Despite its unusual input mechanism via the BceAB transporter, the
441 conformational changes we observed within BceS are therefore consistent with this proposed
442 mode of activation of HAMP-containing histidine kinases.

443 A recent NMR study reported on the structure of the BceS-like kinase NsaS from *S. aureus*,
444 which possesses a much shorter linker region between its transmembrane and DHp domains.
445 This linker region adopts a coiled-coil conformation instead of the HAMP-like fold of BceS
446 [17]. Inspection of kinase sequences in an existing collection of Bce-like resistance systems [5]
447 showed that the vast majority of these proteins contained the larger version of the linker
448 (approximately 60 amino acids), and only a small number possessed short (around 20 amino
449 acid) linkers with a predicted coiled-coil motif. BceS therefore appears to be the more typical
450 subtype of this group of kinases than NsaS. Interestingly however, akin to the alternative
451 helix packing mechanism proposed for HAMP domains [30], activation of NsaS was also
452 proposed to involve switching between helical packing states of the coiled-coil [17]. The
453 molecular principles of activation may therefore be conserved between both subtypes of
454 BceS-like kinases.

455 To further investigate the activation mechanism of BceS, we sought to force each of the
456 conformational changes via amino acid substitutions. Introduction of residues with the same
457 charge at the core of the DHp domain to cause helical rotation consistently led to kinase
458 activation without the need for the physiological stimulus (Fig. 3). However, we were unable
459 to artificially trigger activation through substitutions that should have induced piston
460 displacements in the membrane (Fig. S6) or induced the active conformation of the HAMP
461 domain (Fig. 4 and S4). This is in contrast to studies of other kinases where such
462 substitutions were able to at least partially raise the basal kinase activity [18,34,35]. In canonical
463 histidine kinases, the input signal is detected directly, e.g. through ligand binding. One could
464 therefore speculate that in these proteins, conformational changes have to be smoothly
465 transmitted from each domain to the next, and that this is reflected in the relative ease with
466 which kinase activity can be artificially induced through changes in each individual domain.
467 In contrast, BceS-like proteins are subject to control by the transporter. We do not yet know
468 with which part of the kinase the transporter interacts, although the transmembrane or
469 HAMP-like domains are the most likely. In a kinase that relies on such external control, it is
470 conceivable that small changes in upstream domains are insufficient to trigger rotation of the
471 DHp domain helices, and only manipulation of the DHp domain itself can cause artificial
472 activation. Some support for such a hypothesis may be found in the MD simulations. Our
473 analyses showed that the wild-type DHp domain does not by itself sample the conformational
474 space observed in the active variant (Fig. S3), implying that the transporter is likely required
475 to provide the necessary force for transition to the active state.

476 The use of transporters in accessory control of signaling proteins is not uncommon in
477 bacteria, but BceAB is unusual in that it is an activator of signaling, while most other
478 transporters act as repressors [20]. Repression of signaling and its relief are easily envisaged via
479 complex formation and dissociation between transporter and histidine kinase. For the Bce

480 system, we previously showed that transporter and kinase constitutively form a signaling
481 complex in the membrane, irrespective of the presence or absence of the inducer, bacitracin [2].
482 Considering its activating role and that signaling is proportional to transport activity, the
483 transporter must therefore be able to directly influence kinase activity within this complex.
484 When this was first tested *in vitro*, it was noted that BceAB had a negative effect on the
485 kinase autophosphorylation rate in the OFF state, although we could not explain the
486 observation at the time [2]. In the present study, we now show that the transporter indeed not
487 only controls the ON but also the OFF state of BceS *in vivo* and is able to restore partial
488 control over the high constitutive activity of the DHp domain variant (Fig. 6a). We propose
489 that through controlling both states of the kinase, the transporter is able to exert the level of
490 close control that is needed to explain the enormous dynamic range of the system, where
491 signaling is maintained in proportion to transport activity over more than three orders of
492 magnitude in antibiotic concentrations [1]. A crucial feature of the flux-sensing mechanism is
493 that signaling is immediately reduced if transport activity is low, either because the antibiotic
494 threat is over or because the cell has reached sufficient detoxification capacity [1]. Importantly,
495 the ability of BceAB to actively switch off BceS activity described here explains how this
496 energy-conserving signaling strategy is realized on the molecular level. Moreover, control
497 over the OFF state also allows the transporter to actively prevent any spurious signaling by
498 the two-component system in the absence of the antibiotic. Finally, our mutagenesis analysis
499 of BceAB revealed that the transporter itself has evolved to avoid signaling at very low
500 antibiotic concentrations, highlighting yet another level of cost reduction.

501 Taken together, we here present a molecular level model for activation of antibiotic
502 resistance based directly on the cell's need for protection, using a flux-sensing signaling
503 strategy that employs multiple layers of active energy conservation to minimize any fitness
504 costs of resistance. The system studied here is centered on two-component signaling, one of

505 the most important regulatory features of bacteria, and employs a histidine kinase of rather
506 typical domain composition. Our findings should therefore have wide relevance and serve as
507 a model for understanding, in general, how analog dials can control fine-tuned gene
508 expression in bacteria to achieve an optimal cost-benefit balance. In turn, the design
509 principles of an analog dial, we suggest, could usefully be employed in the context of
510 synthetic biology.

511 **METHODS**

512 **Bacterial strains and growth conditions.** All strains used in this study are listed in table S1
513 and were routinely grown in Lysogeny Broth (LB; 10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, 5 g l^{-}
514 ^{1} NaCl) at 37 ºC with aeration. Solid media contained 15 g l^{-1} agar. Selective media contained
515 chloramphenicol (5 µg ml^{-1}), macrolide-lincosamide-streptogramin B (erythromycin; 1 µg
516 ml^{-1}, lincomycin; 25 µg ml^{-1}), spectinomycin (100 µg ml^{-1}), kanamycin (10 µg ml^{-1}), or
517 ampicillin (100 µg ml^{-1}).

518 **DNA manipulation and strain construction.** All primers and plasmids used in this study
519 are given in tables S2 and S3, respectively, with the use of each primer specified in table S3.
520 Site-directed mutagenesis was carried out according to the manufacturer's instructions for the
521 QuikChange II site-directed mutagenesis kit (Agilent Technologies), or by PCR-overlap
522 extension [38]. *Bacillus subtilis* transformations were carried out via natural competence as
523 previously described [39]. DNA for transformation was either provided as linearised plasmid
524 DNA, or as genomic DNA from a *B. subtilis* strain already carrying the desired genetic
525 construct.

526 To allow manipulation of both *bceS* and *bceAB* sequences, an unmarked deletion strain
527 lacking all three genes (Δ*bceSAB*, SGB377) was constructed as described previously [40],

528 employing plasmid pAK102. Strains lacking *bceS* only (Δ*bceS*; TMB1036) or *bceAB* only
529 (*bceAB*::kan; TMB035 [24]) were available from previous work. Signaling activity was
530 assessed in strains harbouring a chromosomally integrated transcriptional reporter construct
531 of the target promoter P_{bceA}, fused to either *luxABCDE* (pSDlux101 [37]) or *lacZ* (pER603 [24]).
532 Details of the assays used are given below.

533 Genetic manipulation of *bceS* was performed using previously constructed plasmids as
534 template. For Western blot detection, the construct pSD2E01 encoding BceS with a C-
535 terminal octa-histidine tag (P_{xyl}-*bceS*^{WT}-His_8 [2]) was employed. Un-tagged constructs were
536 derived from pAS719 (P_{xyl}-*bceS*^{WT}). Genetic manipulation of *bceAB* was performed using the
537 previously constructed plasmid pFK727, encoding BceAB with a C-terminal triple FLAG-tag
538 on the permease (P_{xyl}-*bceAB*-$FLAG_3$ [37]). All constructs used in this study integrate stably and
539 as a single copy into the *B. subtilis* W168 chromosome.

540 **Signaling activity assays.** In strains carrying the P_{bceA}-*lacZ* reporter, signaling was assessed
541 following challenge of exponentially growing cells with Zn^{2+}-bacitracin (bacitracin) and
542 measured as β-galactosidase activity (expressed in Miller Units), as described previously [24].
543 For strains carrying the P_{bceA}-*luxABCDE* reporter, luciferase activities were assayed using a
544 Tecan® Spark® microplate reader controlled by SparkControl™ V2.1 software. Starter
545 cultures were grown at 37 ºC in 2 ml of LB medium overnight, then diluted 1:1000 into fresh
546 medium before adding 100 µl into 96-well plates (black, clear flat bottoms; Corning®).
547 Samples were grown at 37 ˚C with orbital agitation at 180 rpm and allowed to achieve early
548 exponential growth (OD_{600} 0.3 – 0.4), before adding Zn^{2+}-bacitracin to a final concentration
549 of 1 µg ml^{-1} unless otherwise indicated. OD_{600} and relative luminescence units (RLU;
550 integration time 1 s) were measured every 5 min and were background-corrected using values

551 from LB-only wells. RLU was normalised for cell density (RLU OD^{-1}). The reported values
552 are steady-state activities achieved 60-75 min after bacitracin addition.

553 Cells were grown without the addition of xylose, as the basal activity of the promoter P_{xylA} [41],
554 used to drive expression of the ectopic copies of *bceS* and/or *bceAB*, was sufficient to achieve
555 full signaling activity. In strains containing the native copy of *bceAB* (Δ*bceS* background)
556 signaling was induced with 10 µg ml^{-1} of Zn^{2+}-bacitracin. In strains containing only the
557 ectopic construct for BceAB production (Δ*bceSAB* background), signaling was induced with
558 1 µg ml^{-1} of Zn^{2+}-bacitracin. These concentrations were established in our earlier work as
559 eliciting maximum signaling activity in the respective genetic backgrounds [1].

560 **Bacitracin sensitivity assays.** The sensitivity of *B. subtilis* strains to bacitracin was
561 determined as described [37], with minor modifications. Serial two-fold dilutions of Zn^{2+}-
562 bacitracin from 32 µg ml^{-1} to 0.5 µg ml^{-1} were prepared in Mueller-Hinton medium
563 containing 0.2 % (w/v) xylose to ensure maximal production of BceAB. For each
564 concentration, 100 µl volumes in 96-well plates were inoculated 1:400 from overnight
565 cultures grown in Mueller-Hinton medium with xylose and selective antibiotics. Each culture
566 was scored for growth after 20 to 24 h of incubation at 37°C with aeration. The MIC was
567 determined as the lowest bacitracin concentration where no visible growth was detected.

568 **Cysteine cross-linking *in vivo*.** Cysteine cross-linking was assessed in strains carrying the
569 Δ*bceSAB* deletion complemented with xylose-inducible ectopic copies of *bceAB*-$FLAG_3$ and
570 *bceS*-His_8 with single-cysteine substitutions and the P_{bceA}-*lux* reporter (SGB369 and
571 derivatives). Starter cultures were grown in 2 ml of LB medium overnight at 37 ºC with
572 agitation, then diluted 1:500 into 55 ml of fresh medium supplemented with 0.02% (w/v)
573 xylose. In mid-exponential growth (OD_{600} 0.5 – 0.7), oxidative cross-linking of cysteine
574 residues was triggered by addition of 250 µM of iodine for 15 min under continued

575 incubation. Where required, cells were induced with 60 µg ml^{-1} Zn^{2+}-bacitracin for 45 min
576 prior to cross-linking. Cross-linking reactions were stopped with 20 mM N-ethylmaleimide
577 (NEM) for 15 min under continued incubation. Cells were harvested by centrifugation (3,000
578 × *g*, 20 min, 4 ºC) and resuspended in 5 ml of ice cold lysis buffer (100 mM Tris-HCl [pH
579 8.0], 150 mM NaCl, 10% (w/v) glycerol, 20 mM NEM). Cell disruption was achieved by at
580 least three cycles of 15s sonication on ice with 15s rest. Debris was removed by
581 centrifugation (3,000 × *g*, 20 min, 4 ºC), and membranes were collected from supernatants by
582 ultracentrifugation (150,000 × *g*, 1 h, 4 ºC). Membranes were resuspended in 50 µl lysis
583 buffer, and approximately 150 mg protein per sample was mixed with non-reducing sample
584 buffer (1× final concentration; 1.5 M Tris/HCl [pH 6.8], 30 % (w/v) glycerol, 350 mM SDS,
585 0.12 mg ml^{-1} bromophenol blue) for SDS-PAGE and Western blot analysis (see below). For
586 quantification of band intensities Fiji [42] was used to obtain a profile plot of each lane, and the
587 relative intensity of each band (monomer and dimer BceS) within the lane was expressed as a
588 percentage of the total band intensities.

