

**Table S1. Strains, plasmids and oligonucleotides used in this study**

Strains	Characteristics	Source
<i>E. coli</i>		
EC100λpir	<i>supE44</i> (φ80 <i>lacZ</i> Δ <i>M15</i> ) <i>hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i> (λ <i>pirR6K</i> )	Epicenter
SM10λpir	<i>thi-1 thr leu tonA lacY supE recA::RP4-2-Tc::Mu Km<sup>r</sup></i> (λ <i>pirR6K</i> )	Lab collection
BTH101	F <sup>-</sup> , <i>cya-99, araD139, galE15, galK16, rpsL</i> (Str <sup>r</sup> ), <i>hsdR2, mcrA1, mcrB1</i>	
<i>V. cholerae</i>		
JB58	<i>V. cholerae</i> O1 El Tor strain N16961, Sm <sup>R</sup> Δ <i>lacZ</i> ,	Lab collection
DT733	JB58 Δ <i>toxRS</i>	(1)
XBV222	JB58 Δ <i>leuO</i>	(1)
XBV532	JB58 Δ <i>ompU</i>	(2)
HR35	JB58 Δ <i>tnaA</i>	This study
SS4	JB58 <i>toxR</i> <sup>APPD</sup>	(3)
<b>Plasmids</b>		
pTL61T	<i>lacZ</i> transcriptional reporter, Cb <sup>R</sup>	(4)
pXB192	pTL61T containing <i>toxT</i> promoter	(5)
pXB193	pTL61T containing <i>ctxAB</i> promoter	(6)
pXB194	pTL61T containing <i>tcpA</i> promoter	(6)
pXB201	pTL61T containing <i>toxRS</i> promoter	(6)
pXB202	pTL61T containing <i>aphA</i> promoter	(6)
pXB203	pTL61T containing <i>aphB</i> promoter	(6)
pXB266	pTL61T containing <i>leuO</i> promoter	(1)
pXB270	pTL61T containing <i>tcpPH</i> promoter	(5)
pAL144	pTL61T containing <i>ompU</i> promoter	(6)
pWM91Δ <i>tnaA</i>	Allelic exchange vector for in-frame deletion of <i>tnaA</i>	This study
<b>PCR primers</b>		
<b>Sequence (5'→3'):</b>		
VCA0161-F1-SacI	ATGAGCTCTAGTTCAGCCCGGTGTGCCCATC	
VCA0161-F2	TTTCGTATTCGTCCCGTGCTACGCCACTTTACGGCTCGC	
VCA0161-R1 BamHI	AAAGGATCCCAAATAGTGGCGGCTGCGCCTG	
VCA0161-R2	CGTAGCACGGGACGAATACGAAAAGGTTCTGGTAAGTG	
VCA0161-F-SacI	GGGAGCTCTAGGAATACCCAGCATGGAAAATTTTAAACAC	
VCA0161-R-SphI	GGGCATGCTGCACTTTTTCAATACCGTATTAGG	

**References.**

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2. Bina XR, Howard MF, Ante VM, Bina JE. 2016. *Vibrio cholerae* LeuO Links the ToxR Regulon to Expression of Lipid A Remodeling Genes. *Infect Immun* 84:3161-3171.
3. Ante VM, Bina XR, Howard MF, Sayeed S, Taylor DL, Bina JE. 2015. *Vibrio cholerae* leuO Transcription Is Positively Regulated by ToxR and Contributes to Bile Resistance. *J Bacteriol* 197:3499-510.
4. Linn T, St Pierre R. 1990. Improved vector system for constructing transcriptional fusions that ensures independent translation of *lacZ*. *J Bacteriol* 172:1077-84.
5. Bina XR, Provenzano D, Nguyen N, Bina JE. 2008. *Vibrio cholerae* RND family efflux systems are required for antimicrobial resistance, optimal virulence factor production, and colonization of the infant mouse small intestine. *Infect Immun* 76:3595-605.
6. Bina XR, Bina JE. 2010. The cyclic dipeptide cyclo(Phe-Pro) inhibits cholera toxin and toxin-coregulated pilus production in O1 El Tor *Vibrio cholerae*. *J Bacteriol* 192:3829-32.