The broadly conserved regulator PhoP links pathogen virulence and membrane potential in *Escherichia coli*

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Online Supporting Information

Fig. S1-S4
Table S1

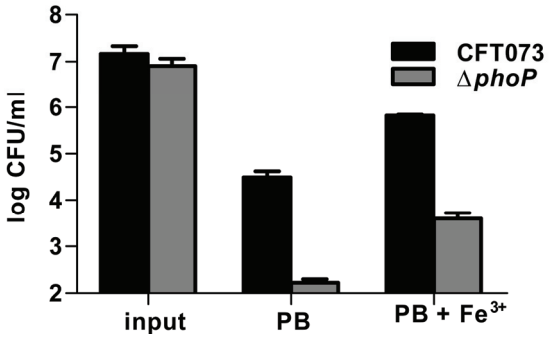

Fig. S1. Loss of *phoP* results in the increased expression of class II and III flagellar transcripts. Quantitative real-time PCR analysis of class I (*flhD*), class II (*fliA*), and class III (*fliC*) flagellar gene expression in Δ*phoP* and parental CFT073 strains. cDNA were prepared from RNA purified from Δ*phoP* and CFT073 grown in LB medium to mid-exponential phase ($O.D._{600nm}$ = 1.0). Transcript levels were normalized to the level of *gapA* (Glyceraldehyde 3-phosphate dehydrogenase A) and changes were determined using CFT073 as the calibrator. Bars represent the fold-change in expression for Δ*phoP* relative to wild-type CFT073.

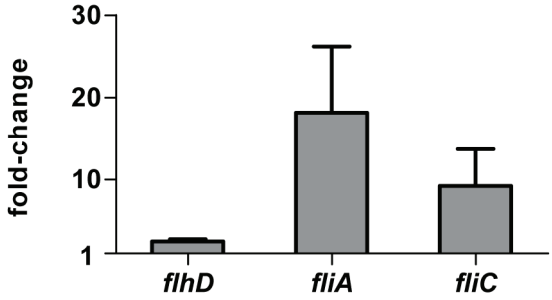

Fig. S2. PhoP-mediates resistance to polymyxin B in *E. coli* independent of [Fe3+]. CFU/ml for CFT073 and Δ*phoP* following 45 min incubation of 10^7 CFU logarithmic phase cells in fresh LB medium containing 10 µM $FeCl_3$ and 2 µg ml^{-1} polymyxin B (PB) or LB medium containing only 2 µg ml^{-1} PB. Viable counts were determined from triplicate experiments by plating serial dilutions on LB agar.

Table S1. Flagellar and motility gene transcription in the absence of *phoP*.

Gene	Function	log 2 fold-change	P-value
flgB	flagellar basal body rod protein	3.9288	0.0004
flgC	flagellar basal body rod protein	3.8482	0.0008
flgE	flagellar hook protein	3.1773	0.0065
flgD	flagellar basal body rod modification protein	2.8490	0.0025
fliL	flagellar basal body-associated protein	2.7998	0.0043
flgG	flagellar component of cell-distal portion of basal-body rod	2.7427	0.0028
flgF	flagellar component of cell-proximal portion of basal-body rod	2.5683	0.0038
flgI	flagellar P-ring protein precursor	2.4815	0.0061
fliN	flagellar motor switch protein	2.1963	0.0182
fliG	flagellar motor switch protein	2.1157	0.0129
flgA	flagellar basal body P-ring biosynthesis protein	2.0892	0.0030
fliK	flagellar hook-length control protein	1.9644	0.0102
flgL	flagellar hook-associated protein	1.9109	0.0024
fliS	flagellar protein FliS	1.8226	0.0174
flgJ	flagellar biosynthesis protein	1.8007	0.0292
fliA	flagellar biosynthesis sigma factor	1.7376	0.0115
flgK	flagellar hook-associated protein	1.6931	0.0480
flgH	flagellar L-ring protein precursor	1.6896	0.0149
fliM	flagellar motor switch protein	1.5893	0.0525
fliZ	regulator of FliA activity	1.5092	0.0316
flgM	anti-sigma factor for FliA (sigma 28)	1.5023	0.0421
flgN	export chaperone for FlgK and FlgL	1.4373	0.0397
cheY	chemotaxis protein cheY	1.3244	0.0140
tap	methyl-accepting protein IV	1.3238	0.0377
fliT	predicted chaperone	1.2788	0.0189
fliC	flagellin structural subunit	1.2340	0.0156
fliI	flagellum-specific ATP synthase	1.2226	0.0418
tar	methyl-accepting chemotaxis protein II	1.2077	0.0492
fliO	flagellar type III export apparatus	1.1855	0.0141
cheB	chemotaxis-specific methylesterase	1.1467	0.0300
cheR	protein-glutamate methyltransferase	1.1450	0.0226
fliJ	flagellar biosynthesis chaperone	1.1376	0.0429
cheA	chemotactic sensory histidine kinase	1.1267	0.0276
cheZ	chemotaxis regulator, protein phosphatase for CheY	1.0869	0.0125
cheW	purine-binding chemotaxis protein	1.0785	0.0180
fliE	flagellar basal body protein	0.7557	0.0225

