Supporting Information

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A

Suppressor mutations imparting wt curli morphology in UTI89ΔqseC	
Gene locus	**Predicted function**
UTI89_C3450, *qseB*	QseB response regulator
UTI89_C2117-18, *sdiA-yecF*	DNA-binding transcriptional activator with autoinducer-binding domain
UTI89_C4706, *pmrB/basS*	**Sensor kinase-phosphotransferase**
UTI89_C2936, *yfiR*	Hypothetical protein
UTI89_C3255-56, *ygeXW*	Predicted transport system, metabolism
UTI89_C0456, *cyoA*	Cytochrome o ubiquinol oxidase subunit II; involved in aerobic respiration
UTI89_C1999, *fadD*	Enzyme involved in small-molecule/fatty acid degradation; contains AMP-binding site
UTI89_C3590, *deaD*	ATP-dependent RNA helicase with a DEAD-box; involved in cold shock response;

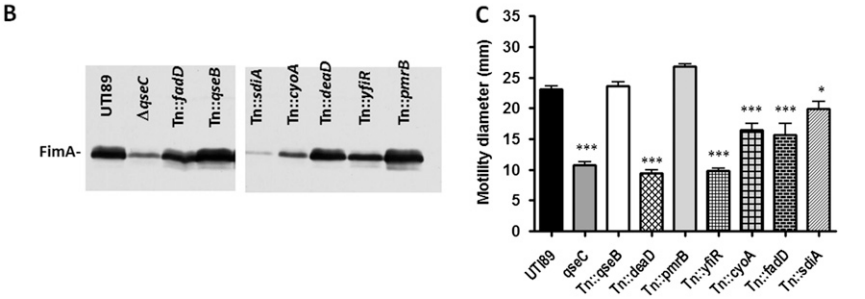

Fig. S1. Random mutagenesis screening identifies suppressors of the *qseC* deletion phenotype. (*A*) Table depicting factors the disruption of which restored wild-type (WT) curli morphology in the *qseC* deletion mutant. Transposon mutagenesis was performed as described in (1). (*B*) Western blot probing for FimA protein abundance in suppressor mutants shown in *A*. A representative of two experiments is shown. (*C*) Bar graph depicting average motility diameters (in mm) of each suppressor mutant compared with WT UTI89 and UTI89Δ*qseC* after 7 h of growth in soft LB-agar (0.25%). Average motility diameters were calculated using data from three independent experiments. Statistics were performed using unpaired, two-tailed Student *t* test. $*P \leq 0.01$, $***P < 0.0001$.

1. Hadjifrangiskou M, et al. (2012) Transposon mutagenesis identifies uropathogenic *Escherichia coli* biofilm factors. *J Bacteriol* 194(22):6195–6205.

