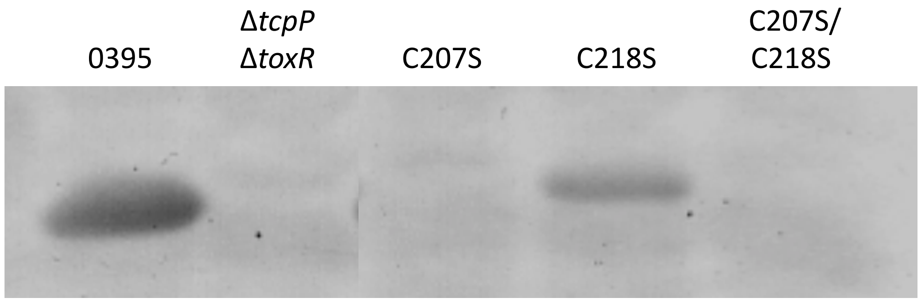

Figure S1. TcpP expression in strains assessed for CT production. Western blot analysis of TcpP expression from 0395, EK459 (Δ*tcpP*Δ*toxR*) and chromosomally-encoded TcpP mutants.

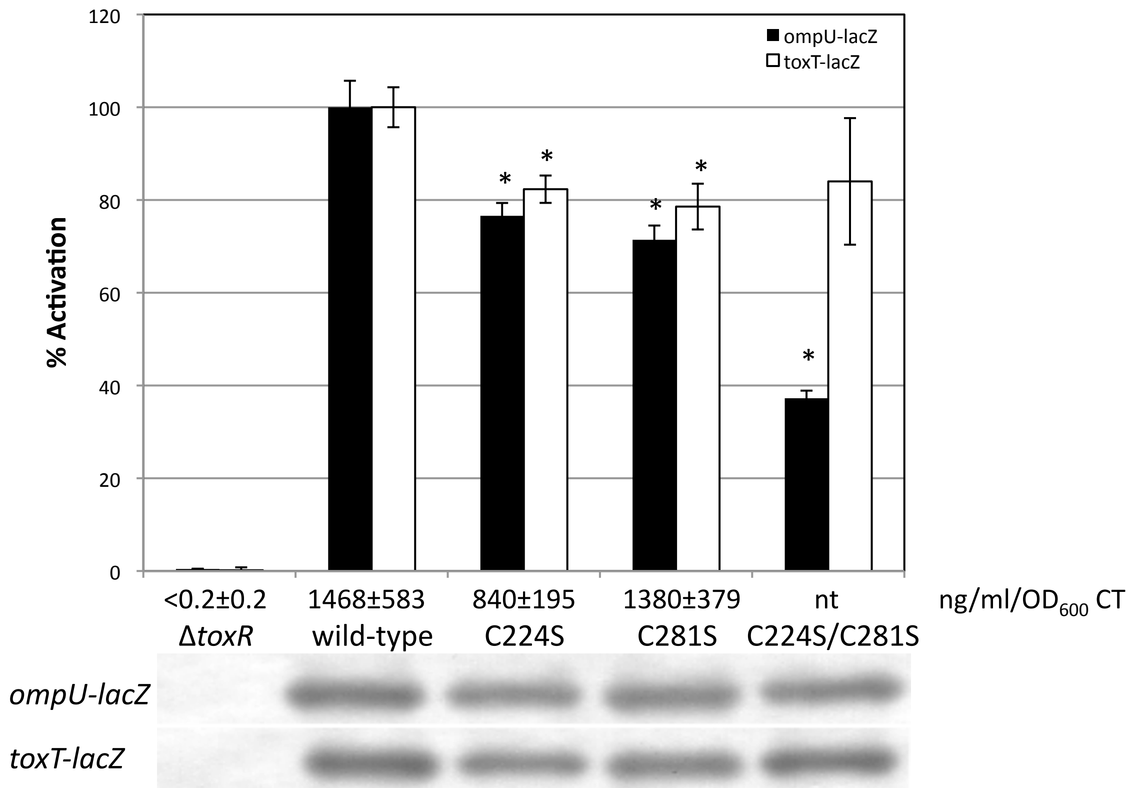

Figure S2: Chromosomally-encoded ToxR periplasmic mutants have modest effects on *ompU* expression and minimal effects on *toxT* expression and CT production. Chromosomally expressed ToxR periplasmic cysteine mutants were assessed for transcription activation of *toxT-lacZ* and *ompU-lacZ* using chromosomal reporters *p < 0.005 relative to wild-type ToxR. The ng/ml/OD_{600} of cholera toxin produced by each strain is designated under the graph as measured by a CT-ELISA assay. No significant difference between CT production of wild-type ToxR and ToxR-C224S or ToxR-C281S was observed. Western blots using anti-ToxR antibody are shown below the graph to indicate stability of these mutants. nt=not tested. Transcription activation was tested at least twice in triplicate (n=6) for each strain. CT assays were performed twice in duplicate (n=4).

Table S1. List of strains and plasmids from this study.

Strains	Genotype/Characteristics	Source
E. coli		
DH5α	*supE44* Δ*lacU169(F80lacZDM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Laboratory collection
DH5α λpir	*supE44* Δ*lacU169(F80lacZDM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1* (λ*pir*)	Laboratory collection