589 **Solvent-accessibility of Cysteine residues *in vivo* (Scan-SCAM).** For solvent-accessibility
590 tests in the second transmembrane helix of BceS, the Δ*bceSAB* deletion strain, complemented
591 with xylose-inducible ectopic copies of *bceAB*-$FLAG_3$ and *bceS*-His_8 and the P_{bceA}-*lux*
592 reporter was employed. The *bceS* complementation construct was made cysteine-free via
593 C45S/C198S/C259S substitutions, followed by introduction of single cysteines to be tested
594 (strain SGB936 and derivatives). The same was done in the E115K/K116E variant to assess
595 transmembrane topology in the active state (strain SGB951 and derivatives). Cultures were
596 grown to mid-exponential phase as described above. Cells were harvested, washed twice with
597 50 mM HEPES buffer (pH 6.8) and resuspended in 5 ml of the same buffer. Water exposed
598 (i.e. cytoplasmic) cysteine residues were masked with 40 mM N-Ethylmaleimide (NEM) for
599 1.5 h at room temperature with shaking or left untreated. Masking reactions were stopped by

600 three washes in cold (4 ˚C) 50 mM HEPES buffer (pH 6.8). Cells were resuspended in 5 ml
601 of ice cold lysis buffer, sonicated and membranes collected as above. Membranes were
602 resuspended in 40 µl accessibility buffer (50 mM HEPES [pH 6.8], 1.3% (w/v) SDS).
603 Cysteines that had not been masked with NEM in the first step were labelled with 5 mM
604 methoxypolyethylene glycol maleimide (PEG-mal; Sigma-Aldrich) for 1.5 h at room
605 temperature with agitation. The reaction was stopped with 1× LDS sample buffer (Expedeon)
606 and 250 mM DTT and rested for 5 min. PEG-mal accessible and non-accessible BceS were
607 analyzed by Western Blot.

608 **Western blot analysis.** Protein samples (~150 µg) from *in vivo* cross-linking and Scan-
609 SCAM were subjected to SDS-PAGE on RunBlue™ TEO-Tricine SDS mini gel in either
610 Tris-Tricine-SDS (Sigma-Aldrich) or Teo-Tricine-SDS (Expedeon) buffer, run for 50 min at
611 120V. Samples were transferred to polyvinylidene fluoride (PVDF) membranes (Merck
612 Millipore, activated in 100 % (v/v) methanol) in 1 × RunBlue SDS Running Buffer
613 (Expedeon), 20 % (v/v) methanol at 75 mA for 70 minutes using a PerfectBlue™ Sedec™
614 Semi-Dry Electroblotter (Peqlab). Membranes were blocked with 7.5 % (w/v) skimmed milk
615 powder in PBST (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 [pH7.4],
616 0.1 % (v/v) Tween 20), washed three times in PBST, and incubated with primary antibody
617 (Penta·His Antibody, QIAGEN®, 1:2000 dilution) in 1% bovine serum albumin in PBST for
618 2h at room temperature. Following three washes in PBST, incubation with secondary
619 antibody (Peroxidase-AffiniPure Goat Anti-Rabbit IgG, Jackson ImmunoResearch Europe
620 Ltd, 1:4000 dilution) was carried out in milk-PBST for 1h at room temperature, followed by
621 three final washes in PBST. Detection of proteins was by chemiluminescence using Pierce™
622 ECL Western Blotting Substrate (Thermo Fisher Scientific Inc.) and a Qiagen CCD camera
623 with Fusion software.

624 **Homology Modelling.** Homology models for the BceS HAMP and DHp domains (positions
625 56 to 174), and for the C-terminal domain of BceAB (from position 507) were generated
626 using I-TASSER with default parameters [26]. The respective homology model with the highest
627 C-score was selected, and the top ranking structural analog from PDB identified by I-
628 TASSER was noted for reference.

629 **Gaussian-accelerated molecular dynamics (GaMD) Simulations.** A homology model of
630 the cytoplasmic region of BceS consisting of the DHp and ATPase domains was constructed
631 using I-TASSER as before [43]. The HAMP-like domain was excluded due to low sequence
632 homology with conventional HAMP domains. The approximate orientation of each BceS
633 monomer with respect to one another was predicted based on cysteine cross-linking data (Fig.
634 2) and further refined using Z-Dock to perform rigid body protein-protein docking [44], with
635 subsequent side chain rotamers optimised using SCWRL4 [45]. The N-terminus of the DHp
636 domain of each monomer was acetylated to cap the α-helix and prevent having a positive
637 charge at the termini. ATP was then docked into each ATP binding site using AutoDock Vina
638 (exhaustiveness set to 32) [46]. The generated model of BceS was then manually modified to
639 construct the E115K/K116E variant. All residues were modelled in their standard protonation
640 states, with His108 and His124 singly protonated on the Nδ1 nitrogen, and all other His
641 singly protonated on the Nε2 nitrogen (as indicated by hydrogen bonding networks). Both
642 systems were solvated in an octahedral water box such that no protein/substrate atom was
643 within 10 Å of the box boundaries, with Na^+ or Cl^- counter ions added as necessary to ensure
644 an overall neutral charge. MD simulations were performed using GPU accelerated Amber16,
645 with the ff14SB force field and TIP3P water model used to describe protein and water
646 molecules respectively. Parameters for ATP were taken from a study by Meagher and
647 colleagues [47]. Following a minimisation, heating, NVT and NPT equilibration procedure
648 (described below), Gaussian accelerated MD (GaMD) simulations were done in the NVT

649 ensemble at 300 K [28], with a boost potential applied to both the total system potential energy
650 as well as the dihedral potential energies (see also GaMD settings in Table S5). To prepare
651 for production GaMD simulations, a 12 ns long conventional MD run was used to obtain
652 maximum, minimum, average and standard deviation of the system potential energies.
653 Following this, a 66 ns long run was used to update the above parameters (updated every 600
654 ps) to ensure converged GaMD production parameters. Production GaMD simulations were
655 then run for 4 × 500 ns, using different random seeds for each replica. MD simulations were
656 run using a 2 fs time step with the SHAKE algorithm applied to any bond containing a
657 hydrogen atom. An 8 Å direct space non-bonded cut-off was applied with long-range
658 electrostatics evaluated using the particle mesh Ewald algorithm [48]. Temperature was
659 regulated using Langevin temperature control (collision frequency of 1 ps^{-1}). All MD and
660 GaMD simulations were performed with a 10 kcal mol^{-1} restraint on the Phi and Psi dihedrals
661 between the acetyl and first N-terminal residue, to prevent the α-helix fraying. Principal
662 component analysis (PCA) and other trajectory analyses were performed within CPPTRAJ [49],
663 on the Cα atoms of the DHp domain residues. PCA was performed on the trajectories after
664 constructing an average structure of all four simulations combined. Reweighting of PCA and
665 distance measurements (to obtain PMFs) was performed using the 'PyReweighting' toolkit [50].
666 To equilibrate structures for GaMD simulations, the following procedure was performed.
667 Minimisation of all hydrogen atoms and solvent molecules was done using 500 steps of
668 steepest descent followed by 500 steps of conjugate gradient. To keep all other atoms (i.e. the
669 protein heavy atoms) in place during the minimisation, 10 kcal mol^{-1} $Å^{-1}$ positional restraints
670 were applied. Retaining the positional restraints on all protein heavy atoms, the system was
671 then heated rapidly from 50 K to 300 K in the NVT ensemble over the course of 200 ps. This
672 system was again minimised for a further 500 steps of steepest descent followed by 500 steps
673 of conjugate gradient, this time only applying positional restraints (of size 5 kcal mol^{-1} $Å^{-1}$)

674 to the Cα carbon atoms. These Cα restraints were retained as the system was again heated
675 from 25 K to 300 K over the course of 50 ps in the NVT ensemble. Simulations were then
676 performed in the NPT ensemble (1 atm, 300 K), first gradually reducing the 5 kcal mol^{-1} $Å^{-1}$
677 Cα carbon restraints over the course of 50 ps of simulation time. This was done in five steps
678 (5, 4, 3, 2, 1 kcal mol^{-1} $Å^{-1}$) of 10 ps each. A final 20 ns long MD simulation was then
679 performed, in which no restraints were used, to equilibrate the box size. All dynamics steps
680 used the SHAKE algorithm. Simulations performed in the NVT ensemble used Langevin
681 temperature control (with a collision frequency of 1 ps^{-1}) and a simulation time-step of 1 fs.
682 Simulations performed in the NPT ensemble again used Langevin temperature control
683 (collision frequency of 1 ps^{-1}) and a Berendsen barostat (1 ps pressure relaxation time), with
684 a simulation time-step of 2 fs.

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AUTHOR CONTRIBUTIONS

AK and MJG carried out all laboratory work and data analysis; MVdK and CRP performed and analyzed the MD simulations; SG supervised all laboratory work and coordinated the research project; SG, CRP and AK wrote the manuscript.

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925 **LIST OF SUPPLEMENTAL FILES**

926 **Figure S1.** Cysteine scanning and site-directed mutagenesis of the BceS DHp domain.

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

928 **Figure S3.** Equilibration and principle component analysis of Gaussian accelerated molecular
929 dynamics simulations of BceS DHp domain conformation.

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

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

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

933 **Figure S7.** Signaling phenotypes of BceAB variants.

934

935 **Table S1.** Strains used in this study

936 **Table S2.** Primers used in this study

937 **Table S3.** Plasmids used in this study

938 **Table S4.** Minimal inhibitory concentration of bacitracin in strains carrying BceAB variants

939 **Table S5.** Parameters used for GaMD simulations

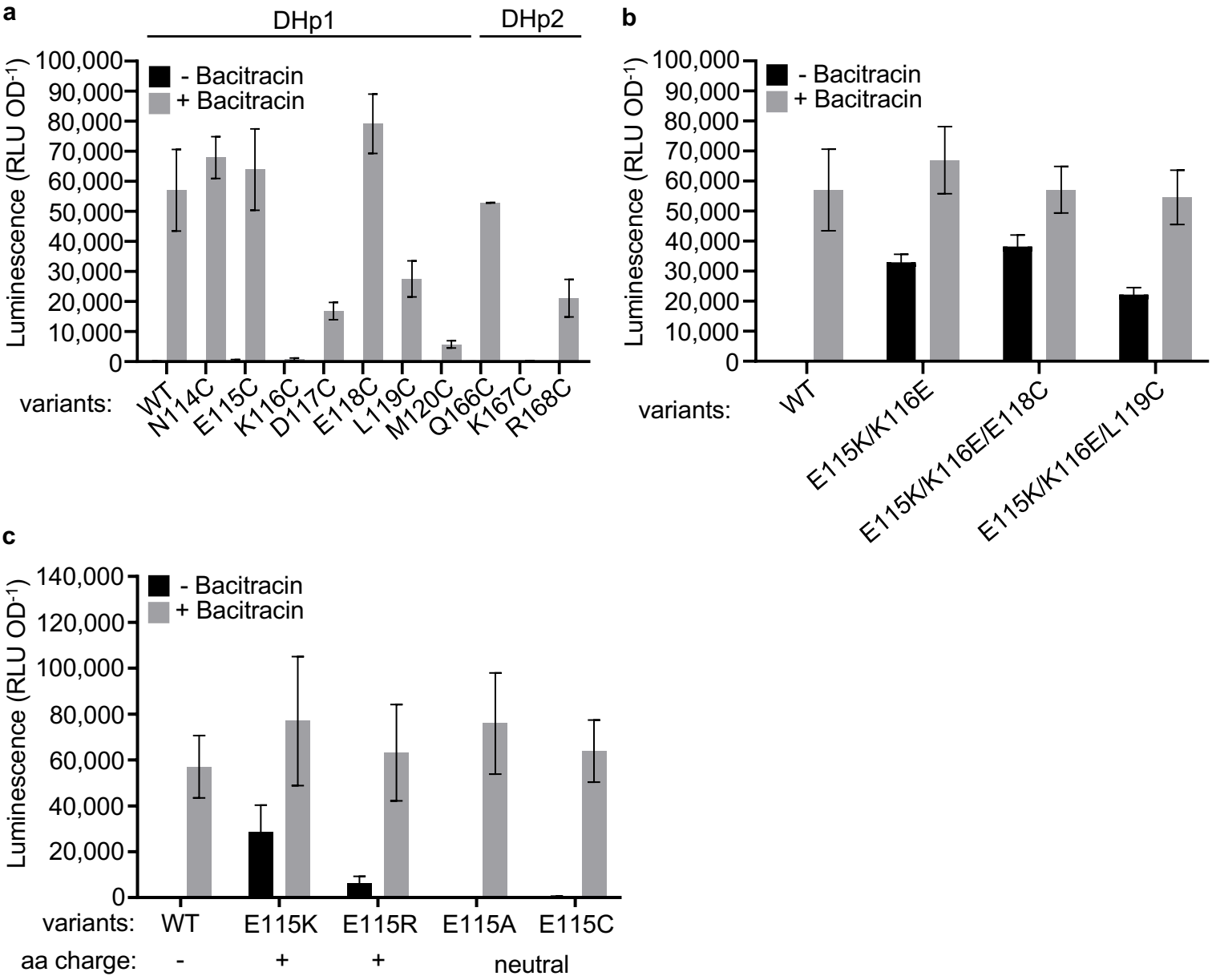

Figure S1. Cysteine scanning and site-directed mutagenesis of the BceS DHp domain. BceS signalling activity measured in strains harbouring P_{bceA}-*luxABCDE* and P_{xyl}-*bceS* wild-type (WT) or variants as indicated. Amino acid substitutions were introduced in the parent construct *bceS*^{WT}-His_8 (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 µg ml^{-1} of bacitracin (grey) or left unchallenged (black). Data are shown as mean ± SD from 4-22 biological repeats.