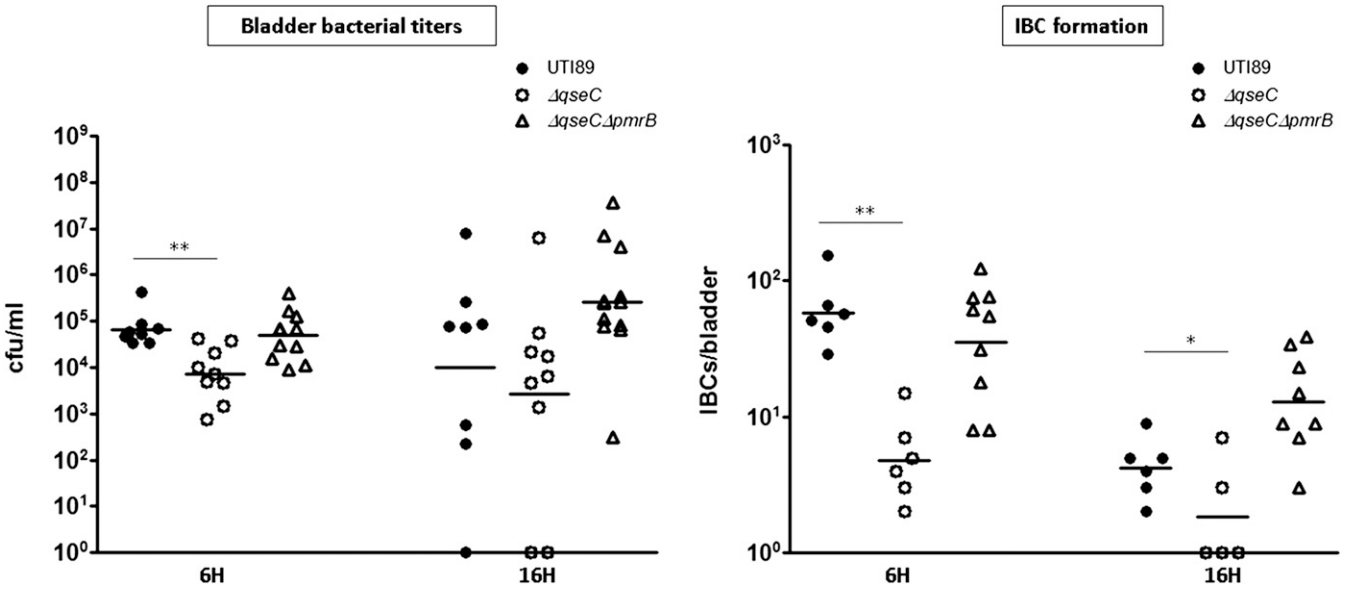

Fig. S2. UTI89Δ*pmrB*Δ*qseC* is restored for virulence during acute urinary tract infection (UTI). Graphs showing bladder titers and IBC numbers recovered at 6 and 16 h post infection. for WT UTI89, UTI89Δ*qseC*, and UTI89Δ*qseC*Δ*pmrB*. $*P \leq 0.027$; $**P \leq 0.0024$ as determined by two-tailed Mann–Whitney. Female C3H/HeN mice (Harlan), 7–9 wk old, were transurethrally infected with 10^7 bacteria carrying the plasmid pANT4 as previously described (1). IBC enumeration was performed using confocal microscopy as described by Kostakioti et al. (1). Experiments were repeated three times.

1. Kostakioti M, Hadjifrangiskou M, Pinkner JS, Hultgren SJ (2009) QseC-mediated dephosphorylation of QseB is required for expression of genes associated with virulence in uropathogenic *Escherichia coli*. *Mol Microbiol* 73(6):1020–1031.

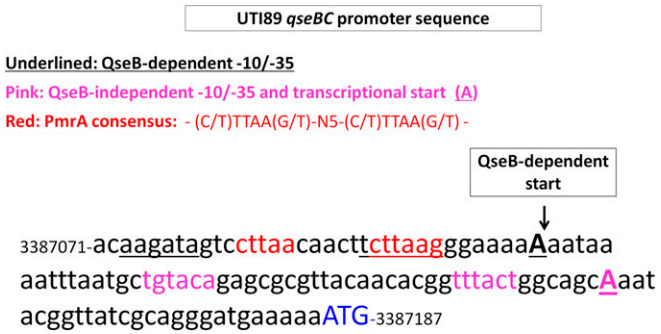

Fig. S3. The *qseBC* promoter region harbors a PmrA binding site. Depicted is the UTI89 promoter sequence (spanning nt 3387071–3387110). The PmrA binding consensus (red) precedes the previously described (1) QseB-dependent start site.

1. Clarke MB, Sperandio V (2005) Transcriptional autoregulation by quorum sensing *Escherichia coli* regulators B and C (QseBC) in enterohaemorrhagic *E. coli* (EHEC). *Mol Microbiol* 58(2): 441–455.

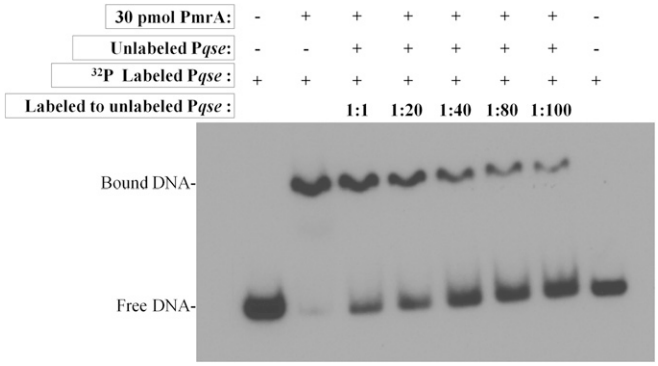

Fig. S4. PmrA regulates *qseBC* expression. Depiction of competition assays using increasing concentrations of unlabeled *qseBC* promoter DNA to validate specificity of interaction. A representative of four independent experiments is shown.