SM10 λpir	*thi-1 thr leu tonA lacY supE recA::RP4-2-Tc::Mu Km*^{R} (λ*pir*)	Laboratory collection
EK3034	Rosetta (DE3) pLysS: F$^-$ *ompT hsdS*$_B$(R$_B^-$ m$_B^-$) *gal dcm* λ(DE3 [*lacI lacUV5*-T7 gene1 *ind1 sam7 nin5*]) + pLysSRARE + pET30b+*toxR*cyt2-TEV	this study
V. cholerae		
O395	0395, wild-type Classical biotype	Laboratory collection
RY1	0395 Δ*tcpP*	(1)
EK459	O395 Δ*toxR*Δ*tcpP*	(2)
EK307	0395 Δ*toxR*	(2)
EK813	0395 Δ*tcpP toxT-lacZ*	(3)
EK1490	0395 Δ*tcpP* Δ*toxR toxT-lacZ*	(3)
SM269	0395 *tcpP*-C207S	this study
SM492	0395 *tcpP*-C218S	this study
SM495	0395 *tcpP*-C207S/C218S	this study
JM84	0395 Δ*yaeL*	(4)
JM128	0395 Δ*degS*	(4)
NB43	0395 Δ*ptd*	(5)
JM160	0395 Δ*tcpP* Δ*yaeL*	this study
SM1651	0395 Δ*tcpP* Δ*degS*	this study
SM1664	0395 Δ*tcpP* Δ*ptd*	this study
SM1833	0395 Δ*tcpP* Δ*degS* Δ*ptd*	this study
EK3434	0395 Δ*tcpP* Δ*tsp*	this study
EK3428	0395 Δ*tcpP* Δ*tsp* Δ*degS* Δ*ptd*	this study
EK410	0395 Δ*toxR ompU-lacZ*	(6)
EK383	0395 *ompU-lacZ*	(6)
SM1301	0395 *ompU-lacZ toxR*-C224S	this study
SM1264	0395 *ompU-lacZ toxR*-C281S	this study
SM1303	0395 *ompU-lacZ toxR*-C224S/C281S	this study
EK733	0395 *toxT-lacZ*	(7)
EK1072	0395 Δ*toxR toxT-lacZ*	(8)
SM1299	0395 *toxT-lacZ toxR-C224S*	this study
SM1260	0395 *toxT-lacZ toxR-C281S*	this study
SM1263	0395 *toxT-lacZ toxR-C224S/C281S*	this study
SM488	0395 *toxR-C224S*	this study

