

**Table S1: Strains and plasmids**

Strain	Description	Reference
E. coli SM10 $\lambda$ pir	thi thr leu tonA lacY supE recA::RP4-2-Tc::Mu ( $\lambda$ pirR6K) Kmr	1
Vibrio cholerae strains		
PW357	MO10 lacZ::vpsLp -->lacZ Sm <sup>r</sup>	2
PW836	MO10 lacZ::vpsLp -->lacZ $\Delta$ EIIA <sup>Glc</sup> Sm <sup>r</sup>	3
PW985	MO10 lacZ::vpsLp -->lacZ $\Delta$ EI $\Delta$ HPr $\Delta$ EIIA <sup>Glc</sup>	3
PW1875	MO10 lacZ::vpsLp -->lacZ $\Delta$ 16EIIA <sup>Glc</sup> Sm <sup>r</sup>	This study
PW1876	MO10 lacZ::vpsLp -->lacZ FL-EIIA <sup>Glc</sup> Sm <sup>r</sup>	This study
PW1877	MO10 lacZ::vpsLp -->lacZ K6P-EIIA <sup>Glc</sup> Sm <sup>r</sup>	This study
PW1878	MO10 lacZ::vpsLp -->lacZ EIIA <sup>Glc</sup> -MinD Sm <sup>r</sup>	This study
PW1879	MO10 lacZ::vpsLp -->lacZ 1XMreB-EIIA <sup>Glc</sup> Sm <sup>r</sup>	This study
PW1880	MO10 lacZ::vpsLp -->lacZ 2XMreB-EIIA <sup>Glc</sup> Sm <sup>r</sup>	This study
PW1881	MO10 lacZ::vpsLp -->lacZ FL EIIA <sup>GlcH91A</sup> Sm <sup>r</sup>	This study
PW1882	MO10 lacZ::vpsLp -->lacZ $\Delta$ 16 EIIA <sup>GlcH91A</sup> Sm <sup>r</sup>	This study
PW1889	MO10 lacZ::vpsLp -->lacZ FL EIIA <sup>Glc</sup> MshH-TAPtag::pGP704 Sm <sup>r</sup> Ap <sup>r</sup>	This study
PW1890	MO10 lacZ::vpsLp -->lacZ $\Delta$ 16EIIA <sup>Glc</sup> MshH-TAPtag::pGP704 Sm <sup>r</sup> Ap <sup>r</sup>	This study
PW1891	MO10 lacZ::vpsLp -->lacZ $\Delta$ EIIA <sup>Glc</sup> MshH-TAPtag::pGP704 Sm <sup>r</sup> Ap <sup>r</sup>	This study
PW2003	MO10 lacZ::vpsLp -->lacZ $\Delta$ EIIA <sup>Glc</sup> AC-TAPtag:: pGP704 Sm <sup>r</sup> Ap <sup>r</sup>	This study
PW2004	MO10 lacZ::vpsLp -->lacZ $\Delta$ 16EIIA <sup>Glc</sup> AC-TAPtag:: pGP704 Sm <sup>r</sup> Ap <sup>r</sup>	This study
PW2005	MO10 lacZ::vpsLp -->lacZ FL EIIA <sup>Glc</sup> AC-TAPtag:: pGP704 Sm <sup>r</sup> Ap <sup>r</sup>	This study
PW1974	MO10 lacZ::vpsLp -->lacZ FL EIIA <sup>Glc</sup> -V5-His $\Delta$ EIIBC <sup>Glc</sup> $\Delta$ MshH $\Delta$ cyaA	This study