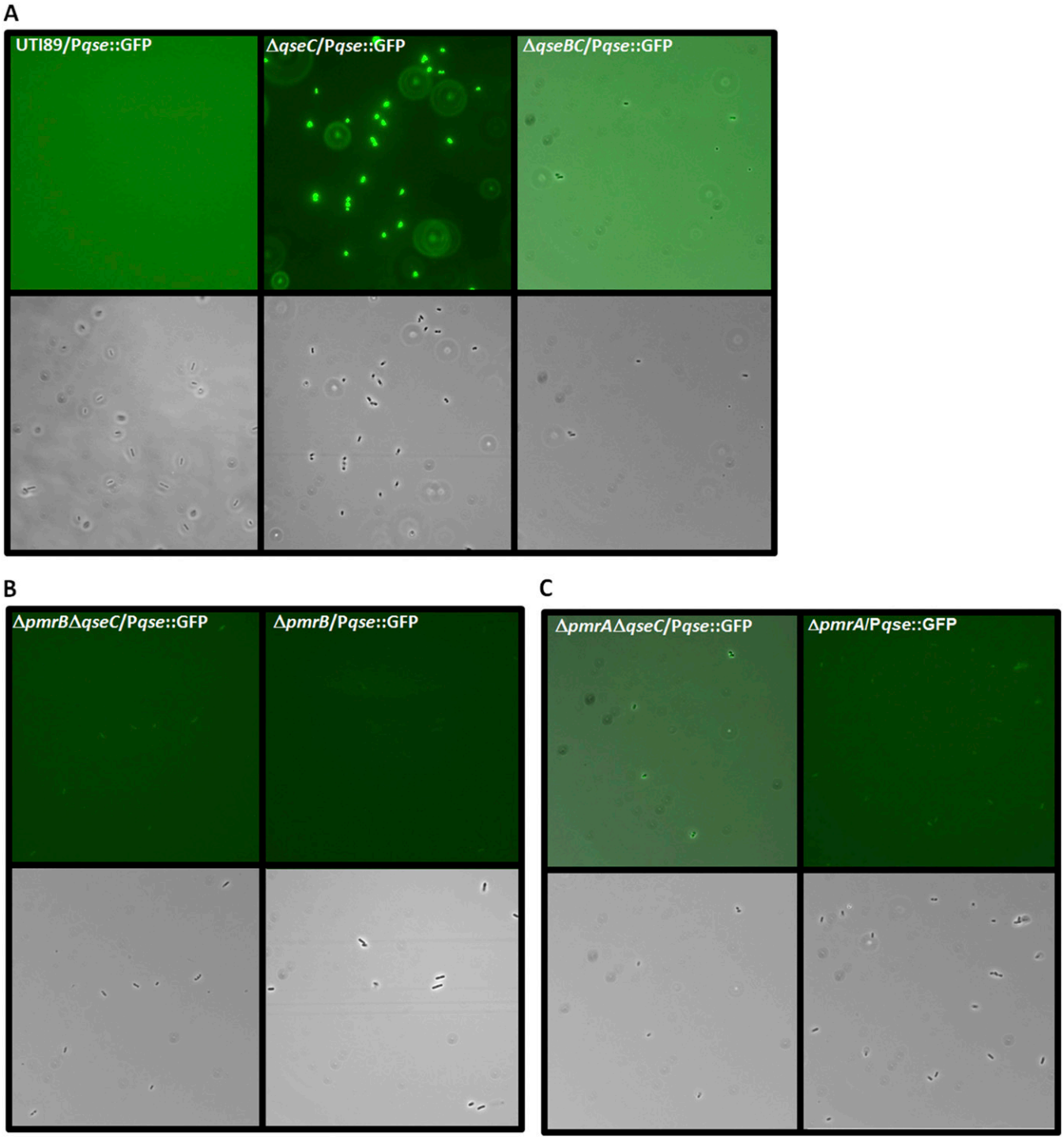

Fig. S5. Q*seBC* up-regulation in the absence of QseC involves both PmrB and PmrA. Fluorescence microscopy tracking *qseBC* promoter-driven GFP expression. (*A*) The *qseBC* promoter is active only in the absence of QseC. Deletion of *pmrB* (*B*) or *pmrA* (*C*) significantly reduces *qseBC* activity in the absence of QseC. Duplicate slides per strain were scanned for fluorescence per experiment. Data shown are representative of three independent experiments. Images shown are representative of the bacterial populations sampled per slide. For fluorescence microscopy, bacteria were grown without shaking, in modified *N*-minimal media (5 mM KCl, 7.5 mM $(NH_4)_2SO_4$, 0.5 mM K_2SO_4, 1 mM KH_2PO_4, 0.1 mM Tris-HCl pH 7.4, 10 mM or 10 mM $MgCl_2$, 0.2% glucose, 0.1% Casamino acids, 38 mM glycerol/L) pH 7.6 and visualized using a Zeiss fluorescence microscope.