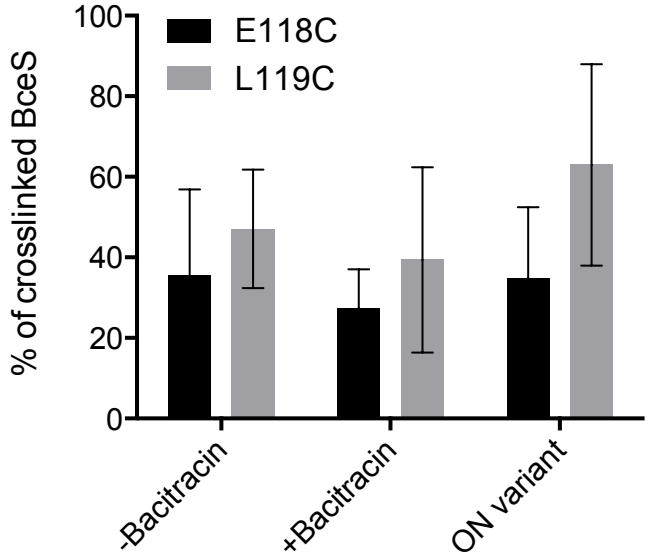

Figure S2. Cysteine cross-linking following activation of signalling. The percentage of crosslinking for BceS Cys variants E118C (black) and L119C (grey) was calculated from relative band intensities between monomer and dimer within each lane, following Western blot detection of BceS-His_8 as described 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.

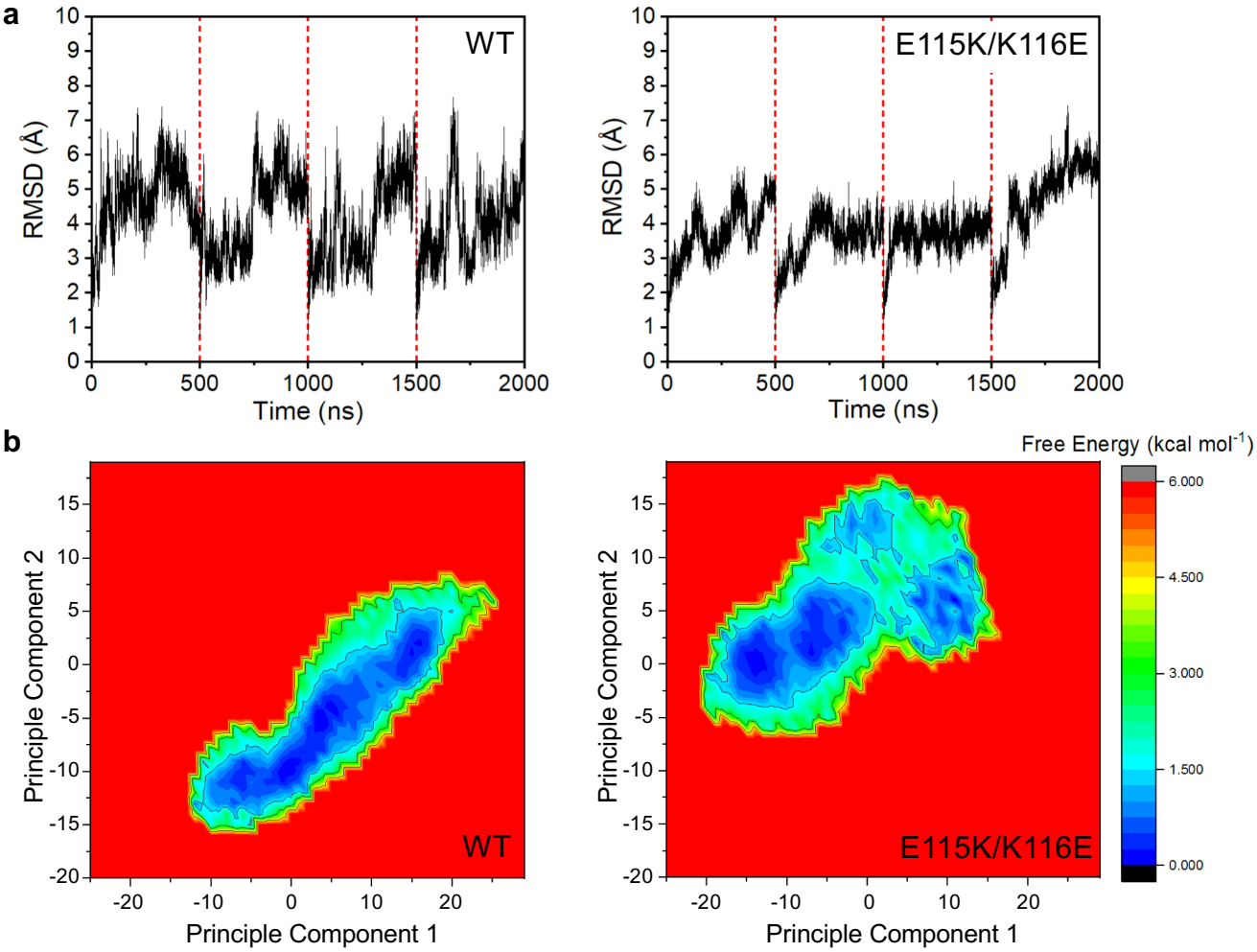

Figure S3. Equilibration and principle component analysis of Gaussian accelerated molecular dynamics (GaMD) simulations of BceS DHp domain conformation. a, Cα RMSD over the course of GaMD simulations for simulations of wild type (WT, left) and the E115K/K116E variant (right) protein. The red dotted lines 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 Cα carbon of the DHp domain residues.

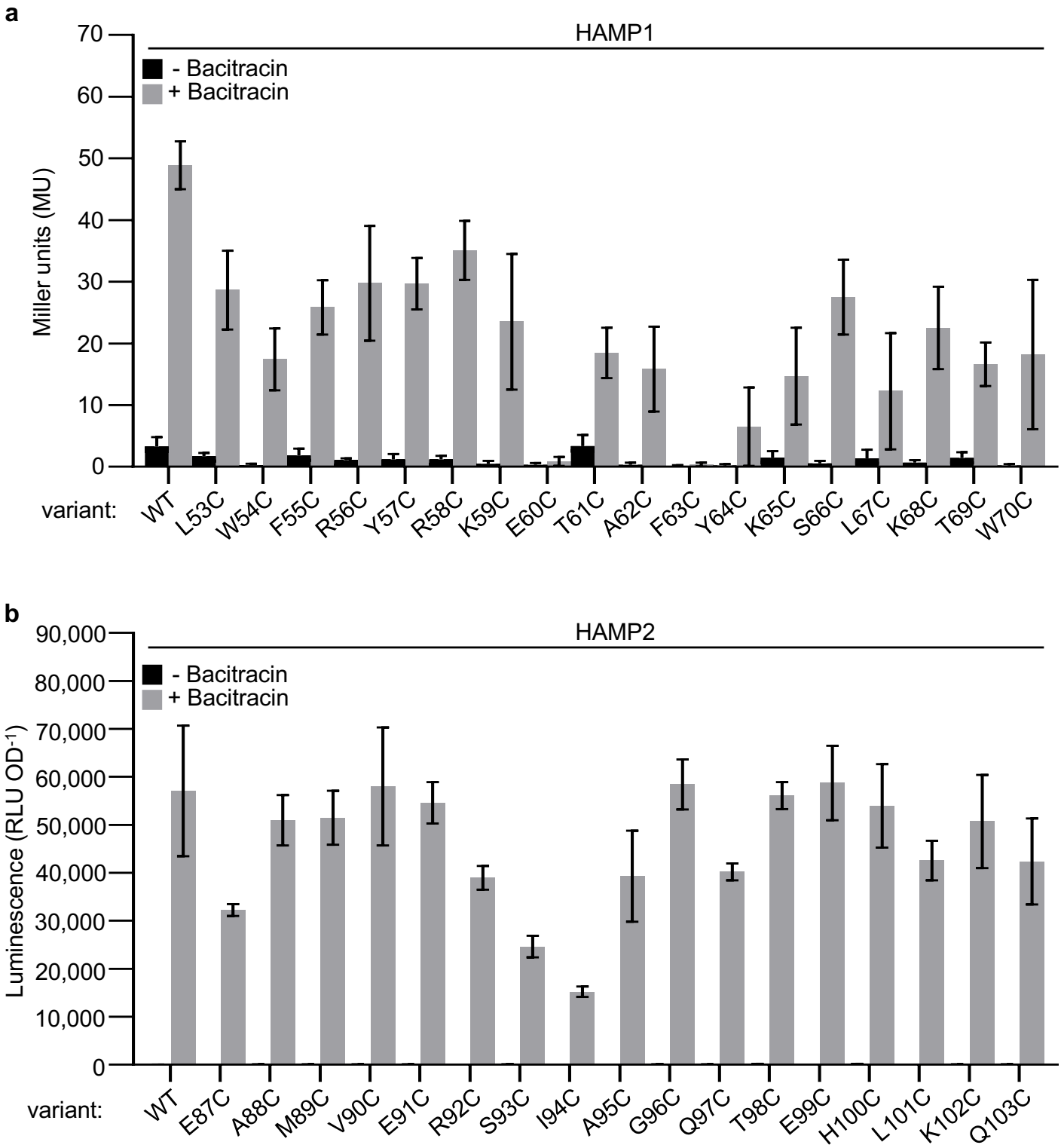

Figure S4: Cysteine scanning mutagenesis of the BceS HAMP-like domain. Amino acid substitutions were introduced into the P_{xyl}-*bceS*-His_8 parent construct, pSD2E01. Cells were challenged with 1 µg ml^{-1} of bacitracin (grey) or left unchallenged (black). **a,** Cys scanning of the helix HAMP1. Signalling was assessed by β-galactosidase activity (Miller Units) in cells carrying the P_{bceA}-*lacZ* reporter (SGB401 and derivatives). **b,** Cys scanning of helix HAMP2. Signalling was assessed by luminescence (RLU OD^{-1}) in cells carrying the P_{bceA}-*luxABCDE* reporter (SGB369 and derivatives). Data are shown as mean ± SD from 3-22 independent repeats.

