# **Supporting Information**

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#### **SI Materials and Methods**

Purification of QseE-His and Reconstitution into Liposomes. A total of 500 mL of NR33 cells was grown in LB medium with the appropriate antibiotic at 37 °C to anf optical density at 600 nM ( $OD_{600}$ ) of 0.3, and induced with 0.5 M IPTG. Cells were grown for 4 more hours at 37 °C, then harvested by centrifugation at  $10,000 \times g$  for 15 min. Inclusion bodies were isolated by resuspending the cells in 10 mL of BPERII Bacterial Protein Extraction Reagent (Pierce) and allowed to shake for 10 min. Cells were centrifuged at 10,000  $\times g$  for 15 min, and the pellet was resuspended in 10 mL of BPERII. Lysozymes to a final concentration of 200  $\mu$ g/mL and 50  $\mu$ L of His-tag protease inhibitor mixture (Sigma) were added, and cells were incubated at room temperature for 10 min. A total of 50 mL of diluted BPERII (1:20) was added to sample, and then cells were centrifuged for 15 min at  $10,000 \times g$ . Cells were washed again with 50 mL of diluted BPERII and spun. Cells were washed a total of 3 times, and each time the supernatant was saved for analysis. The final pellet contained purified inclusion bodies and QseE-His. Liposomes were reconstituted as described previously (1, 2).

**Phosphorylation of QseE-His in Liposomes.** A total of 20  $\mu$ L of the QseE-His-loaded liposomes was adjusted to 10 mM MgCl<sub>2</sub>, 1 mM DTT, and various concentrations of signal molecules. Liposomes were frozen and thawed 3 times in liquid nitrogen, and kept light protected at room temperature for 1 h, allowing the loading of signals and the reformation of the liposomes. [ $\gamma^{32}$ P]dATP (0.5  $\mu$ L; 110 TBq/mmol) was added to each reaction, and tubes were incubated for 5 min. After 5 min, 20  $\mu$ L of SDS-loading dye containing 20% SDS was added to each reaction, samples were subject to SDS/PAGE analysis without boiling, and phosphorylation was visualized by using a Storm PhosphorImager. Bands were quantified by using IMAGE-QUANT version 5.0 software (Amersham Pharmacia).

Identification of Proteins by Mass Spectrometry. Kinase assays were completed as detailed above, but  $\gamma^{32}$ ATP was replaced with cold ATP. Samples were subjected to SDS/PAGE, and gels were stained with Coomassie stain. Bands corresponding to the size of bands from autoradiography detailed above were excised and submitted to the University of Texas Southwestern Protein Chemistry Core Facility. Briefly, in-gel bands were subject to trypsin digestion and reversed-phase, non-high-performance liquid chromatography/ion trap mass spectrometry. Resulting data sets were used to search against the EHEC EDL933 genome.

**RNA Extraction.** Cultures of 8624, NR01, NR02, and NR03 were grown aerobically in LB medium at 37 °C overnight and then were diluted 1:100 in low-glucose red DMEM and allowed to grow at 37° until they reached  $OD_{600}$  of 1.0. RNA was extracted from 3 replicates of each strain by using a RiboPure bacterial RNA isolation kit (Ambion) according to the manufacturer's instructions.

**Real-Time qRT-PCR.** Primers used in qRT-PCR analysis were designed by using Primer Express v 1.5 (Applied Biosystems) and are listed in Table S2. qRT-PCR analysis was conducted by using Applied Biosystems ABI 7500 sequence detection system using a 1-step reaction. Each primer set was checked for amplification efficiency by standard curves resulting from using varying concentrations of RNA template. To ensure template specificity, products were heated to 95 °C for 15 seconds, cooled to 60 °C,

and heated to 95 °C while fluorescence was monitored. To analyze gene expression in NR01, NR02, and NR03 compared with 86–24 *Escherichia coli*, relative quantification analysis was used. Parameters for cDNA generation and amplification were as follows: 1 cycle at 48 °C for 30 min, 1 cycle at 95 °C for 10 min, and 40 cycles at 95° for 15 seconds and 60 °C for 1 min. The RNA polymerase subunit Z, *rpoZ*, was used as an endogenous control. In each reaction of 20  $\mu$ L, 10  $\mu$ L of 2× SYBR master mix, 0.1  $\mu$ L of Multiscribe reverse transcriptase (Applied Biosystems), and 0.1  $\mu$ L of RNase inhibitor (Applied Biosystems) were added.

