Table S1: Plasmids used in this study

Plasmid	Description	Reference
pBBRlux	*luxABCDE* containing promoter-less plasmid, Cam^R	Lab collection
pEVS143	*pTac* overexpression vector, Kan^R	(1)
pKAS32	Sucide vector for mutant construction, Amp^R	(2)
pBRP333	Control expression vector, Kan^R	BRP Paper
6:C9	*tag1* promoter in pBBlux, Cam^R	(3)
pALN17	*tag2* promoter in pBBRlux, Cam^R	This study
pDS129	*tag3* promoter in pBBRlux, Cam^R	This study
pNF022	*tag4* promoter in pBBRlux, Cam^R	This study
pBRP1	*qrgB** (inactive DGC) cloned into expression vector pMMB67Eh, Amp^R	BRP paper
pBRP2	*qrgB* (active DGC) cloned into expression vector, pMMB67Eh, Amp^R	BRP paper
pCMW131	*vpsR* in pEVS143, Kan^R	This study
pCMW132	*vpsT* in pEVS143, Kan^R	This study
pNF011	*vpsT* in pET28b (C-terminal HIS-tag), Kan^R	
pDS138	pKAS32 *tag* deletion construct, Amp^R	This study
pDS139	*tag* in pEVS143, Kan^R	This study

Table S2: Strains used in this study

Strain	Description	Reference
E. coli		
DH10b	F$^-$ *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139Δ(*ara*, *leu*)7697 *gal*U *gal*K λ$^-$ *rps*L *nup*G	ThermoFisher Scientific
S17-λpir	Tpr Smr *recA thi pro hsdR17*($r_K^- m_K^+$) *RP4::2-Tc*::Mu Km Tn7 λpir	Lab stock
BL21(DE3)	F$^-$ *ompT hsdSB*($r_B^- m_B^+$) *gal dcm* (DE3)	Invitrogen
V. cholerae		
C6706 Str2	Wild Type	Lab Stock
NF02	C6706Δ*tag*	This Study
CW2034	C6706 Str2 Δ*vpsL*	(4)
JC1195	CW2034Δ*vpsT*	(3)
WN310	CW2034Δ*vpsR*	(5)
DS12	CW2034Δ*flrA*	(6)
DS14	CW2034Δ*tag*	This study

Table S3: Primers used in this study

Construct/ Purpose	Forward (5' → 3')*	Reverse (5' → 3')*
pALN17	gcggccgctctagaa**CGGTGCCTACAACATCCG**	cggccgcaactagag**GGATCATACCGAGTA**
pDS129	gcggccgctctagaa**CTTTGTTTAAAACCATGC**	cggccgcaactagag**GCTTATCCTTCTTCATTC**
pNF022	gcggccgctctagaa**CGAGCAAGGCCATAAACC**	cggccgcaactagag**GCTTCTTGAGTCTTTCGG**
pNF011	ctttaagaaggagatatac**ATGAAAGATGAAAACAAACT**	cagtggtggtggtggtg**CTCAGAATTGACTTCCTCAAT**
pDS138 - Region 1	cgggccctatatatggatcc**TCGGTGAAGGAGTCGC**	**AGCTCTTGGTGAATCAGCTTATCCTTCTTCAT**
pDS138 – Region 2	**ATGAAGAAGGATAAGCTGATTCACCAAGAGCT**	gctgatatcgatcgcgcgca**GCCAGCATGAACAAAA**
pDS139	tttagcttccttagctcctg**AGGAGCTAAGGAAGCTAAAATGATGAATGCGGAACA**	gcttgctcaatcaatcaccg**TCAGAGCTTGTCTGC**
tag qPCR primers	**CGTTACAACAGGAGTTCGGC**	**CGGGTACATCGCTCATGC**
gyrA qPCR primers	**TGGCCAGCCAGAGATCAAG**	**ACCCGCAGCGGTACGA**
GSP1-*pBBRlux*	**GTCATTCAATATTGGCAGGT**	
GSP2-*pBBRlux*	**AATGGATTGCACTAAATC**	
GSP3-*pBBRlux*	**TTTTAGTCATATTTGCCA**	
EMSA Primers#	FAM- **ATTTTGCGGCCGCAACTAGA**	FAM- **CCGCGGTGGCGGCCGCTCTA**
WT Competitor (5)	**AAAGTAAACTAAAGTTTATTTT**	**AAAATAAACTTTAGTTTACTTT**
MT Competitor (5)	**AAAAGGTCTAAAACTAGGTT**	**AACCTAGTTTTAGACCTTTT**