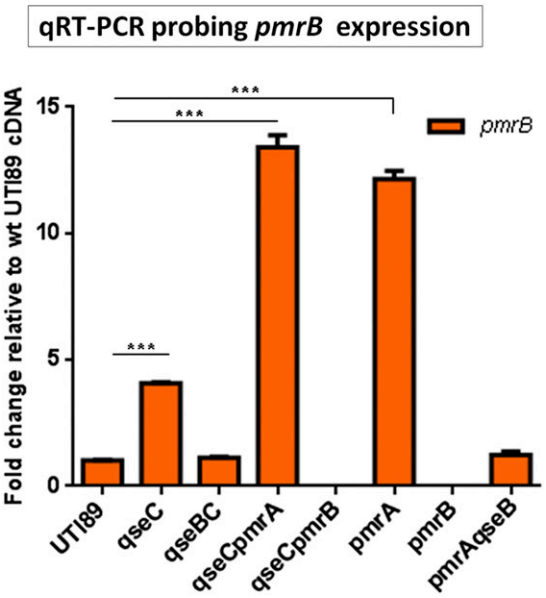

Fig. S6. Expression of *pmrB* is affected by QseB. Relative-fold change of *pmrB* in WT UTI89, UTI89Δ*qseC,* UTI89Δ*qseBC,* UTI89Δ*qseC*Δ*pmrA*, UTI89Δ*qseC*Δ*pmrB*, UTI89Δ*pmrA*, UTI89Δ*pmrB*, and UTI89Δ*qseB*Δ*pmrA* measured by qRT-PCR. Quantitative real-time PCR was executed in triplicate using multiplexed TaqMan minor groove binder chemistry. Abundance of *pmrB* transcripts in UTI89 isogenic mutant strains was calculated using the ΔΔCT method, with each transcript signal normalized to the abundance of the *gyrB* internal control. Error bars for both graphs represent SEM. Statistical analyses were performed by two-tailed unpaired *t* test, where $*P < 0.03$; $**P \leq 0.0053$.