**Detection, Quantification, and Statistical Analysis.** Applied Biosystems ABI Sequence Detection 1.3 software was used for initial collection of data. Values were normalized to *rpoZ* and analyzed by using the comparative critical threshold ( $C_T$ ) value as previously described (3). Expression is shown in graphs as *n*-fold change in expression level compared with wild-type levels at late exponential growth. Error bars represent the standard deviations of the  $\Delta\Delta C_T$  value. The Student *t* test was performed to assess statistical significance. A *P* value of less than 0.05 was considered significant.

Membrane Preparation and Sucrose Density Gradient Centrifugation. Membrane separation methodology was adapted from previously published methods for isolation of outer membranes from Gram-negative bacteria (4-6). A total of 500 mL of 86-24 and NR05 were grown in LB at 37 °C until they reached  $OD_{600}$  of 0.2. Cells were induced by the addition of 0.2% arabinose and harvested once they reached  $OD_{600}$  of 1.0 by centrifugation at  $10,000 \times g$  for 15 min. Cells were resuspended in 10 mL of 0.75 M sucrose in 5 mM Tris, pH 7.5. While stirring on ice, 40 mL of 10 mM EDTA-tetrasodium in 5 mM Tris, pH 7.5, was added, and cells were incubated on ice while stirring for 30 min. Lysozyme was added to a final concentration of 200  $\mu$ g/mL, and cells were incubated while stirring at room temperature for 30 min. Cells were osmotically lysed by the addition of 4.5 volumes or 240 mL of ice-cold dH<sub>2</sub>O, and they were incubated on ice for 30 min while swirling. Debris was collected by centrifuging cells at  $10,000 \times g$  for 30 min. To collect total membranes, supernatants from the previous spin were divided into 8 tubes and ultracentrifuged for 2 h at  $200,000 \times g$  at 4 °C. Total membrane pellets were resuspended each in 1 mL of resuspension solution [25% sucrose in 5 mM Tris, 30 mM MgCl<sub>2</sub>, 1 EDTA-free MiniComplete (Bio-Rad), 15  $\mu$ L of Benzonase (Invitrogen), and 13  $\mu$ L of His-tag protease inhibitor mixture (Sigma)]. Tubes were incubated, shaking at room temperature for 30 min to degrade DNA. Aliquots were taken, and total protein per membrane prep was determined by using the Bradford assay (Bio-Rad). Linear sucrose gradients were poured in  $14 \times 99$  mm Beckman ultracentrifuge tubes by layering 1.8 mL each of 55%, 50%, 45%, 40%, 35%, and 30% sucrose, and crude membrane preparations were layered on top of the 30% sucrose. Sucrose gradients were ultracentrifuged overnight for 17 h in an SW-40 Beckman rotor at 256,000  $\times$  g at 4 °C. Approximately 20 fractions of 500  $\mu$ L each were collected from each sucrose gradient by puncturing the bottom of the tube with an 18-gauge needle and letting fractions leave the tube by gravity flow. The refractive indexes of each fraction were determined by using a refractometer (Fisher Scientific), and the density of each fraction in grams per milliliter was calculated based on the refractive index (7). Fractions were diluted in SDS-loading dye, subjected to SDS/PAGE, and immunoblotted as described above to detect QseG.