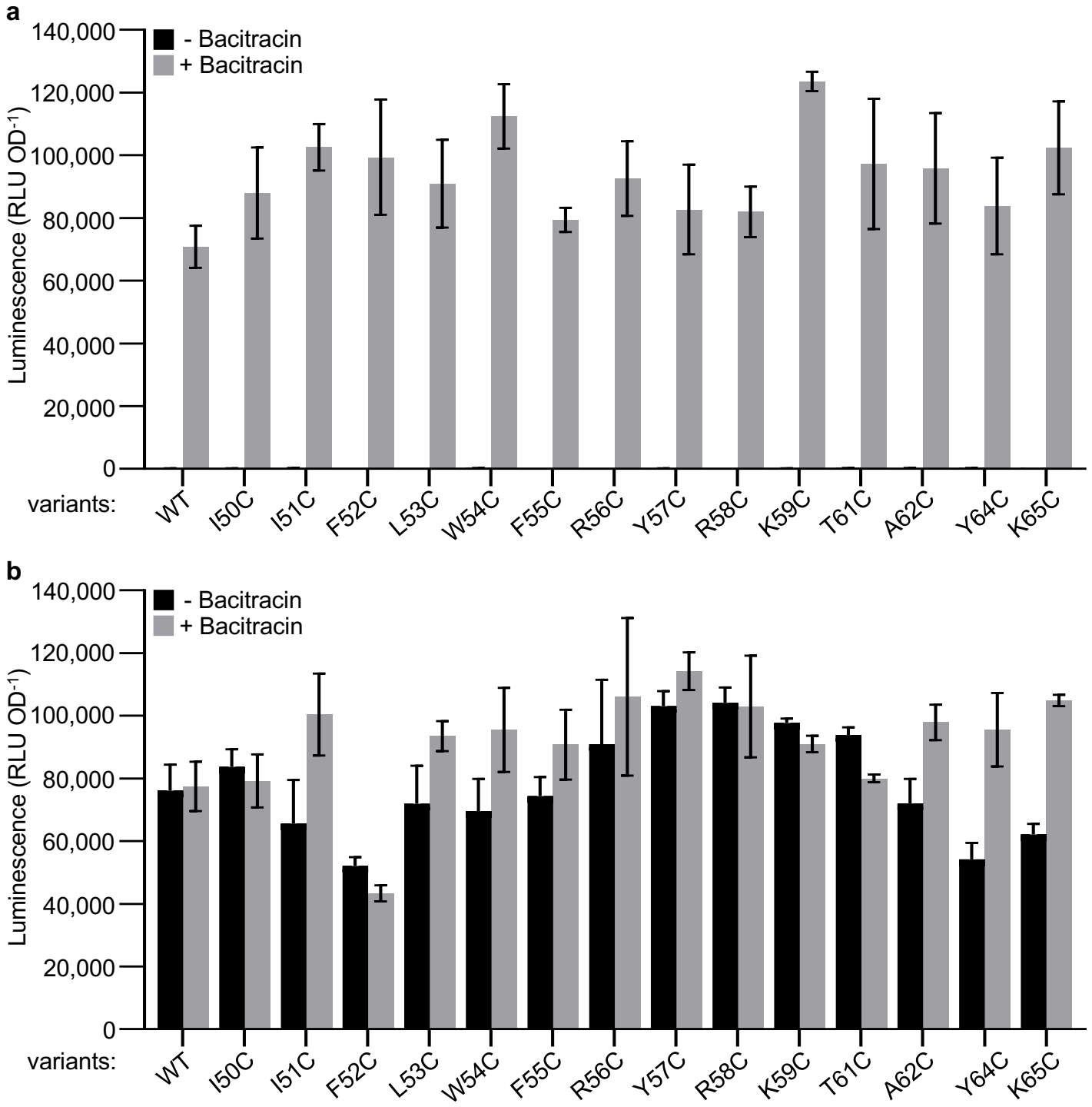

Figure S5. Cysteine scanning mutagenesis of the second transmembrane helix of BceS. BceS signaling activity was assessed in cells harbouring the P_{bceA}-*luxABCDE* reporter and P_{xyl}-*bceS*-His_8. Cells were challenged with 1 µg ml^{-1} of bacitracin (grey) or left unchallenged (black), and signalling 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 ± SD from 3 independent repeats.

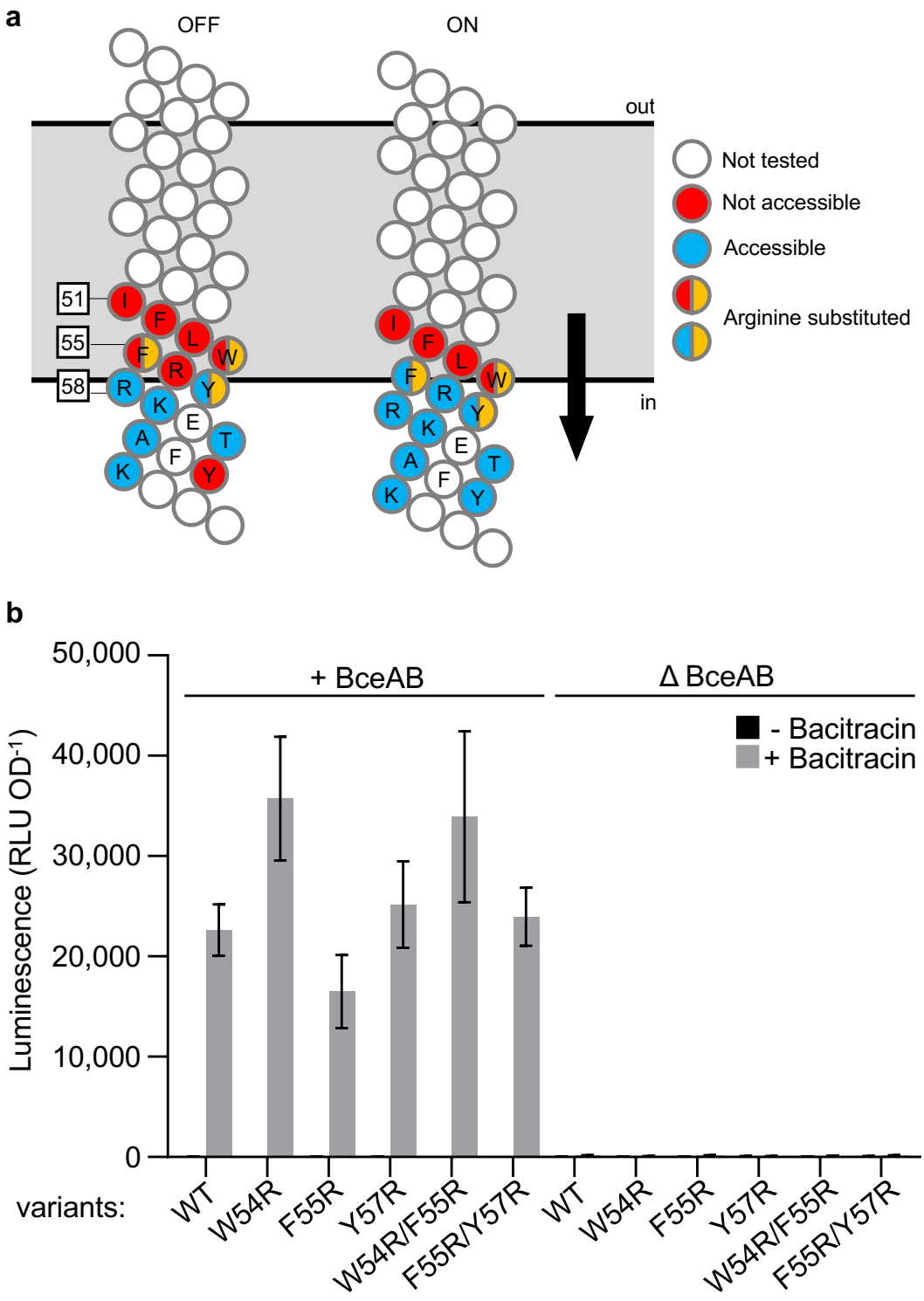

Figure S6. Arginine substitutions in transmembrane helix 2 of BceS. a, Schematic representation of Arg substitutions within transmembrane helix 2, shown in the OFF (left) and ON (right) states. Solvent-accessible Cys residues are indicated in blue, inaccessible Cys residues in red. The introduced Arg substitution are shown as yellow half-circles. **b,** Signalling activity of BceS Arg variants. Amino acid substitutions were introduced into the parent construct P_{xyl}-*bceS*-His_8, in a strain background carrying a Δ*bceS* deletion (+BceAB; SGB792), or a Δ*bceSAB* deletion (ΔBceAB; SGB818). Cells were challenged with 10 µg 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 ± SD from 7-12 independent repeats.

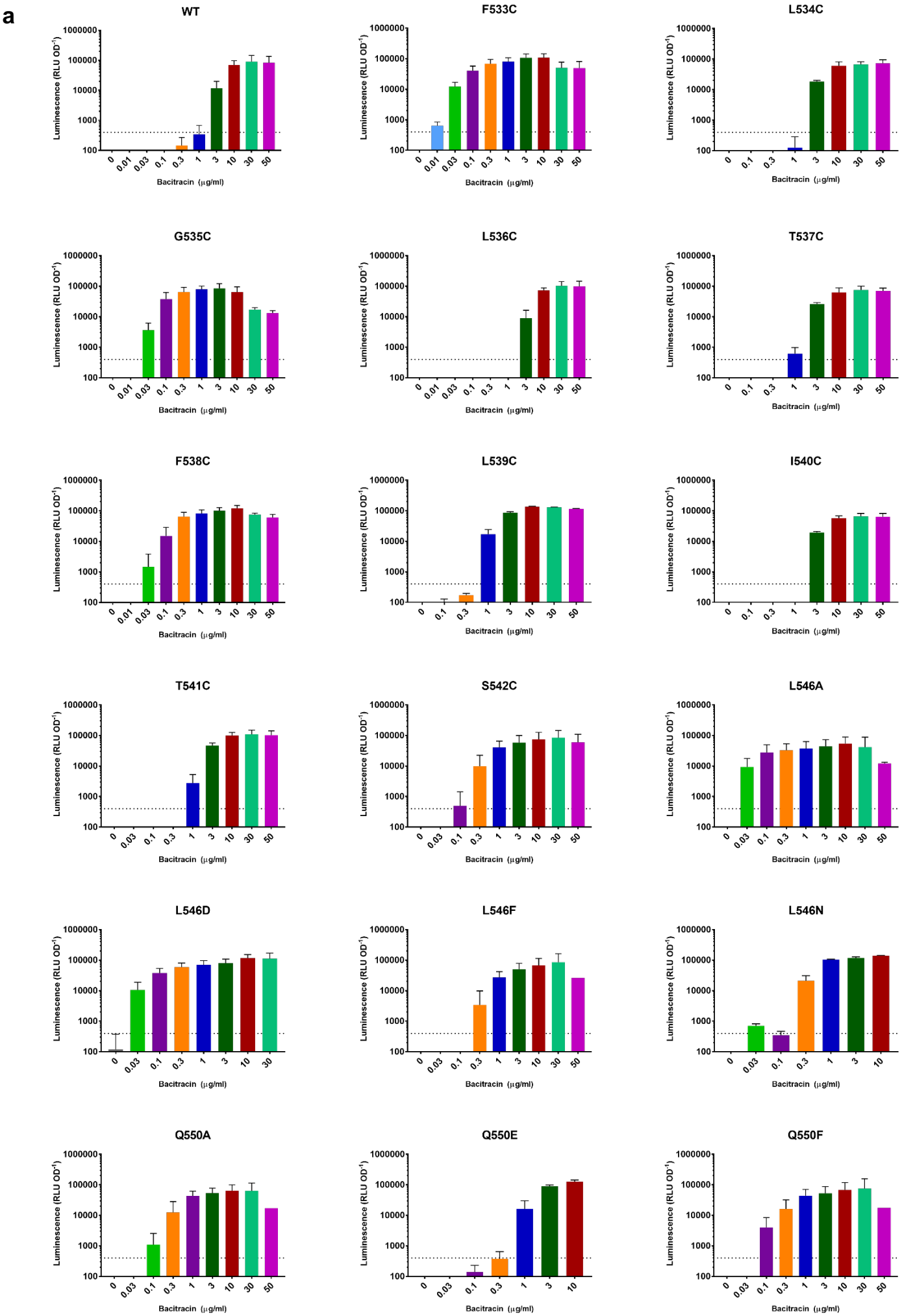

Figure S7. Signalling phenotypes of BceAB variants. *complete legend after panel c.*