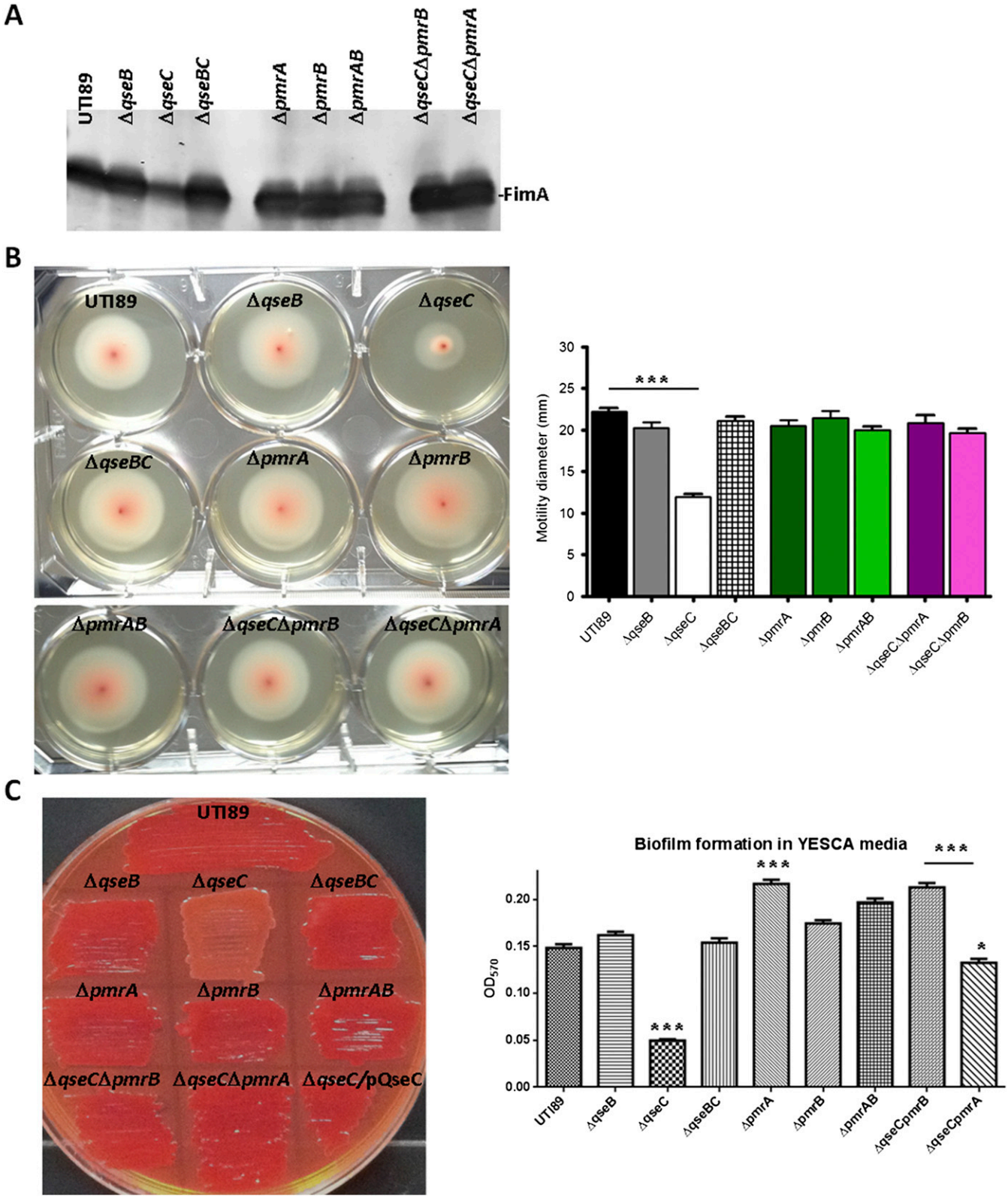

Fig. S7. Deletion of *pmrA* abolishes some of the *qseC* deletion defects. (*A*) FimA Western blot probing total cell lysates from a panel of *qse*/*pmr* mutants. Blot was imaged on an Odyssey infrared imaging system (Li-Cor Biosciences) in the 700-nm channel. (*B*) Motility phenotypes of the *qse*/*pmr* mutants; bacteria were inoculated into 0.25% LB agar/0.001% 2,3,5-triphenyltetrazolium chloride and incubated at 37 °C for 7 h. Experiment was repeated two times with triplicate plates/strain. ***$P \leq 0.0001$, determined by two-tailed unpaired Student *t* test. (*C*) (*Left*) Yeast Extract/Casamino Acid (YESCA) agar plate supplemented with Congo Red (CR) dye serving as a proxy to curli fiber expression. Curli positive bacteria appear bright red on these media, while curli-negative bacteria appear white and smooth. (*Right*) Biofilm formation after 48 h in YESCA media. Quantitation of biomass was performed using the colorimetric method of O'Toole (1).

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Experiment was repeated twice with three technical replicates per biological repeat. *$P < 0.05$, ***$P \leq 0.0001$, as determined by two-tailed unpaired Student t test. Asterisks directly above bars depicting significance compared with WT UTI89.

1. Hadjifrangiskou M, et al. (2012) Transposon mutagenesis identifies uropathogenic Escherichia coli biofilm factors. *J Bacteriol* 194(22):6195–6205.