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#### Table S1. Strains and plasmids used in this study

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Strains     Sta2 and EMEC strain (scrotype 0157:H7)     (1)       96-24     96-24 qce nopolar mutant     (2)       NN02     96-24 qce nopolar mutant     (2)       NN03     96-24 qce nopolar mutant     This study       NN04     NN03 complemented with plasmid pNN03     This study       NN05     NN03 complemented with plasmid pNN03     This study       NN26     NN03 complemented with plasmid pNN03     (2)       NN26     NN26     (2)       NN27     pK (271 in NR02     (2)       NN28     pK (271 in NR02     (2)       NN23     pETS in NR02     (2)       NN23     pETS in NR02     (2)       NN23     pETS in NR02     (2)       NN23     pSS in NR02     (2)       NN33     pAPA in RE32 Cells     This study       NN34     pOpa in RE32     (3)       NN35     pOpa in geG <sup>-1</sup> This study       NN34     pOpa in geG <sup>-1</sup> This study       NN43     pOpa in geG <sup>-1</sup> This study       NN44     pDE2CFP in geG <sup>-1</sup> This study	Plasmid or strain	Relevant genotype	Reference or source
66-24     St2 and EHEC strain (serotype 0157:H7)     (1)       NR01     66-24 gef nonpolar mutant     (2)       NR03     86-24 gef nonpolar mutant     (2)       NR04     NR01 complemented with plasmid pNR01     (2)       NR05     NR03 complemented with plasmid pNR02     (2)       NR05     pK187 in NR02     (2)       NR05     pK187 in NR02     (2)       NR30     pNR15 in NR02     (2)       NR33     pNR15 in NR02     (2)       NR34     popa in geG -     (2)       NR33     pN31 in geG -     (2)       NR34     popa in geG -     (3)       NR34     popa in geG -     (4)       NR34     popa in geG -     (4)       NR34     popa in geG -     This study       NR34     popa-tirin geG -     This	Strains		
NNR1     86-24 qee nonpolar mutant     (2)       NNR03     86-24 qee nonpolar mutant     (2)       NNR04     NR01 complemented with plasmid pNR01     (2)       NNR05     NR03 complemented with plasmid pNR03     This study       NNR05     NR03 complemented with plasmid pNR03     (2)       NR26     PK187 in NR02     (2)       NR27     PK471 in NR02     (2)       NR28     PK183 + in NR02     (2)       NR28     PK183 + in NR02     (2)       NR28     PK183 + in NR02     (2)       NR28     PK15 in NR02     (2)       NR30     PK15 in NR02     (2)       NR31     PG15 in NR02     (2)       NR33     PK36 in NR02     (2)       NR34     PG24 in S24     This study       NR35     Pg3 in Ge2 <sup>-1</sup> This study       NR43     PG24 in Ge2 <sup>-1</sup> This study       NR44     PG24FP in S624     This study       NR45     PG24FP in S64     This study       NR46     PDE26FP in ge6 <sup>-1</sup> This study       NR46 <td>86–24</td> <td>Stx2 and EHEC strain (serotype O157:H7)</td> <td>(1)</td>	86–24	Stx2 and EHEC strain (serotype O157:H7)	(1)
NR02     B6-24 gef nonpolar mutant     (2)       NR03     B6-24 gef nonpolar mutant     This study       NR04     NR01 complemented with plasmid pNR01     (2)       NR05     NR03 complemented with plasmid pNR03     This study       NR05     NR03 complemented with plasmid pNR02     (2)       NR05     RR04 gef polar mutant     (2)       NR26     pK187 in NR02     (2)       NR27     pK6471 in NR02     (2)       NR38     pK187 in NR02     (2)       NR30     pK1815 in NR02     (2)       NR31     pK181 in NR02     (2)       NR33     pK31 in B624     This study       NR34     pcya in ge67     (3)       NR35     pcya in ge67     This study       NR36     pcya in ge67     This study       NR41     pcya in ge67     This study       NR43     pcya-tir in ge66'     This study       NR44     pD22CFP in ge67     This study       NR45     pD22CFP in ge67'     This study       NR46     pD22CFP in ge66'     (3)	NR01	86–24 qseE nonpolar mutant	(2)