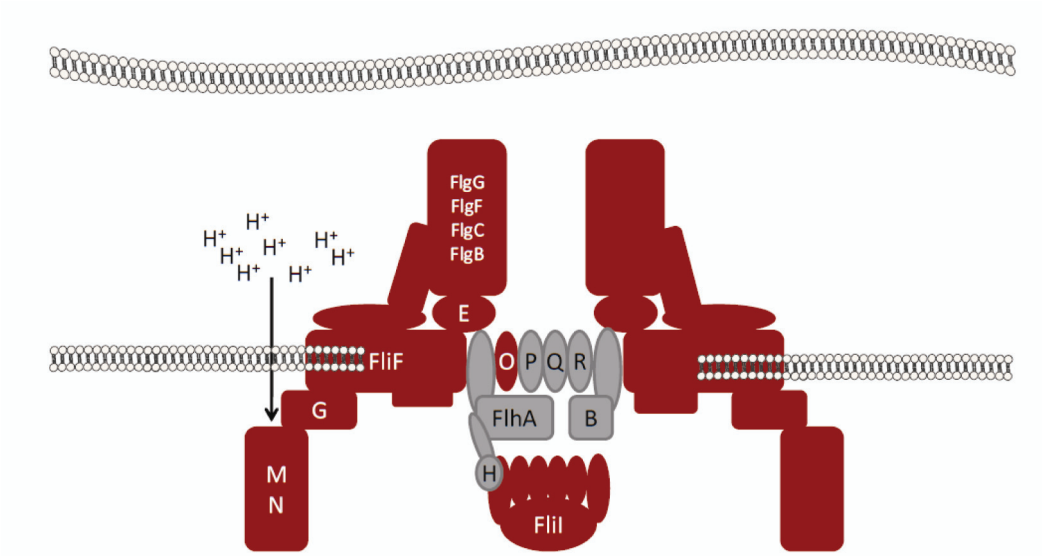

Fig. S3. The precise PhoP-mediated control of motility genes specifically excludes the type III protein export apparatus components. Individual genes that have significantly increased transcription in the absence of *phoP* are colored red. Gene expression levels that were not considered significant are colored grey. Rotation of the flagellum is dependent on proton translocation (arrow) through the MotAB stator (not shown).

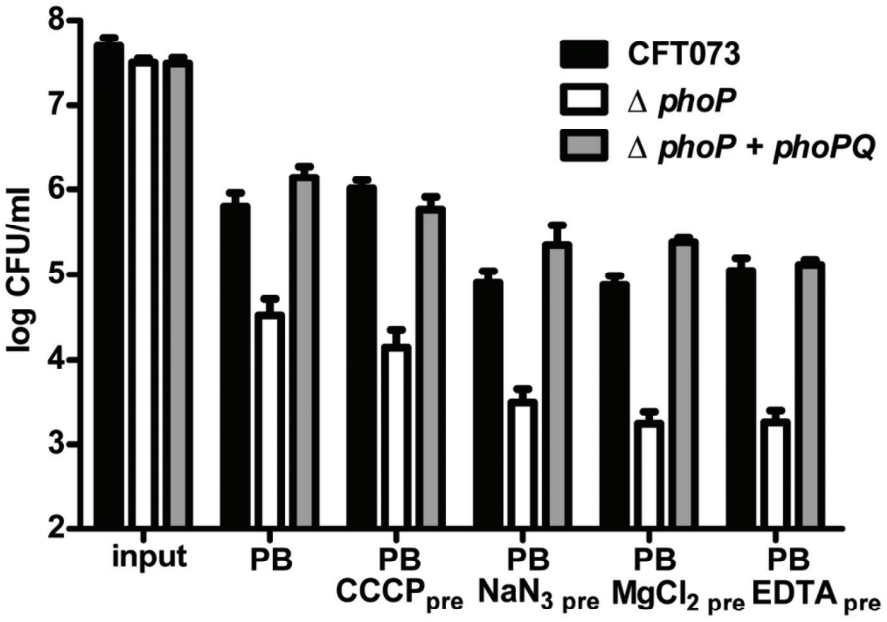

Fig. S4. PhoP-dependent μH^+-based resistance to polymyxin B is an active response and not a preformed state of the membrane. Viable counts of CFT073 and Δ*phoP* following 45 min incubation in 2 μg ml^{-1} PB with and without pre-treatment in 50 μM *m*-chlorophenyl carbonyl cyanide hydrozone (CCCP), 0.1% sodium azide (NaN_3), 0.5 mM EDTA or 10 mM $MgCl_2$. CFT073, Δ*phoP*, and Δ*phoP*+*phoPQ* cells grown to OD_{600} = 0.8 were diluted 1:10 (input) and pre-treated with CCCP or NaN_3 for 1 h. Following pre-treatment, cells were washed with LB medium and incubated in fresh LB medium containing 2 μg ml^{-1} PB for 45 min. Untreated cells incubated in LB medium containing 2 μg ml^{-1} PB for 45 min were included as a reference. Viable counts were determined from triplicate experiments by plating serial dilutions on LB agar.