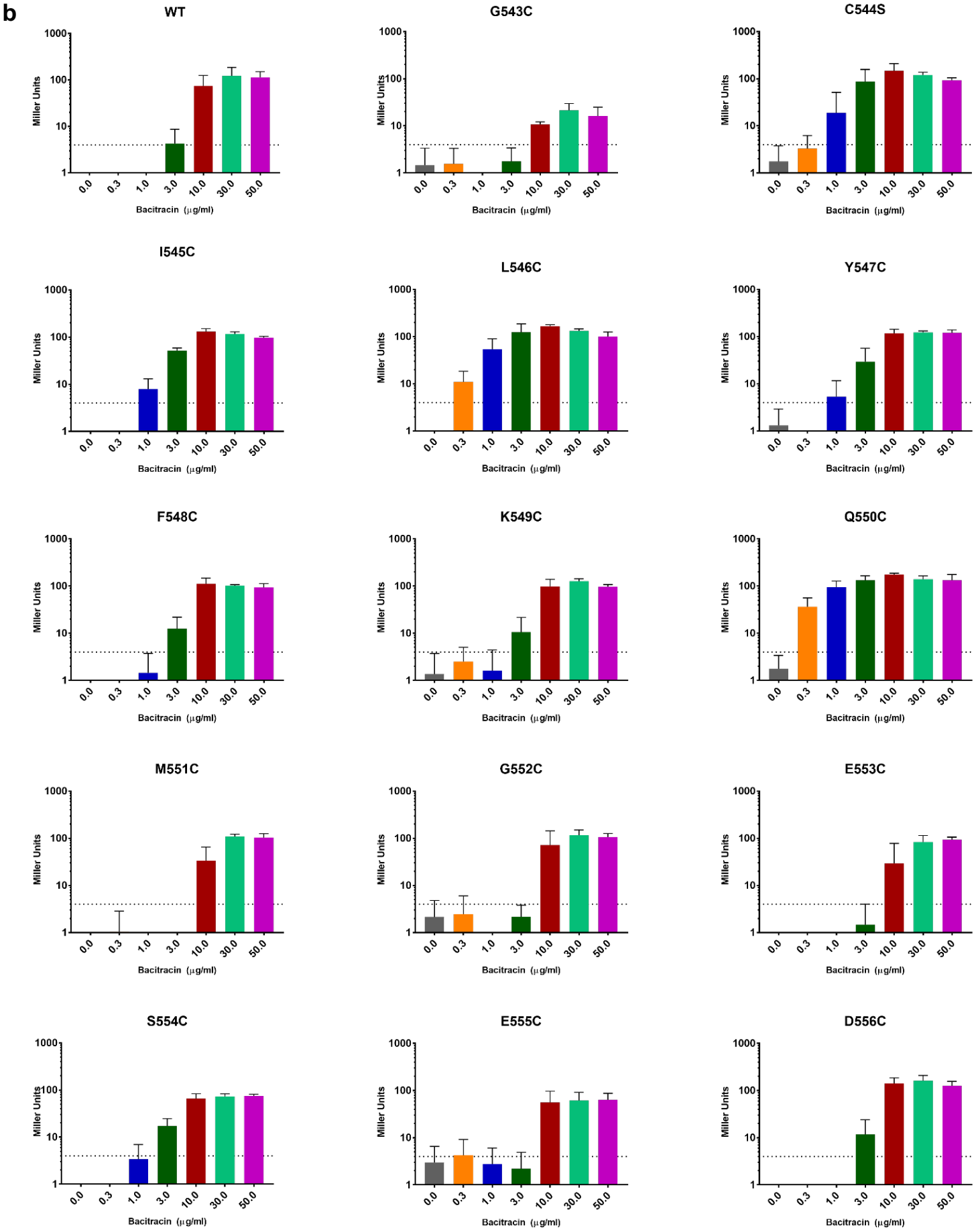

Figure S7. Signalling phenotypes of BceAB variants. *complete legend after panel c.*

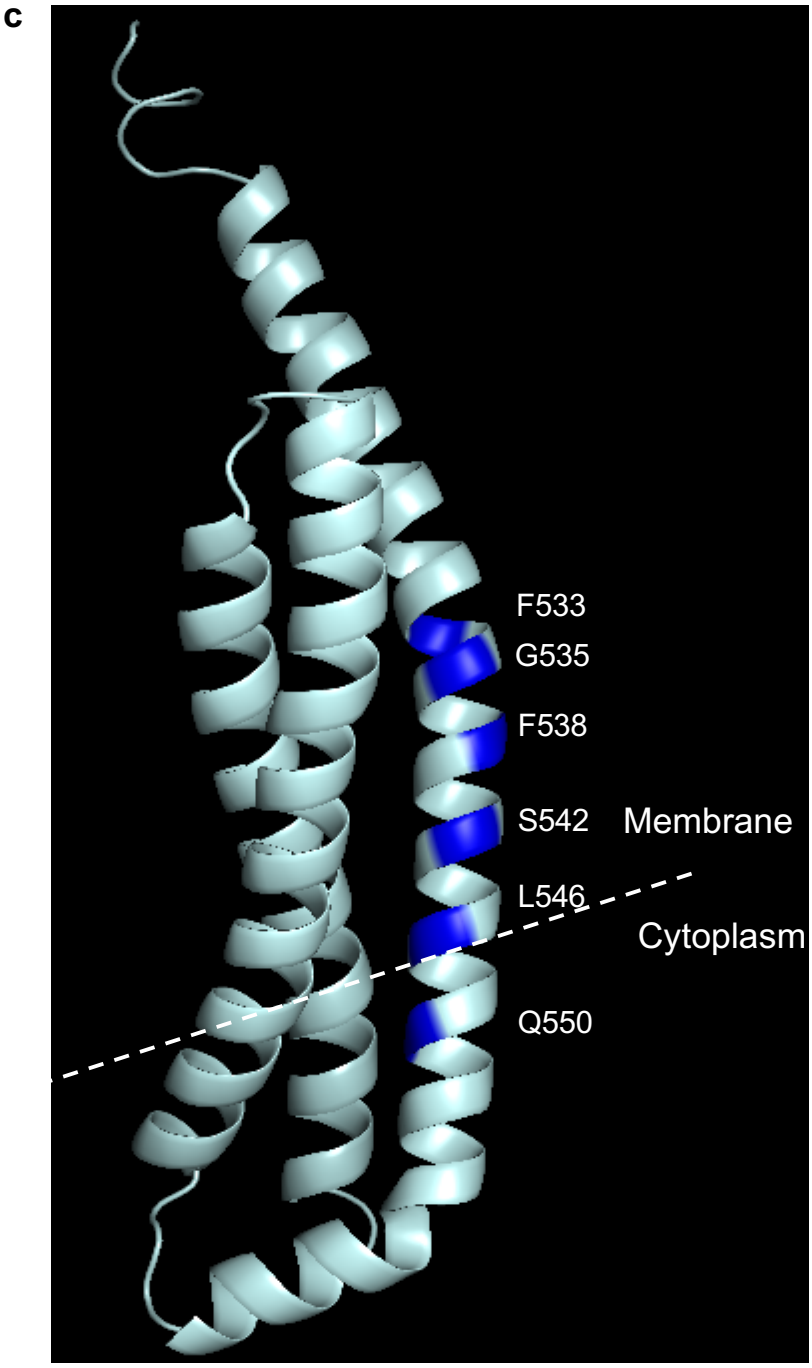

Figure S7. Signalling phenotypes of BceAB variants. Amino acid substitutions were introduced into the P_{xyl}-*bceAB*-$FLAG_3$ parent construct, pFK727. Cells were challenged with a series of bacitracin concentrations as indicated. **a,** Signalling was assessed by luminescence (RLU OD^{-1}) in cells carrying the P_{bceA}-*luxABCDE* reporter (SGB731 and derivatives). **b,** Signalling was assessed by b-galactosidase activity (Miller Units) in cells carrying the P_{bceA}-*lacZ* reporter (SGB370 and derivatives). Data are shown as mean ± 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. **c,** Homology model of the N-terminal three transmembrane helices of BceB (from amino acid 507, C-score -0.66) generated using I-TASSER; the top structural analog was MacB of *Acinetobacter baumanii* (PDB 5WS4). Positions where amino acid substitutions cause increased signalling sensitivity are highlighted in blue. The predicted membrane-cytoplasm interface is indicated by a white dashed line.

Table S1. Strains used in this study

Strain	Genotype	Parent strain[a]	Plasmid/ Strain used[b]	Source
SGB16	*W168 bceAB::kan amyE::bceA-lacZ*	TMB035	pER603	this work
SGB79	*W168 bceAB::kan sacA::PbceA-luxABCDE*			(Kallenberg *et al.*, 2013)
SGB176	*W168 bceAB::kan thrC::Pxyl-bceAB-FLAG*			(Kallenberg *et al.*, 2013)
SGB276	*W168 ΔbceS sacA::PbceA-luxABCDE lacA::Pxyl-bceS-His8*			(Dintner *et al.*, 2014)
SGB334	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{E553C}*-FLAG*	SGB16	pMG707	this work
SGB337	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{L546C}*-FLAG*	SGB16	pAM703	this work
SGB338	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{D556C}*-FLAG*	SGB16	pJL705	this work
SGB340	*W168 sacA::PbceA-luxABCDE*	W168	SGB276	this work
SGB341	*W168 sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG*	SGB340	SGB176	this work
SGB345	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{Y547C}*-FLAG*	SGB16	pMG704	this work
SGB352	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{K549C}*-FLAG*	SGB16	pMG713	this work
SGB357	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG*	SGB341	pAK102	this work
SGB359	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{E115K}*-His8*	SGB357	pAK2E02	this work
SGB369	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{WT}*-His8*	SGB357	pSD2E01	this work
SGB370	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB-FLAG*	SGB16	pFK727	this work
SGB371	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{G543C}*-FLAG*	SGB16	pMG718	this work
SGB372	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{I545C}*-FLAG*	SGB16	pMG719	this work
SGB373	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{F548C}*-FLAG*	SGB16	pMG720	this work
SGB374	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{Q550C}*-FLAG*	SGB16	pMG721	this work
SGB375	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{M551C}*-FLAG*	SGB16	pMG722	this work
SGB376	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{G552C}*-FLAG*	SGB16	pMG723	this work
SGB377	*W168 ΔbceSAB*	W168	pAK102	this work
SGB378	*W168 ΔbceSAB sacA::PbceA-luxABCDE*	SGB340	pAK102	this work
SGB379	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{C544S}*-FLAG*	SGB16	pMG724	this work
SGB380	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{E115A}*-His8*	SGB357	pAK2E12	this work
SGB381	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{E115R}*-His8*	SGB357	pAK2E13	this work
SGB382	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{E115C}*-His8*	SGB357	pAK2E14	this work

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

SGB431	*W168 bceAB::kan amyE::PbceA-lacZ thrC:: pXT-BceAB*^{E555C}*-FLAG*	SGB16	pMG739	this work
SGB433	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{K116E}*-His8*	SGB357	pAK2E26	this work
SGB436	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{K167D}*-His8*	SGB357	pAK2E29	this work
SGB440	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{E118C}*-His8*	SGB357	pAK2E33	this work
SGB441	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E}$*-His8*	SGB357	pAK2E34	this work
SGB444	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{I94C}*-His8*	SGB357	pAK2E37	this work
SGB445	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{A95C}*-His8*	SGB357	pAK2E38	this work
SGB446	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{G96C}*-His8*	SGB357	pAK2E39	this work
SGB447	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{Q97C}*-His8*	SGB357	pAK2E40	this work
SGB448	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{T98C}*-His8*	SGB357	pAK2E41	this work
SGB449	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{E99C}*-His8*	SGB357	pAK2E42	this work
SGB450	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{H100C}*-His8*	SGB357	pAK2E43	this work
SGB451	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{L101C}*-His8*	SGB357	pAK2E44	this work
SGB452	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{K102C}*-His8*	SGB357	pAK2E45	this work
SGB453	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{Q103C}*-His8*	SGB357	pAK2E46	this work
SGB454	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{E87C}*-His8*	SGB357	pAK2E47	this work
SGB455	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{A88C}*-His8*	SGB357	pAK2E48	this work
SGB456	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{M89C}*-His8*	SGB357	pAK2E49	this work
SGB457	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{V90C}*-His8*	SGB357	pAK2E50	this work
SGB458	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{E91C}*-His8*	SGB357	pAK2E51	this work
SGB459	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{R92C}*-His8*	SGB357	pAK2E52	this work
SGB460	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{S93C}*-His8*	SGB357	pAK2E53	this work
SGB465	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{L67C}*-His8*	SGB357	pAK2E58	this work
SGB470	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{D117C}*-His8*	SGB357	pAK2E63	this work
SGB473	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{K167D/K116E}$*-His8*	SGB357	pAK2E66	this work
SGB513	*W168 ΔbceSAB amyE::PbceA-lacZ thrC::Pxyl-bceAB*^{WT}*-FLAG lacA::Pxyl-bceS*^{E115K}*-His8*	SGB400	pAK2E02	this work
SGB532	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K167D}$*-His8*	SGB357	pAK2E118	this work
SGB562	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{L119C}*-His8*	SGB357	pAK2E134	this work
SGB565	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*^{M120C}*-His8*	SGB357	pAK2E137	this work