NR03     86-24 geG nonpolar mutant     This study       NR04     NR04 complemented with plasmid pNR03     This study       NR05     NR03 complemented with plasmid pNR03     This study       NR06     NR04 complemented with plasmid pNR03     (2)       KR17     86-24 geE polar mutant     (2)       NR26     pK187 in NR02     (2)       NR27     pKC471 in NR02     (2)       NR33     pK187 in NR02     (2)       NR34     pK187 in NR02     (2)       NR33     pK187 in NR02     (2)       NR34     pK187 in NR02     (2)       NR35     pK187 in NR02     (2)       NR34     pK187 in NR02     (2)       NR35     pK187 in NR02     (2)       NR36     pcya in geG <sup>-1</sup> This study       NR37     pcya in geG <sup>-1</sup> This study       NR37     pcya in geG <sup>-1</sup> This study       NR48     pcya in geG <sup>-1</sup> This study       NR44     pC2CPP in geG <sup>-1</sup> This study       NR45     pDE2CPP in geG <sup>-1</sup> This study	NR02	86–24 qseF nonpolar mutant	(2)
NR04     NR01 complemented with plasmid pNR03     (2)       NR05     NR02 complemented with plasmid pNR02     (2)       KR17     8-74 get polar mutant     (2)       NR05     pK187 in NR02     (2)       NR26     pK187 in NR02     (2)       NR27     pK471 in NR02     (2)       NR28     pK185 4 in BL21 DE3 cells     (3)       NR30     pK181 in NR02     (2)       NR31     pE16 in NR02     (2)       NR33     pK181 in NR02     (2)       NR33     pK181 in NR02     (2)       NR34     pC41 in R02     (2)       NR33     pK181 in SL21 DE3 cells     This study       NR34     pC3 in GeC <sup>-</sup> This study       NR35     pC3 in GeC <sup>-</sup> This study       NR44     pC3 in GeC <sup>-</sup> This study       NR45     pDE2CFP in GeC <sup>-</sup> This study       NR46     pDE2CFP in GeC <sup>-</sup> This study       NR46     pDE2CFP in GeC <sup>-</sup> This study       NR47     pE348/69 espA mutant     (3)       VD451 <td< td=""><td>NR03</td><td>86–24 qseG nonpolar mutant</td><td>This study</td></td<>	NR03	86–24 qseG nonpolar mutant	This study
ΝR05     NR02 complemented with plasmid pNR03     This study       NR06     NR02 complemented with plasmid pNR02     (2)       KRJ7     86-24 quee polar mutant     (2)       NR26     pK1871 in NR02     (2)       NR27     pKC471 in NR02     (2)       NR28     pK1354 in BL21 DE3 cells     (3)       NR30     pAR15 in NR02     (2)       NR31     pE035 in NR02     (2)       NR33     p635 in NR02     (2)       NR34     poja in 8624     This study       NR35     poja in 6824     This study       NR36     poja in qeeG <sup>-</sup> This study       NR43     poja viti in 6824     This study       NR44     p6267 Pi n 6824     This study       NR45     pDE267P in qeeG <sup>-</sup> This study       NR46     pDE267P in qeeG <sup>-</sup> This study       NR45     pDE267P in qeeG <sup>-</sup> This study       NR46     pE267P in qeeG <sup>-</sup> This study       NR46     pE267P in qeeG <sup>-</sup> This study       NR46     pE267P in qeeG <sup>-</sup> This study <td>NR04</td> <td>NR01 complemented with plasmid pNR01</td> <td>(2)</td>	NR04	NR01 complemented with plasmid pNR01	(2)