SM486	0395 *toxR-C281S*	this study
Plasmids		**Source**
pEK41 (pMMB207-*tcpP*-HSV)		(2)
pMMB207		(9)
pMMB207-*tcpP*-HSV-C207S		this study
pMMB207-*tcpP*-HSV-C218S		this study
pMMB207-*tcpP*-HSV-C207S/C218S		this study
pSK-*toxR*-HA		(8)
pACYC184 Tet^{S}		(5)
pACYC184-*tcpH*		(5)
pTLI2 (pTL61T-*toxT*$_{\mathrm{pro}}$)		(10)
pEK32 (pMMB207-*tcpPH*)		(2)
pBAD18 (Kan^{R})		(11)
pGOOD=pBAD18 (Kan^{R}) with modified polylinker		this study
pGOOD-*tcpP* (wt)		this study
pGOOD-*tcpP*-C207S		this study
pGOOD-*tcpP*-C218S		this study
pGOOD-*tcpP*-C207S/C218S		this study
pGOOD-*tcpPH* (wt)		this study
pGOOD-*tcpPH*-C207S		this study
pGOOD-*tcpPH*-C218S		this study
pGOOD-*tcpPH*-C207S/C218S		this study
pGOOD-*tcpP*(wt)-*H*-C114S		this study
pKAS32		(12)
pKAS32-*tcpP*-C207S		this study
pKAS32-*tcpP*-C218S		this study
pKAS32-*tcpP*-C207S/C218S		this study
pKAS32-Δ*yaeL*		(4)
pKAS32-Δ*tcpP*		(2)
pKAS32-Δ*degS*		(4)
pKAS32-Δ*tsp*		(13)
pET30b+*toxR*cyt2-TEV		this study

Table S2. List of primers used in this study.

tcpP C207S top	CTATTGATCAACATCAGTCTTCCGTGAATTATG
tcpP C207S bottom	CATAATTCACGGAAGACTGATGTTGATCAATAG
tcpP C218S top	CAGAAGACATTAGAATCCACAAAAAATGCCCC
tcpP C218S bottom	GGGGCATTTTTTGTGGATTCTAATGTCTTCTG
tcpP C218S chrom top	CAGAAGACATTAGAATCCACAAAAAATTAAAAGC
tcpP C218S chrom bottom	GCTTTTAATTTTTTGTGGATTCTAATGTCTTCTG
tcpH C114S top	CAACTCGGCAAAGGTTCTTTTCTCGCCTTCCC
tcpH C114S bottom	GGGAAGGCGAGAAAAGAACCTTTGCCGAGTTG
toxR C224S top	CGTCAATCGAACTGTCCGTTAAAAAATACAATG
toxR C224S bottom	CATTGTATTTTTTAACGGACAGTTCGATTGACG
toxR C281S top	GATGCCATCAAAGTGTCTGAGCTCGAGTACCC
toxR C281S bottom	GGGTACTCGAGCTCAGACACTTTGATGGCATC
toxR C281S chrom top	GATGCCATCAAAGTGTCTGAGTAGGATCTTGC
toxR C281S chrom bottom	GCAAGATCCTACTCAGACACTTTGATGGCATC
pBAD NoBAMH1 top	GATTAGCGGATCGTACCTGACGCTTTTTATC
pBAD NoBamH1 bottom	GATAAAAAGCGTCAGGTACGATCCGCTAATC
pBAD NoNhe1 top	ACCCGTTTTTTTGGGCAAGCGAATTCGAGC
pBAD NoNhe1 bottom	GCTCGAATTCGCTTGCCCAAAAAAACGGGT
TEV site for pET30b+ BOTTOM	CCAGATCTGGGTACCGAGAACCTGTACTTCCAGGGCG CCATGGCGATATCGG
TEV site for pET30b+ BOTTOM	CCGATATCGCCATGGCGCCCTGGAAGTACAGGTTCTC GGTACCCAGATCTGG
NdeI-ToxR orf-1 Forw	GGAATTCCATATGAGTCATATTGGTACTAAATTC
KpnI-ToxR orf-170 Rev	GGGGTACCTCGATTCCCCAAGTTTGGAG

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