SGB693	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{L67F}-His8*	SGB357	pAK2E154	this work
SGB696	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{L67G}-His8*	SGB357	pAK2E157	this work
SGB707	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{I94G}-His8*	SGB357	pAK2E168	this work
SGB709	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS^{I94F}-His8*	SGB357	pAK2E170	this work
SGB718	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{T541C}-FLAG*	SGB79	pMG759	this work
SGB719	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{S542C}-FLAG*	SGB79	pMG760	this work
SGB721	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{L546A}-FLAG*	SGB79	pMG762	this work
SGB722	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{L546F}-FLAG*	SGB79	pMG763	this work
SGB723	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{Q550A}-FLAG*	SGB79	pMG764	this work
SGB724	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{Q550F}-FLAG*	SGB79	pMG765	this work
SGB731	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB-FLAG*	SGB79	pFK727	this work
SGB732	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{F538C}-FLAG*	SGB79	pMG712	this work
SGB765	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{L546N}-FLAG*	SGB79	pMG770	this work
SGB766	*W168 bceAB::kan sacA::PbceA-luxABCDE thrC::pXT-BceAB^{Q550E}-FLAG*	SGB79	pMG771	this work
SGB771	*W168 ΔbceS sacA::PbceA-luxABCDE*	TMB1036	SGB378	this work
SGB790	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS$^{E115K/K116E/E118C}$-His8*	SGB357	pAK2E176	this work
SGB791	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS$^{E115K/K116E/L119C}$-His8*	SGB357	pAK2E177	this work
SGB792	*W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{WT}*	SGB771	pAKXT03	this work
SGB795	*W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{E115K}*	SGB771	pAKXT06	this work
SGB807	*W168 bceAB::kan sacA::PbceA-lux thrC::pXT-BceAB^{L546D}-FLAG*	SGB79	pMG772	this work
SGB818	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{WT}*	SGB378	pAKXT03	this work
SGB820	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{E115K}*	SGB378	pAKXT06	this work
SGB868	*W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS$^{E115K/K116E}$*	SGB771	pAKXT13	this work
SGB869	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS$^{E115K/K116E}$*	SGB378	pAKXT13	this work
SGB886	*W168 ΔbceSAB sacA::PbceA-luxABCDE lacA::Pxyl-bceS^{L67F}-His8*	SGB378	pAK2E154	this work
SGB895	*W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{W54R}*	SGB771	pAKXT15	this work
SGB896	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{W54R}*	SGB378	pAKXT15	this work
SGB897	*W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{F55R}*	SGB771	pAKXT22	this work
SGB898	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{F55R}*	SGB378	pAKXT22	this work
SGB899	*W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-BceS^{Y57R}*	SGB771	pAKXT20	this work
SGB900	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS^{Y57R}*	SGB378	pAKXT20	this work
SGB901	*W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-BceS$^{W54R/F55R}$*	SGB771	pAKXT16	this work
SGB902	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS$^{W55R/F55R}$*	SGB378	pAKXT16	this work
SGB903	*W168 ΔbceS sacA::PbceA-luxABCDE thrC::Pxyl-BceS$^{F55R/Y57R}$*	SGB771	pAKXT21	this work

SGB904	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceS*$^{F55R/Y57R}$	SGB378	pAKXT21	this work
SGB936	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S}$*-His8*	SGB357	pAK2E185	this work
SGB937	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/I50C}$*-His8*	SGB367	pAK2E186	this work
SGB938	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/I51C}$*-His8*	SGB357	pAK2E187	this work
SGB939	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/F52C}$*-His8*	SGB357	pAK2E188	this work
SGB940	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/L53C}$*-His8*	SGB357	pAK2E189	this work
SGB941	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/W54C}$*-His8*	SGB357	pAK2E190	this work
SGB942	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/F55C}$*-His8*	SGB357	pAK2E191	this work
SGB943	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/R56C}$*-His8*	SGB357	pAK2E192	this work
SGB944	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/Y57C}$*-His8*	SGB357	pAK2E193	this work
SGB945	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/R58C}$*-His8*	SGB357	pAK2E194	this work
SGB946	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/K59C}$*-His8*	SGB357	pAK2E195	this work
SGB947	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/T61C}$*-His8*	SGB357	pAK2E196	this work
SGB948	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/A62C}$*-His8*	SGB357	pAK2E197	this work
SGB949	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/Y64C}$*-His8*	SGB357	pAK2E198	this work
SGB950	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{C45S/C198S/C259S/K65C}$*-His8*	SGB357	pAK2E199	this work
SGB951	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S}$*-His8*	SGB357	pAK2E202	this work
SGB952	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/I50C}$*-His8*	SGB357	pAK2E203	this work
SGB953	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/I51C}$*-His8*	SGB357	pAK2E204	this work
SGB954	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/F52C}$*-His8*	SGB357	pAK2E205	this work
SGB955	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/L53C}$*-His8*	SGB357	pAK2E206	this work
SGB956	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/W54C}$*-His8*	SGB357	pAK2E207	this work
SGB957	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/F55C}$*-His8*	SGB357	pAK2E208	this work
SGB958	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/R56C}$*-His8*	SGB357	pAK2E209	this work
SGB959	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/Y57C}$*-His8*	SGB357	pAK2E210	this work
SGB960	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/R58C}$*-His8*	SGB357	pAK2E211	this work
SGB961	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/K59C}$*-His8*	SGB357	pAK2E212	this work
SGB962	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/T61C}$*-His8*	SGB357	pAK2E213	this work
SGB963	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/A62C}$*-His8*	SGB357	pAK2E214	this work
SGB964	*W168 ΔbceSAB sacA::PbceA-luxABCDE thrC::Pxyl-bceAB-FLAG lacA::Pxyl-bceS*$^{E115K/K116E/C45S/C198S/C259S/Y64C}$*-His8*	SGB357	pAK2E215	this work

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

References

Dintner, S., Heermann, R., Fang, C., Jung, K., and Gebhard, S. (2014) A sensory complex consisting of an ATP-binding cassette transporter and a two-component regulatory system controls bacitracin resistance in *Bacillus subtilis*. *J Biol Chem* **289**: 27899–27910.

Kallenberg, F., Dintner, S., Schmitz, R., and Gebhard, S. (2013) Identification of regions important for resistance and signalling within the antimicrobial peptide transporter BceAB of *Bacillus subtilis*. *J Bacteriol* **195**: 3287–3297.

Rietkötter, E., Hoyer, D., and Mascher, T. (2008) Bacitracin sensing in *Bacillus subtilis*. *Mol Microbiol* **68**: 768–785.

Table S2. Primers used in this study

Primer	Sequence (5'→3')
SG0068	CCTTTATTTTTGTCAAATGGGTGAAAGTGAAGATGAAAAACCGAG
SG0069	CACCCATTTGACAAAAATAAAGGATACAACCTGATGTAATCAGG
SG0109	TGAAAGTGAATGTGAAAAACCGAGCTATACAATTTTAAGAAAACTCGG
SG0110	TCGGTTTTTCACATTCACTTTCACCCATTTGTTTAAAATAAAGGATAC
SG0117	AGGTTGTATCTGTTATTTTAAACAAATGGGTGAAAGTGAAGATGAAAAACC
SG0118	GTTTAAAATAACAGATACAACCTGATGTAATCAGGAACGTTAACCC
SG0119	ACAAATGGGTTGTAGTGAAGATGAAAAACCGAGCTATACAATTTTAAG
SG0120	CATCTTCACTACAACCCATTTGTTTAAAATAAAGGATACAACCTGATG
SG0127	CATCGCTTAGCATTGGAAAATAAAAAAGATG
SG0128	CCATGCCATCAGCTCATCTTTTTTATTTTC
SG0175	GTATCCTTTGTTTTAAACAAATGGGTGAAAGTGAAGATGAAAAACCGAGC
SG0176	CATTTGTTTAAAACAAAGGATACAACCTGATGTAATCAGGAACGTTAACCC
SG0179	AATTTAATTTCCATGGCTTGTCAACCTGGCCGTTCCGATG
SG0180	AATTTAATTTGAATTCCTGCTGAAACAAAAACGCGGCAAT
SG0181	AATTTAATTTGAATTCCTGTCAGTTCTTTATTATAAGAAG
SG0182	AATTTAATTTGGATCCGAAATGGTATTAGCATTCTTAGGT
SG0197	GGGTTAACGTGCCTGATTACATCAGGTTGTATCCTTTATTTTAAAC
SG0198	GTAATCAGGCACGTTAACCCTAGAAGCCGACGATG
SG0215	CGGCATCGCTTAGCATTGGAAAATGCTAAAGATGAG
SG0216	GATCCATGCCATCAGCTCATCTTTAGCATTTTCC
SG0217	CGGCATCGCTTAGCATTGGAAAATCGTAAAGATGAG
SG0218	GATCCATGCCATCAGCTCATCTTTACGATTTTCC
SG0219	GGCATCGCTTAGCATTGGAATGCGAAAAAG
SG0220	GCCATCAGCTCATCTTTTTCGCATTCCAATG
SG0221	CATCGCTTAGCATTGGAAAATTGCAAAGATG
SG0222	CCATGCCATCAGCTCATCTTTGCAATTTTC
SG0223	GCTTAGCATTGGAAAATGAATGCGATGAG
SG0224	GATCCATGCCATCAGCTCATCGCATTCAT
SG0225	CTTCTTGATCAGCAGCTTCATTGCAAACG
SG0226	GTTTTCAATAAATGAAATGCGTTTGCAATGAAG
SG0227	CTTGATCAGCAGCTTCATCAATGCCGCATTTC
SG0228	GTCGTTTTCAATAAATGAAATGCGGCATTGATG
SG0229	GATCAGCAGCTTCATCAAAAATGCATTTC
SG0230	GGTCGTTTTCAATAAATGAAATGCATTTTTG
SG0231	CATCATGTTGTATCCTTTATTTTAAACAAATGGGTG
SG0232	GATACAACATGATGTAATCAGGAACGTTAACC
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	CATATTCTGTTGGTTCCGCTATCGGAAAGAAAC
SG0248	CGGAACCAACAGAATATGATAAAAAACAAGATGCAC
SG0249	GTGCATCTTGTTTTTTATCATATTCCTTTGCTTCCGC
SG0250	GCTGTTTCTTTCCGATAGCGGAAGCAAAGG
SG0251	TTTGGTGCCGCTATCGGAAAGAAACAGCGTTTTATAAAAGC
SG0252	CCGATAGCGGCACCAAAGGAATATGATAAAAAACAAGATGC
SG0253	TGGTTCTGCTATCGGAAAGAAACAGC
SG0254	CCGATAGCAGAACCAAAGGAATATGATAAA
SG0255	CGCTGTCGGAAAGAAACAGCGTTTTATAAAAGC
SG0256	CTTTCCGACAGCGGAACCAAAGGAATATGATAAA
SG0257	CCGCTATTGTAAAGAAACAGCGTTTTATAAAAGC
SG0258	GTTTCTTTACAATAGCGGAACCAAAGGAATATG
SG0259	TATCGGTGCGAAACAGCGTTTTATAAAAGCTTGAAAACATGG
SG0260	CGCTGTTTCACAGCGATAGCAGAACCAAAGG
SG0261	GGAAATGCACAGCGTTTTATAAAAGCTTGAAAACATGGG
SG0262	GCTGTGCATTTCCGATAGCGGAACCAAAGG
SG0263	CGGAAAGAATGCGCGTTTTATAAAAGCTTGAAAACATGG
SG0264	AACGCGCATTCTTTGCGATAGCAGAACCAAAGG
SG0265	GAAACATGCTTTTATAAAAGCTTGAAAACATGGGAGAAC
SG0266	TAAAAGCATGTTTCTTTCCGATAGCGGAACCAAAG
SG0267	GCTTAGCATTGGAAAATGAAGAAGATGAG
SG0268	GATCCATGCCATCAGCTCATCTTCTTCAT
SG0275	CTTGATCAGCAGCTTCATCAAGACCGCATTTC
SG0276	GTCGTTTTCAATAAATGAAATGCGGTCTTGATG
SG0283	CTTAGCATTGGAAAATGAAAAATGCGAGCTG
SG0284	GGATCCATGCCATCAGCTCGCATTTTTC
SG0285	GCATTGGAAAATGAAAAAGATTGCCTGATG
SG0286	CTCATGGATCCATGCCATCAGGCAATCTTT
SG0289	CGCTTAGCATTGGAAAATAAAGAAGATGAG
SG0290	GATCCATGCCATCAGCTCATCTTCTTTATTTTC
SG0295	GAAGCAATGGTTGAAAGAAGCTGCGCCGGG
SG0296	CAAGTGTTCTGTTTGCCCGGCGCAGCTTC
SG0297	GCAATGGTTGAAAGAAGCATTTGCGGGCAA
SG0298	CTTCAAGTGTTCTGTTTGCCCGCAAATGC
SG0299	CAATGGTTGAAAGAAGCATTGCCTGCCAAAC
SG0300	CTGCTTCAAGTGTTCTGTTTGGCAGGCAATG