NR06     NR02 complemented with plasmid pNR02     ()       KRL7     86-4 qee polar mutant     ()       NR26     pK187 in NR02     ()       NR27     pKC471 in NR02     ()       NR28     pK187 in NR02     ()       NR30     pNR15 in NR02     ()       NR31     pET16 in NR02     ()       NR33     pK107 in BL21 DE3 cells     ()       NR33     pK107 in R02     ()       NR33     pK107 in BL21 DE3 cells     ()       NR34     proj in 6824     This study       NR35     proj an ge6 <sup>-</sup> This study       NR36     proj in ge6 <sup>-</sup> This study       NR41     proj in ge6 <sup>-</sup> This study       NR44     pDE2GFP in ge6 <sup>-</sup> This study       NR45     pDE2GFP in ge6 <sup>-</sup> This study       NR46     pDE2GFP in ge6 <sup>-</sup> This study       NR46     pDE2GFP in ge6 <sup>-</sup> This study       NR47     pE32469 espA mutant     ()       VDM072     E2348/69 espA mutant     ()       PR3551     ()	NR05	NR03 complemented with plasmid pNR03	This study
KRJ7     86-24 qee polar mutant     (2)       NR26     KR187 in NR02     (2)       NR27     pKC471 in NR02     (2)       NR28     pKH354 in BL21 DE3 cells     (3)       NR30     pKH35 in NR02     (2)       NR31     pET16 in NR02     (2)       NR33     pK195 in NR02     (2)       NR34     pQ3 in 8624     This study       NR35     pQ4 in 6624     This study       NR36     pQ3 in qe66 <sup>-</sup> This study       NR47     pQ657 in qe66 <sup>-</sup> This study       NR48     pQ24rLin ge66 <sup>+</sup> This study       NR44     pD22GFP in qe67 <sup>-</sup> This study       NR45     pD22GFP in qe67 <sup>-</sup> This study       NR46     pD22GFP in qe67 <sup>-</sup> This study       NR47     pD22GFP in qe67 <sup>-</sup> This study       NR48     pD22GFP in qe67 <sup>-</sup> This study       NR46     pD22GFP in qe67 <sup>-</sup> This study       NR47     pD22GFP in qe67 <sup>-</sup> This study       NR485     pD22GFP in qe67 <sup>-</sup> This study       NR47 <td>NR06</td> <td>NR02 complemented with plasmid pNR02</td> <td>(2)</td>	NR06	NR02 complemented with plasmid pNR02	(2)
N226     pK187 in N802     (2)       N827     pK4374 in N802     (2)       N828     pKH354 in BL21 DE3 cells     (3)       N830     pNR15 in NR02     (2)       NR33     pR15 in NR02     (2)       NR33     pR33 in BL21 DE3 cells     (3)       NR34     pc35 in NR02     (2)       NR35     pR30 in BL21 DE3 cells     This study       NR36     pcy in qeeG <sup>-</sup> This study       NR37     pcy-itr in 6824     This study       NR38     pcy-itr in qeeG <sup>-</sup> This study       NR41     pcy-air qeeG <sup>-</sup> This study       NR43     pc2CFP in qeeG <sup>-</sup> This study       NR44     pC2CFP in qeeG <sup>-</sup> This study       NR45     pDE2CFP in qeeG <sup>-</sup> This study       NR46     pC2CFP in qeeG <sup>-</sup> (6)       pMR01     qeef in pBadMycHis     (2)       pNR02     qeef in pBadMycHis     (2)       pNR03     qeef in pBadMycHis     (2)       pNR04     qeF1 cac in pR5551     (2)       pNR05     qeefi	KRL7	86–24 gseE polar mutant	(2)
NP27     pKC471 in NP02     (2)       NP28     pKH354 in BL21 DE3 cells     (3)       NR30     pKH15 in NP02     (2)       NR31     pET16 in NP02     (2)       NR33     pKB35 in NR02     (2)       NR33     pKB35 in NR02     (2)       NR34     pcg in B624     This study       NR35     pcg in GeG     This study       NR36     pcg in qseG     This study       NR37     pcg-tri in B624     This study       NR41     pcg in qseG f     This study       NR43     pcg-tri in geG f     This study       NR44     pDE2CFP in geG f     This study       NR45     pDE2CFP in geG f     This study       NR46     pDE2CFP in geG f     This study       NR45     pDE2CFP in qseG f     This study       NR46     pDE2CFP in geG f     This study       NR46     pDE2CFP in qseG f     This study       NR47     pE2GPE in qseG f     This study       NR46     pDE2CFP in qseG f     This study       NR46     pDE2GP	NR26	pK187 in NR02	(2)
