SUPPLEMENTARY MATERIAL

Supplementary Materials & Methods:

Phosphatase assays. Membranes were prepared from UTI89Δ*qseC*/pQseCmyc-His or UTI89Δ*qseC*/pBADmyc-HisA, previously constructed (2). Beads were prepared and used to *in vitro* phosphorylate QseB according to Kato and Groisman (1). QseB~P (0.2 nmol, equal to 9000 cpm) was incubated at room temperature with 7 μg of membrane vesicles in 1x TBS/0.5 mM DTT/0.5 mM $MgCl_2$ in the presence or absence of LED209, ranging from 5pM to 0.5mM in concentration. Aliquots (10 ml) were withdrawn from the reaction master-mix and treated as described above. Gels were dried, exposed to Phosphor Imaging plates and developed using the BAS-5000 scanner (Fujifilm).

LED209 *in vivo* studies. 2 ml of UTI89 bacteria normalized to 10^8 cfu/ml in PBS, were pre-incubated with 260 μl of 10mM LED209 (Formal name: 1-phenyl-3-[4-(phenylsulfamoyl)phenyl]thiourea; CAS number: 245342-14-7) in DMSO bringing the final LED209 concentration to 1.3mM. A range of LED209 concentrations was tested to reflect concentrations similar or higher to the ones previously used by Rasko *et al.* (3). A similar effect was observed with all concentrations. We report the outcome of the infection study performed with the highest LED209 concentration. In parallel, a control UTI89 2ml sample was pre-incubated with 260 μl DMSO alone, to account for any DMSO vehicle effects. Bacteria were then incubated at 37°C for 1h and 20min. prior to inoculation. Mice were infected with 10^7 UTI89/LED209 or 10^7 UTI89/DMSO and euthanized at 16 h.p.i. bladder cfu enumeration.

Supplementary Figure 1:

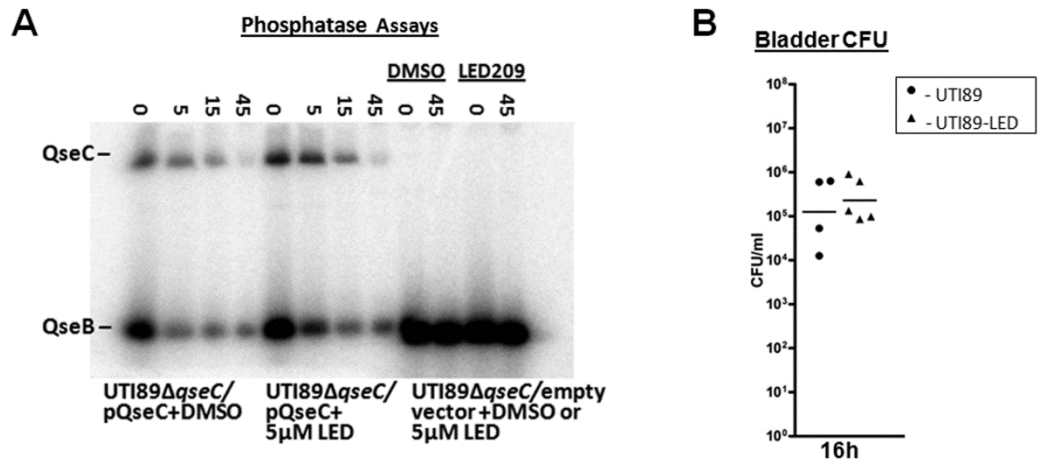

FIG S1. A) Incubation with LED209 does not impede the ability of QseC to de-phosphorylate QseB. QseB was phosphorylated *in vitro* prior to the phosphatase assays, using PmrB-loaded beads as described in Supplementary Materials and Methods. Numbers on top panel indicate time of incubation in minutes. Similar results were obtained with all LED209 concentrations tested (range 5 pM to 0.5 mM); results with 5μM LED209 are shown. B) Treatment with LED209 does not impact UPEC bladder titers. A representative from 3 independent experiments is shown.

1. **Kato, A., and E. A. Groisman.** 2004. Connecting two-component regulatory systems by a protein that protects a response regulator from dephosphorylation by its cognate sensor. Genes Dev **18:**2302-2313.
2. **Kostakioti, M., M. Hadjifrangiskou, J. S. Pinkner, and S. J. Hultgren.** 2009. QseC-mediated dephosphorylation of QseB is required for expression of genes associated with virulence in uropathogenic Escherichia coli. Mol Microbiol **73:**1020-1031.
3. **Rasko, D. A., C. G. Moreira, R. Li de, N. C. Reading, J. M. Ritchie, M. K. Waldor, N. Williams, R. Taussig, S. Wei, M. Roth, D. T. Hughes, J. F. Huntley, M. W. Fina, J. R. Falck, and V. Sperandio.** 2008. Targeting QseC signaling and virulence for antibiotic development. Science **321:**1078-1080.