SG0301	GTTGAAAGAAGCATTGCCGGGTGCACAGAAC
SG0302	GTCTGCTTCAAGTGTTCTGTGCACCCGGC
SG0303	GAAAGAAGCATTGCCGGGCAATGCGAACAC
SG0304	GCGGTCTGCTTCAAGTGTTCGCATTGCCC
SG0305	GAAGCATTGCCGGGCAAACATGCCACTTG
SG0306	GCTGCGGTCTGCTTCAAGTGGCATGTTTG
SG0307	GCATTGCCGGGCAAACAGAATGCTTGAAG
SG0308	CGTGCTGCGGTCTGCTTCAAGCATTCTG
SG0309	CATTGCCGGGCAAACAGAACACTGCAAGCAG
SG0310	GCCGTGCTGCGGTCTGCTTGCAGTGTTC
SG0311	CCGGGCAAACAGAACACTTGTGCCAGACC
SG0312	GATGCCGTGCTGCGGTCTGGCACAAGTG
SG0313	GGGCAAACAGAACACTTGAAGTGCACCGC
SG0314	TAAGCGATGCCGTGCTGCGGTGCACTTCAA
SG0315	GAACCGGAAACGCCGTTTTGCGCAATG
SG0316	CAATGCTTCTTTCAACCATTGCGCAAAACGG
SG0317	GAACCGGAAACGCCGTTTGAATGCATGGTTG
SG0318	CGGCAATGCTTCTTTCAACCATGCATTCAAAC
SG0319	CCGGAAACGCCGTTTGAAGCATGCGTTGAA
SG0320	CCCGGCAATGCTTCTTTCAACGCATGCTTC
SG0321	GAAACGCCGTTTGAAGCAATGTGCGAAAG
SG0322	GCCCGGCAATGCTTCTTTCGCACATTGC
SG0323	ACGCCGTTTGAAGCAATGGTTTGCAGAAGC
SG0324	CTGTTTGCCCGGCAATGCTTCTGCAAACCAT
SG0325	CCGTTTGAAGCAATGGTTGAATGCAGCATTG
SG0326	GTTCTGTTTGCCCGGCAATGCTGCATTCAAC
SG0327	GTTTGAAGCAATGGTTGAAAGATGCATTGCCG
SG0328	GTGTTCTGTTTGCCCGGCAATGCATCTTTC
SG0329	CGCTATCGGAAAGAAACAGCGTGCTATAAAAG
SG0330	CTCCCATGTTTTCAAGCTTTTATAGCACGCTGT
SG0331	GCTATCGGAAAGAAACAGCGTTTTGCAAAAGC
SG0332	CTCCCATGTTTTCAAGCTTTTGCAAAACGC
SG0333	CGGAAAGAAACAGCGTTTTATTGCAGCTTG
SG0334	GTTCTCCCATGTTTTCAAGCTGCAATAAAAC
SG0335	TCGGAAAGAAACAGCGTTTTATAAATGCTTGAAAAC
SG0336	GAGATTGTTCTCCCATGTTTTCAAGCATTTATA
SG0337	GAAACAGCGTTTTATAAAAGCTGCAAAACATG
SG0338	GAGATTGTTCTCCCATGTTTTGCAGCTTTTA
SG0339	GAAACAGCGTTTTATAAAAGCTTGTGCACATGG
SG0340	CATCGAGATTGTTCTCCCATGTGCACAAGCT
SG0341	GCGTTTTATAAAAGCTTGAAATGCTGGGAGA
SG0342	CACATCGAGATTGTTCTCCCAGCATTTCAAG
SG0343	GCGTTTTATAAAAGCTTGAAAACATGCGAGAAC
SG0344	GTCACATCGAGATTGTTCTCGCATGTTTTC

SG0356	GGTGAATGTGAAGATGAAAAACCGAGCTATAC
SG0357	CATCTTCACATTCACCCATTTGTTTAAAATAAAGGATAC
SG0358	GAAAGTTGCGATGAAAAACCGAGCTATACAATTTTAAG
SG0359	TTCATCGCAACTTTCACCCATTTGTTTAAAATAAAGGATAC
SG0400	CAGCGTGTTATAAAAGCTTGAAAACATGGGAGAACAATCTCG
SG0401	CTTTTATAACACGCTGTTTCTTTCCGATAGCGGAACCAAAG
SG0402	GCGTTTTGTAAAAGCTTGAAAACATGGGAGAACAATCTCGATGTGAC
SG0403	GCTTTTACAAAACGCTGTTTCTTTCCGATAGCGGAACCAAAGG
SG0404	GTTTTATTGTAGCTTGAAAACATGGGAGAACAATCTCGATGTGACAGC
SG0405	CAAGCTACAATAAAACGCTGTTTCTTTCCGATAGCGGAACC
SG0480	TGAGTGCATGGCATGGATCCATGAGGTC
SG0481	GCCATGCACTCATCTTTTTCATTTTCC
SG0486	GCTGTGCGCATGGATCCATGAGGTCAAA
SG0487	ATGCGCACAGCTCATCTTTTTCATTTTC
SG0534	AAGCTTCAAAACATGGGAGAACAATCTCGATG
SG0535	GTTTTGAAGCTTTTATAAAACGCTGTTTCTTTC
SG0542	AAGCGGTAAAACATGGGAGAACAATCTCGATG
SG0543	GTTTTACCGCTTTTATAAAACGCTGTTTCTTTC
SG0579	GAAGCGGTGCCGGGCAAACAGAACACTTGAAG
SG0580	CCGGCACCGCTTCTTTCAACCATTGCTTCAAAC
SG0583	GAAGCTTCGCCGGGCAAACAGAACACTTGAAG
SG0584	CCGGCGAAGCTTCTTTCAACCATTGCTTCAAAC
SG0614	GTATCGCTTATTTTAAACAAATGGGTG
SG0615	AATAAGCGATACAACCTGATGTAATCAG
SG0616	GTATCTTTTATTTTAAACAAATGGGTG
SG0617	AATAAAAGATACAACCTGATGTAATCAG
SG0618	TTGTATCCTTTATTTTAAAGCAATGGGTG
SG0619	CATCTTCACTTTCACCCATTGCTTTAA
SG0620	TTGTATCCTTTATTTTAAATTTATGGGTG
SG0621	CATCTTCACTTTCACCCATAAATTTAA
SG0622	GATTACATGCGGTTGTATCCTTTATTTTAA
SG0623	CAACCGCATGTAATCAGGAACGTTA
SG0624	CTGATTTGTTCAGGTTGTATCCTTTA
SG0625	ACCTGAACAAATCAGGAACGTTAAC
SG0659	GAAAATAAAGAAGATTGCCTGATGGCATGGATCCATGAG
SG0660	CATCAGGCAATCTTCTTTATTTTCCAATGCTAAGCGATG
SG0661	GAAAATAAAGAAGATGAGTGCATGGCATGGATCCATGAGGTC
SG0662	TGCCATGCACTCATCTTCTTTATTTTCCAATGCTAAGCGATG
SG0671	AGTCGGTCTCGGATCCTACATCGCGAAGGAAGAGG
SG0672	AGTCGGTCTCGAATTCGGGCTTTTCCTTCGATACGG
SG0675	GTATCAATTATTTTAAACAAATGGGTG
SG0676	AATAATTGATACAACCTGATGTAATCAG
SG0677	TTGTATCCTTTATTTTAAAGAAATGGGTG
SG0678	CATCTTCACTTTCACCCATTTCTTTAA

SG0715	GTATCGATTATTTTAAACAAATGGGTG
SG0716	AATAATCGATACAACCTGATGTAATCAG
SG0727	TTTTTGCATATTCCTTTGGTTCCGCTAT
SG0729	TATCTGCTTCCTTTGGTTCCGCTATCGG
SG0731	CATATGCCTTTGGTTCCGCTATCGGAAA
SG0733	ATTCTGCTGGTTCCGCTATCGGAAAGAA
SG0734	ACCAGCAGAATATGATAAAAAACAAGAT
SG0735	CCTTTGCTTCCGCTATCGGAAAGAAACA
SG0736	GGAAGCAAAGGAATATGATAAAAAACAA
SG0737	TTGGTGCCGCTATCGGAAAGAAACAGCG
SG0738	AGCGGCACCAAAGGAATATGATAAAAAA
SG0739	GTTCTGCTATCGGAAAGAAACAGCGTTT
SG0740	GATAGCAGAACCAAAGGAATATGATAAA
SG0741	CCGCTGCCGGAAAGAAACAGCGTTTTAT
SG0742	TCCGGCAGCGGAACCAAAGGAATATGAT
SG0743	CTATTGCAAAGAAACAGCGTTTTATAAA
SG0744	CTTTGCAATAGCGGAACCAAAGGAATAT
SG0745	TCGGTGCGAAACAGCGTTTTATAAAAGC
SG0746	TTTCGCACCGATAGCGGAACCAAAGGAA
SG0779	GTGGTCTATCCAAAAAGGGATCGGTTTT
SG0780	GGATAGACCACGATTGTAAATCTTTGAT
SG0781	AGATTCTGGCAGGGGCATTGATCCAAAA
SG0782	TGCCAGAATCTTTCACTTGGAGCTGCGT
SG0783	ATATGCAAAAAAACAAGATAGACAAATA
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	TCCGCCGTCGGAAAGAAACAGCGTTTTAT
SG0898	TCCGACGGCGGAACCAAAGGAATATGAT
SG899	TTGGCGTCGCCGTCGGAAAGAAACAGCGTTTTAT
SG0900	TCCGACGGCGACGCCAAAGGAATATGATAAAAAAC
SG0932	CGTCGGCTGCTTAGGGTTAACGTTCCTG
SG0933	CCCTAAGCAGCCGACGATGAACATCACC
SG0934	CGGCTTCTGTGGGTTAACGTTCCTGATTAC
SG0935	GTTAACCCACAGAAGCCGACGATGAACATCACC

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

Table S3. Plasmids used in this study.

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

pAK2E66	*bla lacA*::(P_{xyl}-*bceS*$^{K167D/K116E}$-*His8*) *mls*	pAK2E26	SG0245/SG0276 & SG0246/SG0275	this work
pAK2E118	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K167D}$-*His8*) *mls*	pAK2E29	SG0245/SG0128 & SG0246/SG0127	this work
pAK2E134	*bla lacA*::(P_{xyl}-*bceS*^{D117C}-*His8*) *mls*	pSD2E01	SG0480/SG0481(QC)	this work
pAK2E137	*bla lacA*::(P_{xyl}-*bceS*^{M120C}-*His8*) *mls*	pSD2E01	SG0486/SG0487 (QC)	this work
pAK2E154	*bla lacA*::(P_{xyl}-*bceS*^{L67F}-*His8*) *mls*	pSD2E01	SG0534/SG0535 (QC)	this work
pAK2E157	*bla lacA*::(P_{xyl}-*bceS*^{L67G}-*His8*) *mls*	pSD2E01	SG0542/SG0543 (QC)	this work
pAK2E168	*bla lacA*::(P_{xyl}-*bceS*^{I94G}-*His8*) *mls*	pSD2E01	SG0579/SG0580 (QC)	this work
pAK2E170	*bla lacA*::(P_{xyl}-*bceS*^{I94F}-*His8*) *mls*	pSD2E01	SG0583/SG0584 (QC)	this work
pAK2E176	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/E118C}$-*His8*) *mls*	pSD2E01	SG0659/SG0660 (QC)	this work
pAK2E177	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/L119C}$-*His8*) *mls*	pSD2E01	SG0661/SG0662 (QC)	this work
pAK2E183	*bla lacA*::(P_{xyl}-*bceS*^{C45S}-*His8*) *mls*	pSD2E01	SG0245/SG0829 & SG0246/SG0828	this work
pAK2E184	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S}$-*His8*) *mls*	pAK2E183	SG0245/SG0780 & SG0246/SG0779	this work
pAK2E185	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S}$-*His8*) *mls*	pAK2E184	SG0245/SG0782 & SG0246/SG0781	this work
pAK2E186	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/I50C}$-*His8*) *mls*	pAK2E185	SG0245/SG0783 & SG0246/SG0727	this work
pAK2E187	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/I51C}$-*His8*) *mls*	pAK2E185	SG0245/SG0784 & SG0246/SG0729	this work
pAK2E188	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/F52C}$-*His8*) *mls*	pAK2E185	SG0245/SG0785 & SG0246/SG0731	this work
pAK2E189	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/L53C}$-*His8*) *mls*	pAK2E185	SG0245/SG0734 & SG0246/SG0733	this work
pAK2E190	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/W54C}$-*His8*) *mls*	pAK2E185	SG0245/SG0736 & SG0246/SG0735	this work
pAK2E191	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/F55C}$-*His8*) *mls*	pAK2E185	SG0245/SG0738 & SG0246/SG0737	this work
pAK2E192	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/R56C}$-*His8*) *mls*	pAK2E185	SG0245/SG0740 & SG0246/SG0739	this work
pAK2E193	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/Y57C}$-*His8*) *mls*	pAK2E185	SG0245/SG0742 & SG0246/SG0741	this work
pAK2E194	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/R58C}$-*His8*) *mls*	pAK2E185	SG0245/SG0744 & SG0246/SG0743	this work
pAK2E195	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/K59C}$-*His8*) *mls*	pAK2E185	SG0245/SG0746 & SG0246/SG0745	this work
pAK2E196	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/T61C}$-*His8*) *mls*	pAK2E185	SG0245/SG0264 & SG0246/SG0263	this work
pAK2E197	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/A62C}$-*His8*) *mls*	pAK2E185	SG0245/SG0266 & SG0246/SG0265	this work
pAK2E198	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/Y64C}$-*His8*) *mls*	pAK2E185	SG0245/SG0403 & SG0246/SG0402	this work
pAK2E199	*bla lacA*::(P_{xyl}-*bceS*$^{C45S/C198S/C259S/K65C}$-*His8*) *mls*	pAK2E185	SG0245/SG0405 & SG0246/SG0404	this work
pAK2E200	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S}$-*His8*) *mls*	pAK2E34	SG0245/SG0829 & SG0246/SG0828	this work
pAK2E201	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S}$-*His8*) *mls*	pAK2E200	SG0245/SG0780 & SG0246/SG0779	this work
pAK2E202	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S}$-*His8*) *mls*	pAK2E201	SG0245/SG0782 & SG0246/SG0781	this work
pAK2E203	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/I50C}$-*His8*) *mls*	pAK2E202	SG0245/SG0783 & SG0246/SG727	this work
pAK2E204	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/I51C}$-*His8*) *mls*	pAK2E202	SG0245/SG0784 & SG0246/SG0729	this work
pAK2E205	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/F52C}$-*His8*) *mls*	pAK2E202	SG0245/SG0785 & SG0246/SG0731	this work