NR28     PKH35-4 in BL21 DE3 cells     (2)       NR30     pKH35-4 in BL21 DE3 cells     (2)       NR31     pFT16 in NR02     (2)       NR32     p635 in NR02     (2)       NR33     pR80 in BL21 DE3 cells     This study       NR34     p0ya in ge6-     This study       NR37     p0ya-tri in 8624     This study       NR37     p0ya-tri in ge6-     This study       NR41     p0ya in ge6-     This study       NR43     p0ya-tri in ge6-     This study       NR44     pDE2GFP in ge6-     This study       NR45     pDE2GFP in ge6-     This study       NR46     pDE2GFP in ge6-     This study       VLM872     E2348/69 egpA mutant     (6)       pR551     laC reporter gene fusion vector     (6)       pNR01     qeE in pBadMycHis     This study       pV5254     qeE in pBadMycHis     (7)       pX551     laC reporter gene fusion vector     (6)       pNR03     pAH75 derivative containing FRT-flanked chloramphenicol resistance     (7)       pX524     qeE in	NR27	, pKC471 in NR02	(2)
N830     pRTS in NR02     (2)       NR31     pCTI 6 in NR02     (2)       NR32     pG35 in NR02     (2)       NR33     pNR30 in BL21 DE3 cells     This study       NR34     pCya in 8524     This study       NR36     pCya in 9567     This study       NR36     pCya in 9567     This study       NR37     pCya in 9567     This study       NR39     pCya in 9567     This study       NR41     pCya in 9567     This study       NR43     pCya in 9567     This study       NR44     pDE2GFP in 9567     This study       NR45     pDE2GFP in 9567     This study       NR46     pDE2GFP in 9567     This study       UM0872     E2348/69 espA mutant     (5)       Plasmids     (2)     pNR01     qeE in pBadMycHis     C+terminal Myc-His-tag cloning vector     Invitrogen       pS551     lac2 reporter gene fusion vector     (6)     pNR01     qeE in pBadMycHis     (2)       pNR03     pG4Fi in 924674     RPS551     (2)     pNR02     (2)	NR28	pKH35-4 in BL21 DE3 cells	(3)
NB31     PETIG in NR02     (2)       NR32     pG35 in NR02     (2)       NR33     DNR00 in BL21 DE3 cells     This study       NR34     poya in sec0     This study       NR35     poya in sec0     This study       NR36     poya in sec0     This study       NR37     poya-tri in sec0     This study       NR41     poya in sec0     This study       NR43     poya-tri in sec0     This study       NR44     pDE2GFP in sec0     This study       NR45     DE2GFP in sec0     This study       NR46     DE2GFP in sec0     This study       NR46     DE2GFP in sec0     This study       UMB872     E238469 espA mutant     (6)       Plasmids     (2)     PhandMycHis     (2)       pS551     lac2 reporter gene fusion vector     (6)       pNR01     qseE in pBadMycHis     (2)       pNR03     qseE in pBadMycHis     (2)       pNR04     pdForistion and thermal induction of FLP synthesis.     (7)       pKM33     pAVTS'derivative containing FRT-flanke	NR30	pNR15 in NR02	(2)
NR32pG35 in NR02(2)NR33pQR30 in BL21 DE3 cellsThis studyNR34pCya in geG7This studyNR36pCya in geG7This studyNR37pCya in geG7This studyNR39pCya in geG7This studyNR41pCya in geG7This studyNR43pCya in geG7This studyNR44pDE2GFP in geG7This studyNR45pDE2GFP in geG7This studyNR46pDE2GFP in geG7This studyNR46pDE2GFP in geG7(6)UMD872E2348/69 espA mutant(2)PBadMycHisC-terminal Myc-His-tag cloning vector(6)pNR01qeE in pBadMycHis(2)pNR03pAES51Ia27 reporter gene fusion vector(2)pNR03pAES51(2)pNR03pAES51(2)pNR03pATS7 derivative containing FRT-flanked chloramphenicol resistance(7)pKD3pATS9(2)pNR03pCT21+ queE(3)pKD3pATS9(2)pNR03pET21+ queE(3)pKD3pATS9(2)pNR03pET21+ queE(3)pR551Ia27 reporter gene fusion vector(6)pNR10esfr in pBadMycHis(2)pNR30pET21+ queEThis studypKB35Ia27 reporter gene fusion vector(6)pNR10esfr in pACYC177(2)pNR30pET21+ queEThis studypKH35-4yfhA in pET214(+)(3) <t< td=""><td>NR31</td><td>pET16 in NR02</td><td>(2)</td></t<>	NR31	pET16 in NR02	(2)