Table S1. PmrA targets affected in the absence of QseC

Gene name	Locus	Fold-change in UTI89Δ*qseC* (1)	PmrA-mediated fold-change in *Salmonella* (2)	Predicted function
yijP	UTI89_C4546	7.1	3.2	Hypothetical
yfbH	UTI89_C2538	2.6	2.1	PgbP operon component; polymyxin B resistance
oppB	UTI89_C1442	−2.0	−2.2	Oligopeptide transport permease protein
deoC	UTI89_C5152	−2.0	2.4	Enzyme; salvage of nucleosides and nucleotides
fadA	UTI89_C4430	−2.0	2.0	Enzyme; degradation of small molecules, fatty acids
udp	UTI89_C4411	−2.3	3.2	Uridine phosphorylase
ybaO	UTI89_C0475	−2.3	3.1	Hypothetical transcriptional regulator

1. Hadjifrangiskou M, et al. (2011) A central metabolic circuit controlled by QseC in pathogenic *Escherichia coli*. *Mol Microbiol* 80(6):1516–1529.
2. Tamayo R, Prouty AM, Gunn JS (2005) Identification and functional analysis of *Salmonella enterica* serovar Typhimurium PmrA-regulated genes. *FEMS Immunol Med Microbiol* 43(2): 249–258.

Table S2. Primers used in this study

Primer	Sequence (5′→3′)	Description
PmrB_KO_L	TTATATCTGGTTTGCCACGTACTGATCTTTTTTCAGCCGTA TCCAGGCCCGTGTAGGCTGGAGCTGCTTC	50 bp at +4605923 with pKD4 left priming site to knock out UTI89_C4706 (*pmrB*)
PmrB_KO_R	TTGAATCTGATGCGTTTTCTGCGCCGACCAATATCG CTGCGCCAACGGCTCATATGAATATCCTCCTTAG	50 bp at -4607023 with pKD4 right priming site to knock out UTI89_C4706 (*pmrB*)
Test_PmrB_KOL	GTGTTCAGCGTGCTGGTGGT	Left primer to verify *pmrB* deletion; anneals at +4605891
Test_PmrB_KOR	TGTCGCGCCTGATGCTTAAA	Right primer to verify *pmrB* deletion; anneals at -4607220
PmrA_KO_L	TTAGTTTTCCTCATTTGCGACCAGCATATAGCCA AATCCGCGCACGGTGCGTGTAGGCTGGAGCTGCTTC	50 bp at +4607024 with pKD4 left priming site to knock out UTI89_C4707 (*pmrA*)
PmrA_KO_R	ATGAAAATTCTGATTGTTGAAGACGATACGCTGT TATTGCAGGGATTGATCATATGAATATCCTCCTTAG	50 bp at -4607692 with pKD4 right priming site to knock out UTI89_C4707 (*pmrA*)
Test_PmrA_KOR	CCTGACTGGCGTTGAGACGA	Right primer to verify *pmrA* deletion; anneals at -4607764
P*pmr*_BamHI_F	CGGGATCCACTATCGGCAGCCACG	Left primer to amplify and clone *pmrAB* promoter in pTRC99A; BamH I site is underlined
P*pmr*_ SmaI _R	TCCCCCGGGTTCACTCACTCTCCTGCA	Right primer to amplify and clone *pmrAB* promoter in pTRC99A; Sma I site is underlined
*pmrB*_SmaI_F	TCCCCCGGGTTGAATCTGATGCGTTTTC	Left primer to amplify and clone *pmrB* in pTRC99A, downstream of the *pmrAB* promoter; Sma I site is underlined
*pmrB*_EcoRI_R	CGGAATTCTTATATCTGGTTTGCCACGTAC	Right primer to amplify and clone *pmrB* in pTRC99A, downstream of the *pmrAB* promoter; EcoR I site is underlined
PmrB_H155A_F	GTTGTTTACCGCTGACGTCGCGGCCGAACTGCGAACGCCACTGG	Left primer to introduce an H→A mutation in PmrB. H encoding CAC was changed to GCC (underlined)
PmrB_H155A_R	CCAGTGGCGTTCGCAGTTCGGCCG CGACGTCAGCGGTAAACAAC	Reverse anti-parallel primer complementary to PmrB_H155A_F. Mutated codon underlined
PmrB_mycHis_F	CATGCCATGGCCAATCTGATGCGTTTTCTGC	Left primer to amplify and clone *pmrB* and *pmrB*_H155A in pBAD-mycHis A, Nco I site is underlined
PmrB_mycHis_R	CCCAAGCTTTTATATCTGGTTTGCCACG	Right primer to amplify and clone *pmrB* and *pmrB*_H155A in pBAD-mycHis A, Hind III site is underlined