PW1975	MO10 lacZ::vpsLp -->lacZ H $\Delta$ 16-EIIA <sup>Glc</sup> -V5-His $\Delta$ EIIBC <sup>Glc</sup> $\Delta$ Msh $\Delta$ cyoA	This study
PW1898	MO10 lacZ::vpsLp -->lacZ EI <sup>H189A</sup> $\Delta$ EIIA <sup>Glc</sup>	This study
PW1892	MO10 lacZ::vpsLp -->lacZ EI <sup>H189A</sup>	This study
PW2010	MO10 lacZ::vpsLp -->lacZ $\Delta$ 16 EIIA <sup>Glc</sup> -V5-6XHis $\Delta$ EIIBC <sup>Glc</sup>	This study
PW2015	MO10 lacZ::vpsLp -->lacZ EI <sup>H189A</sup> $\Delta$ 16 EIIA <sup>Glc</sup> -V5-6XHis $\Delta$ EIIBC <sup>Glc</sup>	This study
PW2008	MO10 lacZ::vpsLp -->lacZ EI <sup>H189A</sup> $\Delta$ 16EIIA <sup>Glc</sup> -V5-6XHis	This study
PW2009	MO10 lacZ::vpsLp -->lacZ EI <sup>H189A</sup> FL EIIA <sup>Glc</sup> -V5-6XHis	This study
PW2006	MO10 lacZ::vpsLp -->lacZ $\Delta$ 16 EIIA <sup>Glc</sup> -V5-His $\Delta$ mlc	This study
PW993	MO10 lacZ::vpsLp -->lacZ $\Delta$ mlc	4
Plasmids for complementation		
PW1464	pBAD-TOPO encoding <i>lacZ</i>	Invitrogen
PW1884	pBAD-TOPO encoding FL-EIIA <sup>Glc</sup> -V5-His	This study
PW1883	pBAD-TOPO encoding $\Delta$ 16EIIA <sup>Glc</sup> -V5-6XHis	This study
PW1885	pBAD-TOPO encoding one MreB amphipathic helix fused to $\Delta$ 16-EIIA <sup>Glc</sup> -V5-6XHis	This study
PW1886	pBAD-TOPO encoding two MreB amphipathic helices (2XMreB) fused to $\Delta$ 16 EIIA <sup>Glc</sup> -V5-6XHis	This study
PW1887	pBAD-TOPO encoding FL EIIA <sup>GlcK6P</sup> -V5-6XHis	This study
PW1888	pBAD-TOPO encoding $\Delta$ 16 EIIA <sup>Glc</sup> -MinD-V5-6XHis	This study
PW2000	pBAD-TOPO carrying the EIIA <sup>Glc</sup> amphipathic helix fused to neon green-V5-6XHis	This study
PW1999	pBAD Neon Green-V5 -6XHis	This study
PW967	pBAD-TOPO encoding EI (Enzyme I)	3
Plasmids used for chromosomal recombination		

pWM91	oriR6K mobRP4 lacI pTac tnp mini-Tn10(Km) Km <sup>r</sup> Ap <sup>r</sup>	<sup>5</sup>
pWM91 FL EIIA <sup>Glc</sup>	Plasmid used to recombine FL EIIA <sup>Glc</sup> onto the chromosome	This study
pWM91 FL EIIA <sup>Glc</sup>	Plasmid used to recombine $\Delta$ 16 EIIA <sup>Glc</sup> -V5-6XHis onto the chromosome	This study
pWM91 1XMreB-EIIA <sup>Glc</sup>	Plasmid used to recombine 1XMreB-EIIA <sup>Glc</sup> -V5-6XHis onto the chromosome	This study
pWM91 2XMreB-EIIA <sup>Glc</sup>	Plasmid used to recombine 2XMreB-EIIA <sup>Glc</sup> -V5-6XHis onto the chromosome	This study
pWM91 K6P-EIIA <sup>Glc</sup>	Plasmid used to recombine EIIA <sup>GlcK6P</sup> -V5-6XHis onto the chromosome	This study
pWM91 EIIA <sup>Glc</sup> -MinD	Plasmid used to recombine EIIA <sup>Glc</sup> -MinD-V5-6XHis onto the chromosome	This study
pGP704::VC0398-TAP	Plasmid used to generate MshH-TAP on the chromosome	<sup>6</sup>
pGP704::cyaA-TAP	Plasmid used to generate AC-TAP on the chromosome	<sup>6</sup>
pWM91 EI H189A	Plasmid used to recombine EI <sup>H189A</sup> onto the chromosome	This study

## Supplementary References

- 1 Miller, V. L. & Mekalanos, J. J. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. *J Bacteriol* **170**, 2575-2583 (1988).
- 2 Haugo, A. J. & Watnick, P. I. *Vibrio cholerae* CytR is a repressor of biofilm development. *Mol Microbiol* **45**, 471-483, doi:3023 [pii] (2002).
- 3 Houot, L. & Watnick, P. I. A novel role for enzyme I of the *Vibrio cholerae* phosphoenolpyruvate phosphotransferase system in regulation of growth in a biofilm. *J Bacteriol* **190**, 311-320, doi:10.1128/JB.01410-07 (2008).
- 4 Pickering, B. S., Lopilato, J. E., Smith, D. R. & Watnick, P. I. The transcription factor Mlc promotes *Vibrio cholerae* biofilm formation through repression of phosphotransferase system components. *J Bacteriol* **196**, 2423-2430, doi:10.1128/JB.01639-14 (2014).
- 5 Metcalf, W. W. *et al.* Conditionally replicative and conjugative plasmids carrying *lacZ* alpha for cloning, mutagenesis, and allele replacement in bacteria. *Plasmid* **35**, 1-13, doi:10.1006/plas.1996.0001 (1996).
- 6 Pickering, B. S., Smith, D. R. & Watnick, P. I. Glucose-specific enzyme IIA has unique binding partners in the *vibrio cholerae* biofilm. *MBio* **3**, e00228-00212, doi:10.1128/mBio.00228-12 (2012).