NR33pNR30 in BL21 DE3 cellsThis studyNR34pcya in ds624This studyNR36pcya in ds667This studyNR37pcya-tri in gs60°This studyNR38pcya-tri in ds64This studyNR39pcya-tri in gs60°This studyNR41pcya-tri in gs60°This studyNR43pcya-tri in gs60°This studyNR44pDE2GFP in ds624This studyNR45pDE2GFP in ds624This studyNR46pDE2GFP in ds624This studyUMD872E2348/69 espA mutant(4)UMD872E2348/69 espA mutant(5)PlasmidsC-terminal Myc-His-tag cloning vector(6)pNR01qs61 in pBadMycHis(2)pNR02qs61 in pBadMycHis(2)pNR03pAES51(2)pNR04qs61 in pBadMycHis(2)pNR05pARTS derivative containing FRT-flanked chloramphenicol resistance(7)pKD3pARTS derivative containing FRT-flanked chloramphenicol resistance(7)pR551lac2 reporter gene fusion vector(6)pRK01espFu in pR551(2)pAR30pET21 + qs6(3)pR551lac2 reporter gene fusion vector(6)pR410espFu in pR551(2)pR551lac2 reporter gene fusion vector(3)pR551lac2 reporter gene fusion vector(3)pR551lac2 reporter gene fusion vector(2)pR551lac2 reporter gene fusion vector(3)pR	NR32	p635 in NR02	(2)
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NR36pcya in qeG <sup>-</sup> This studyNR37pcya-tri in qsG-This studyNR39pcya-tri in qsG-This studyNR41pcya in qsG-This studyNR43pcya-tri in qsG-This studyNR44pDE2GFP in qsG-This studyNR45pDE2GFP in qsG-This studyNR46pDE2GFP in qsG-This studyNR47gsG-This studyNR46pDE2GFP in qsG-This studyVM47gsG-This studyNR46pDE2GFP in qsG-This studyVM57gsC-trin studyPlasmidsC-terminal Myc-His-tag cloning vector(6)pNR01qsE in pBadMycHis(2)pNR02qsE in pBadMycHis(2)pNR03qsG in pBadMycHis(2)pNR03qsG in pBadMycHis(2)pKD3pATS v derivative containing FRT-flanked chloramphenicol resistance(7)pKD3pATS v derivative containing FRT-flanked chloramphenicol resistance(7)pKD3pATS v derivative containing FRT-flanked chloramphenicol resistance(3)pKD3pAFL1 + qsEThis studypKD3pSFL1 + qsEThis studypKD4qsE in pR351(3)pKD3pSFL1 + qsE(3)pKD3pSFL1 + qsE(3)pKD3pSFL1 + qsE(3)pKD4qsE in pACYC177(2)pC720Commercial blunt-end cloning vectorNew England BlolabspS551lac2 reporter gene fusion vector(2)	NR34	prva in 8624	This study
NR37pcya-tir in 8624This studyNR39pcya-tir in geG <sup>-</sup> This studyNR41pcya in geG <sup>+</sup> This studyNR43pcya-tir in geG <sup>+</sup> This studyNR44pDE2GFP in geG <sup>-</sup> This studyNR45pDE2GFP in geG <sup>+</sup> This studyNR46pDE2GFP in geG <sup>+</sup> This studyNR46pDE2GFP in geG <sup>+</sup> (4)UMB72E2348/69 espA mutant(5)Plasmids(5)Plasmids(6)pNR01geE in pBadMycHis(2)pNS254qseE in pBadMycHis(2)pNS03qseF in pBadMycHis(2)pVS254qseF in pBadMycHis(2)pNR01geG in pBadMycHis(2)pNR03qseF in pBadMycHis(7)pKM201\A red recombinase expression plasmid(7)pKM201A red recombinase expression plasmid(2)pNR03pES551laZ reporter gene fusion vector(6)pNR01gseG in pBadMycHis(7)pKM201\A red recombinase expression plasmid(7)pKM201\A red recombinase expression plasmid(2)pNR10espFu in pR551(2)pNR10geFi in pACYCT77(3)pNR15qseF in pACYCT77(2)pNR30pET21 + qseEThis studypNR15qseF in pACYCT77(9)pVS262espFu in OPO(2)TopoCommercial blurt-end cloning vectorNovagenpT16Expression vectorNovagenpT16 <td>NR36</td> <td><math>pcya in cyce^-</math></td> <td>This study</td>	NR36	$pcya in cyce^-$	This study