pAK2E206	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/L53C}$-*His8*) *mls*	pAK2E202	SG0245/SG0734 & SG0246/SG0733	this work
pAK2E207	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/W54C}$-*His8*) *mls*	pAK2E202	SG0245/SG0736 & SG0246/SG0735	this work
pAK2E208	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/F55C}$-*His8*) *mls*	pAK2E202	SG0245/SG0738 & SG0246/SG0737	this work
pAK2E209	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/R56C}$-*His8*) *mls*	pAK2E202	SG0245/SG0740 & SG0246/SG0739	this work
pAK2E210	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/Y57C}$-*His8*) *mls*	pAK2E202	SG0245/SG0742 & SG0246/SG0741	this work
pAK2E211	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/R58C}$-*His8*) *mls*	pAK2E202	SG0245/SG0744 & SG0246/SG0743	this work
pAK2E212	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/K59C}$-*His8*) *mls*	pAK2E202	SG0245/SG0746 & SG0246/SG0745	this work
pAK2E213	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/T61C}$-*His8*) *mls*	pAK2E202	SG0245/SG0264 & SG0246/SG0263	this work
pAK2E214	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/A62C}$-*His8*) *mls*	pAK2E202	SG0245/SG0266 & SG0246/SG0265	this work
pAK2E215	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/Y64C}$-*His8*) *mls*	pAK2E202	SG0245/SG0403 & SG0246/SG0402	this work
pAK2E216	*bla lacA*::(P_{xyl}-*bceS*$^{E115K/K116E/C45S/C198S/C259S/K65C}$-*His8*) *mls*	pAK2E202	SG0245/SG0405 & SG0246/SG0404	this work
pAKXT03	*bla thrC*::(P_{xyl}-*bceS*^{WT}) *spec*	pXT	SG0671/SG0672 (pAS719)	this work
pAKXT06	*bla thrC*::(P_{xyl}-*bceS*^{E115K}) *spec*	pXT	SG0671/SG0672 (PCF705)	this work
pAKXT13	*bla thrC*::(P_{xyl}-*bceS*$^{E115K/K116E}$) *spec*	pXT	SG0671/SG0807 & SG0672/SG0806 (pAS719)	this work
pAKXT15	*bla thrC*::(P_{xyl}-*bceS*^{W54R}) *spec*	pAKXT03	SG0671/SG0813 & SG0672/SG0812 (pAKXT03)	this work
pAKXT16	*bla thrC*::(P_{xyl}-*bceS*$^{W54R/F55R}$) *spec*	pAKXT03	SG0671/SG0817 & SG0672/SG0816 (pAKXT03)	this work
pAKXT20	*bla thrC*::(P_{xyl}-*bceS*^{Y57R}) *spec*	pAKXT03	SG0671/SG0898 & SG0672/SG0897 (pAKXT03)	this work
pAKXT21	*bla thrC*::(P_{xyl}-*bceS*$^{F55R/Y57R}$) *spec*	pAKXT03	SG0671/SG0900 & SG0672/SG0899 (pAKXT03)	this work
pAKXT22	*bla thrC*::(P_{xyl}-*bceS*^{F55R}) *spec*	pAKXT03	SG0671/SG0815 & SG0672/SG0814 (pAKXT03)	this work
pAM703	*bla thrC*::(P_{xyL}bceAB^{L546C}-Flag$_3$) *spec*	pFK727	SG0117/SG0118 (QC)	this work
pAS719	*bla thrC*::(P_{xyl}-*bceS*^{WT}) *spec*	pXT		gift from Mascher lab
pCF705	*bla thrC*::(P_{xyl}-*bceS*^{E115K}) *spec*	pXT		Laboratory stock
pER603	*bla amyE::P$_{bceA}$-lacZ cm*			(Rietkötter *et al.*, 2008)
pFK727	*bla thrC*::(P_{xyL}bceAB^{WT}-Flag$_3$) *spec*	pXT		(Kallenberg *et al.*, 2013)
pJL705	*bla thrC*::(P_{xyL}bceAB^{D556C}-Flag$_3$) *spec*	pFK727	SG0109/SG0110 (QC)	this work

pMAD	*bla bgaB erm*			(Arnaud *et al.*, 2004)
pMG2E01	*bla lacA*::(P_{xyl}-*bceS*^{L53C}-*His8*) *mls*	pBS2E01	SG0247/SG0248 (QC)	this work
pMG2E02	*bla lacA*::(P_{xyl}-*bceS*^{W54C}-*His8*) *mls*	pBS2E01	SG0245/SG0250 & SG0246/SG0249	this work
pMG2E03	*bla lacA*::(P_{xyl}-*bceS*^{F55C}-*His8*) *mls*	pBS2E01	SG0251/ SG0252 (QC)	this work
pMG2E04	*bla lacA*::(P_{xyl}-*bceS*^{R56C}-*His8*) *mls*	pBS2E01	SG0253/ SG0254 (QC)	this work
pMG2E05	*bla lacA*::(P_{xyl}-*bceS*^{Y57C}-*His8*) *mls*	pBS2E01	SG0255/ SG0256 (QC)	this work
pMG2E06	*bla lacA*::(P_{xyl}-*bceS*^{R58C}-*His8*) *mls*	pBS2E01	SG0257/ SG0258 (QC)	this work
pMG2E07	*bla lacA*::(P_{xyl}-*bceS*^{K59C}-*His8*) *mls*	pBS2E01	SG0259/ SG0260 (QC)	this work
pMG2E08	*bla lacA*::(P_{xyl}-*bceS*^{E60C}-*His8*) *mls*	pBS2E01	SG0261/SG0262 (QC)	this work
pMG2E09	*bla lacA*::(P_{xyl}-*bceS*^{T61C}-*His8*) *mls*	pBS2E01	SG0263/SG0264 (QC)	this work
pMG2E10	*bla lacA*::(P_{xyl}-*bceS*^{A62C}-*His8*) *mls*	pBS2E01	SG0265/SG0266 (QC)	this work
pMG2E12	*bla lacA*::(P_{xyl}-*bceS*$^{E60C/E115K}$-*His8*) *mls*	pBS2E01	SG0261/SG0262 (QC)	this work
pMG2E15	*bla lacA*::(P_{xyl}-*bceS*$^{F63C/E115K}$-*His8*) *mls*	pBS2E01	SG0400/SG0401 (QC)	this work
pMG704	*bla thrC*::(P_{xyl}-bceAB^{Y547C}-Flag$_3$) *spec*	pFK727	SG0175/SG0176 (QC)	this work
pMG707	*bla thrC*::(P_{xyl}-bceAB^{E553C}-Flag$_3$) *spec*	pFK727	SG0119/SG0120 (QC)	this work
pMG712	*bla thrC*::(P_{xyl}-bceAB^{F538C}-Flag$_3$) *spec*	pFK727	SG0197/SG0198 (QC)	this work
pMG713	*bla thrC*::(P_{xyl}-bceAB^{K549C}-Flag$_3$) *spec*	pFK727	SG0068/SG0069 (QC)	this work
pMG718	*bla thrC*::(P_{xyl}-bceAB^{G543C}-Flag$_3$) *spec*	pFK727	SG0231/SG0232 (QC)	this work
pMG724	*bla thrC*::(P_{xyl}-bceAB^{C544S}-Flag$_3$) *spec*	pFK727	SG0243/SG0244 (QC)	this work
pMG719	*bla thrC*::(P_{xyl}-bceAB^{I545C}-Flag$_3$) *spec*	pFK727	SG0233/SG0234 (QC)	this work
pMG720	*bla thrC*::(P_{xyl}-bceAB^{F548C}-Flag$_3$) *spec*	pFK727	SG0235/SG0236 (QC)	this work
pMG721	*bla thrC*::(P_{xyl}-bceAB^{Q550C}-Flag$_3$) *spec*	pFK727	SG0237/SG0238 (QC)	this work
pMG722	*bla thrC*::(P_{xyl}-bceAB^{M551C}-Flag$_3$) *spec*	pFK727	SG0239/SG0240 (QC)	this work
pMG723	*bla thrC*::(P_{xyl}-bceAB^{G552C}-Flag$_3$) *spec*	pFK727	SG0241/SG0242 (QC)	this work
pMG738	*bla thrC*::(P_{xyl}-bceAB^{S554C}-Flag$_3$) *spec*	pFK727	SG0356/SG0357 (QC)	this work
pMG739	*bla thrC*::(P_{xyl}-bceAB^{E555C}-Flag$_3$) *spec*	pFK727	SG0358/SG0359 (QC)	this work
pMG759	*bla thrC*::(P_{xyl}-bceAB^{T541C}-Flag$_3$) *spec*	pFK727	SG0624/SG0625 (QC)	this work
pMG760	*bla thrC*::(P_{xyl}-bceAB^{S542C}-Flag$_3$) *spec*	pFK727	SG0622/SG0623 (QC)	this work
pMG762	*bla thrC*::(P_{xyl}-bceAB^{L546A}-Flag$_3$) *spec*	pFK727	SG0614/SG0615 (QC)	this work
pMG763	*bla thrC*::(P_{xyl}-bceAB^{L546F}-Flag$_3$) *spec*	pFK727	SG0616/SG0617 (QC)	this work
pMG764	*bla thrC*::(P_{xyl}-bceAB^{Q550A}-Flag$_3$) *spec*	pFK727	SG0618/SG0619 (QC)	this work
pMG765	*bla thrC*::(P_{xyl}-bceAB^{Q550F}-Flag$_3$) *spec*	pFK727	SG0620/SG0621 (QC)	this work
pMG770	*bla thrC*::(P_{xyl}-bceAB^{L546N}-Flag$_3$) *spec*	pFK727	SG0675/SG0676 (QC)	this work
pMG771	*bla thrC*::(P_{xyl}-bceAB^{Q550E}-Flag$_3$) *spec*	pFK727	SG0677/SG0678 (QC)	this work
pMG772	*bla thrC*::(P_{xyl}-bceAB^{L546D}-Flag$_3$) *spec*	pFK727	SG0715/SG0716 (QC)	this work
pMG775	*bla thrC*::(P_{xyl}-bceAB^{F533C}-Flag$_3$) *spec*	pFK727	SG0932/SG0933 (QC)	this work
pMG776	*bla thrC*::(P_{xyl}-bceAB^{L534C}-Flag$_3$) *spec*	pFK727	SG0934/SG0935 (QC)	this work
pMG777	*bla thrC*::(P_{xyl}-bceAB^{G535C}-Flag$_3$) *spec*	pFK727	SG0936/SG0937 (QC)	this work
pMG778	*bla thrC*::(P_{xyl}-bceAB^{L536C}-Flag$_3$) *spec*	pFK727	SG0938/SG0939 (QC)	this work
pMG779	*bla thrC*::(P_{xyl}-bceAB^{T537C}-Flag$_3$) *spec*	pFK727	SG0940/SG0941 (QC)	this work
pMG780	*bla thrC*::(P_{xyl}-bceAB^{L539C}-Flag$_3$) *spec*	pFK727	SG0942/SG0943 (QC)	this work
pMG781	*bla thrC*::(P_{xyl}-bceAB^{I540C}-Flag$_3$) *spec*	pFK727	SG0944/SG0945 (QC)	this work
pSD2E01	*bla lacA*::(P_{xyl}-*bceS*^{WT}-*His8*) *mls*			(Dintner *et al.*, 2014)

pSDlux101	pAH328-P_{bceA}-*luxABCDE*			(Kallenberg *et al.*, 2013)
pXT	*bla thrC*::(P_{xyl}) *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. *mls*, 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 S4. Minimal inhibitory concentration (MIC) of bacitracin in strains carrying BceAB variants.

Amino acid exchange in BceB	MIC of bacitracin (µg 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 µg ml^{-1}; the MIC of strains lacking *bceAB* was 4 µg ml^{-1}.

Table S5. 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).