*Lowercase: 5' overhang region added for Gibson Assembly
UPPERCASE, EMBOLDENED: Gene specific region
#: FAM (6-carboxyfluorescein)

Supplemental References

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3. Srivastava D, Harris RC, Waters CM. 2011. Integration of cyclic di-GMP and quorum sensing in the control of vpsT and aphA in *Vibrio cholerae*. J Bacteriol 193:6331–6341.
4. Waters CM, Lu W, Rabinowitz JD, Bassler BL. 2008. Quorum sensing controls biofilm formation in *Vibrio cholerae* through modulation of cyclic Di-GMP levels and repression of vpsT. J Bacteriol 190:2527–2536.
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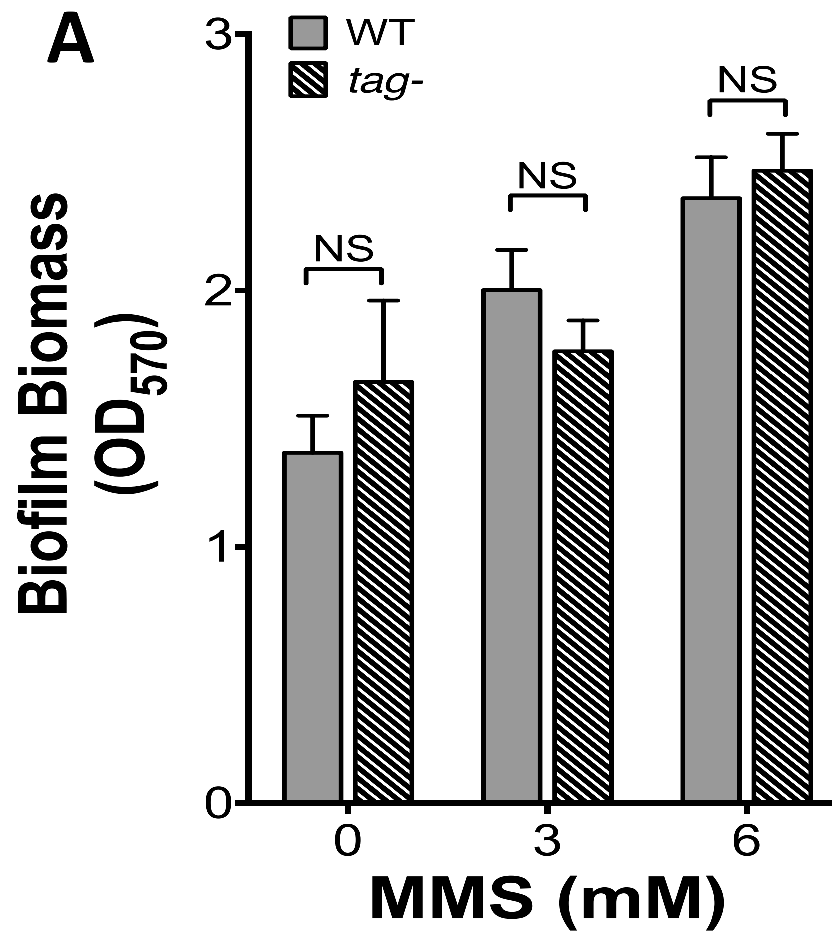
6. Srivastava D, Hsieh M-L, Khataokar A, Neiditch MB, Waters CM. 2013. Cyclic di-GMP inhibits *Vibrio cholerae* motility by repressing induction of transcription and inducing extracellular polysaccharide production. Mol Microbiol 90:1262–1276.



Supplemental Figure 1: *Tag* and MMS do not contribute to biofilm formation in *V. cholerae*. Strains were cultured 1:100 from overnight cultures into wells of a 96-well plate with either 0, 3, or 6 mM MMS added. Biofilms were grown at 35 °C while shaking at 220 RPM for 24 hours. After 24 hours, cultures were removed and a crystal violet assay was carried out using 0.4% crystal violet following the protocol from (O'Toole GA. 2011. Microtiter Dish Biofilm Formation Assay. J Vis Exp 10–11). Data are **A)** average biofilm biomass (absorbance at 570 nm) and from six biological replicates and error bars are standard deviation. Two-Way ANOVA followed by Tukey's Multiple Comparison Test determined all comparisons were significant ($p < .05$) except those marked by brackets and NS (non-significant, $p > .05$).