NR39pcya-tir in geeG*This studyNR41pcya-tir in geeG*This studyNR41pcya-tir in geeG*This studyNR43pcya-tir in geeG*This studyNR44pDE2GFP in 8624This studyNR45pDE2GFP in geeG*This studyNR46pDE2GFP in geeG*This studyVM872E2348/69 espA mutant(4)UMD872E2348/69 espA mutant(5)PlasmidsC <terminal cloning="" myc-his-tag="" td="" vector<="">(6)pN801qseE in pBadMycHis(2)pVS551laC reporter gene fusion vector(6)pN801qseE in pBadMycHis(2)pVS254qseF in pBadMycHis(2)pN803qseG in pBadMycHis(2)pKM201A red recombinase expression plasmid(8)pCP20TS replication and thermal induction of FLP synthesis.(7)pR5551laCz reporter gene fusion vector(6)pNR01espFu in pR5551(2)pNR03pET21 + qseE(3)pR5554laCz reporter gene fusion vector(6)pR101espFu in pR5551(2)pR5554gseF in pACYC177(2)pR5554gseF in pACYC177(2)pR155qseF in pACYC177(2)pACYC177Cloning vectorNovagenpYS524espfu in TOPO(2)pAR15qseF in pACYC177(9)pF176Expression vectorNovagenpF171Expression vectorNovagenpF171pCyaTir tir</terminal>	NR37	pcya-tir in 8624	This study
INR-1pcya in geG*This studyNR41pcya in geG*This studyNR43pcya-tir in geG*This studyNR44pDE2GFP in ds62This studyNR45pDE2GFP in ds65*This studyNR46pDE2GFP in ds66*This studyVD451B6-24 eck mutant(4)UMD872E2348/69 espA mutant(5)Plasmids-(6)pR551laC reporter gene fusion vector(6)pNR01qseF in pBadMycHis(2)pVS254qseF laZ in pR551(2)pNR02qseF in pBadMycHis(2)pK03qseF in pBadMycHis(2)pK043pATSy derivative containing FRT-flanked chloramphenicol resistance(7)pKN201A red recombinase expression plasmid(8)pCP20TS replication and thermal induction of FLP synthesis.(7)pNR10espFu in pR551(2)pNR30pET1+ qseEThis studypNR30pET21+ qseEThis studypNR31qseF in pACYC177(2)pAR15qseF in ACYC177(2)pAR15qseF in ACYC177(2)pAR15qseF in ACYC177(2)pAR15Expression vectorNovagenpYS262espFu in TOPO(2)TopoCommercial blunt-end cloning vectorNovagenpT16Expression vectorNovagenpCYacyaA of Bordetella pertusis cloned into pACYC177(9)pDE2GFPgfo vector driven by the lac promoterClontech	NB39	$p_{cya}$ the model $r$	This study
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pCya-Tirtir-cyaA fusion cloned into pACYC177(9)pDE2GFPgfp vector driven by the lac promoterClontech	рСуа	cyaA of Bordetella pertussis cloned into pACYC177	(9)
pDE2GFP gfp vector driven by the lac promoter Clontech	pCya-Tir	tir-cyaA tusion cloned into pACYC177	(9)
	pDE2GFP	gtp vector driven by the lac promoter	Clontech

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### Table S2. Oligonucleotides used in this study

PNAS PNAS

Primer name	Sequence	Description	
QseE RTR	CGC GCC ATG ATC TTC GA	Real-time analysis	
QseE RTF	CCC TTC ACC GCC CCT TT	Real-time analysis	
QseF RT R	CGT AAG CTG CTG CAA ATT ACC A	Real-time analysis	
QseF RT F	CGC CCC GCC ATT CTC	Real-time analysis	
YfhG-F-Bad	GGT ACC CAA CGA TTA TTG CCC CGA CG	Construction of pNR03	
YfhG-R-Bad	GAA TTC CCG ACC TCA TGG GTG GAT GGC GCG	Construction of pNR03	
yfhG Red P1	ATC CTC ACG TAC GAC ATG TAC GCT CCG GTT TCT CCG	Construction of the qseG isogenic mutant	
	CGC TGT CCA TGT CCG TGT AGG CTG GAG CTG CTT C		
yfhG Red P2	TCC AGT TGC TGC TGT AGA ACG TGA TGT TGC TGG CGC	Construction of the qseG isogenic mutant	
	AAT GTA TCC AGC TCC ATA TGA ATA TCC TCC TTA		
QsePet21 F-final	CGA ATG ACG CAC GGA TCC GAG CCT GCC GT	Construction of pNR30	
QseERpET21	GGG ATA GGC TGT GCG GCC GCT TTC GTG TTT	Construction of pNR30	
RpoZ RTF	TGC AGG TAG GCG GAA AGG	Real-time analysis	
rpoZRTR	GCG CAG CGC GAT TAC AGT	Real-time analysis	
eae RTF	GCT GGC CCT TGG TTT GAT CA	Real-time analysis	
eae RTR	GCG GAG ATG ACT TCA GCA CTT	Real-time analysis	
espA RTF	TCA GAA TCG CAG CCT GAA AA	Real-time analysis	
espA RTR	CGA AGG ATG AGG TGG TTA AGC T	Real-time analysis	
QseG RTF	TGC GCA GGC ATT AAA CGA T	Real-time analysis	
QseG RTR	CTG CGG GCG ATG GAT TGT GCT	Real-time analysis	
ler RTF	CGACCAGGTCTGCCC	Real-time analysis	
ler RTR	GCGCGGAACTCATC	Real-time analysis	
glnBRTF	CACTGGCTGAAGTCGGCATT	Real-time analysis	
glnBRTR	TGGCGGCCAAAACCTTT	Real-time analysis	