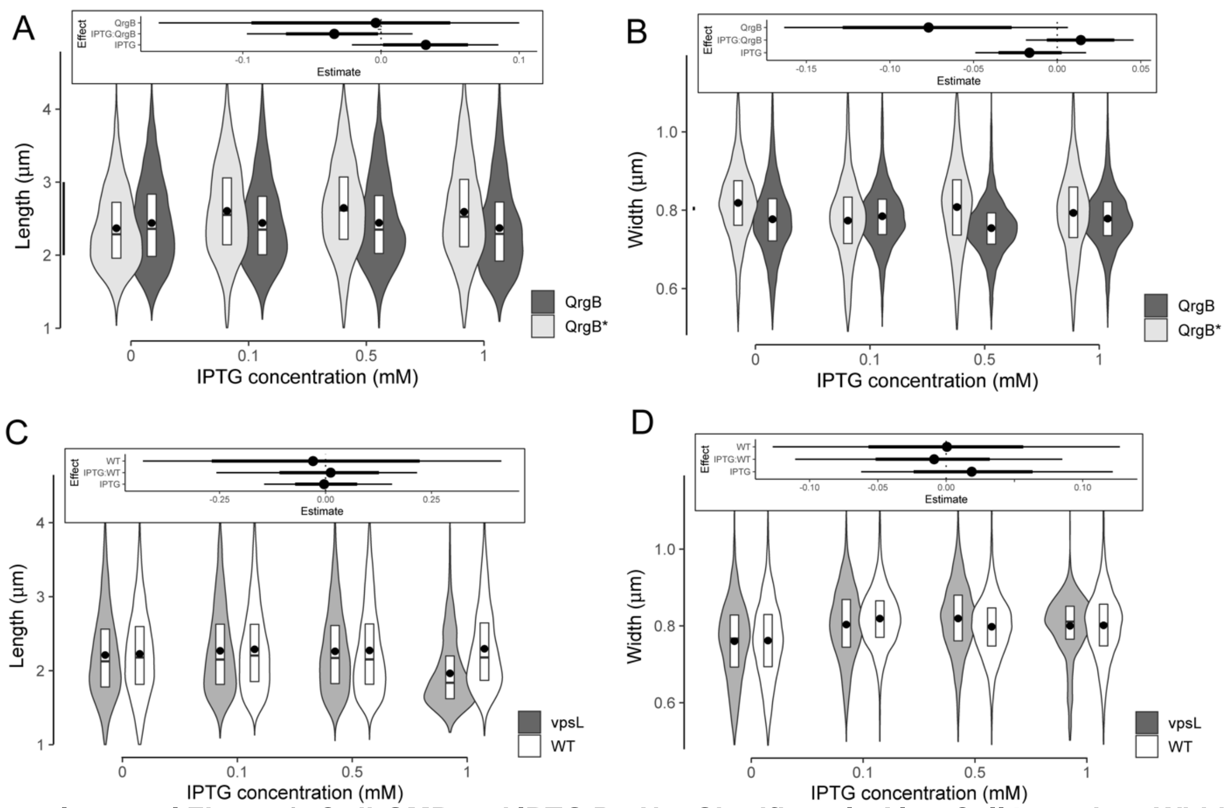
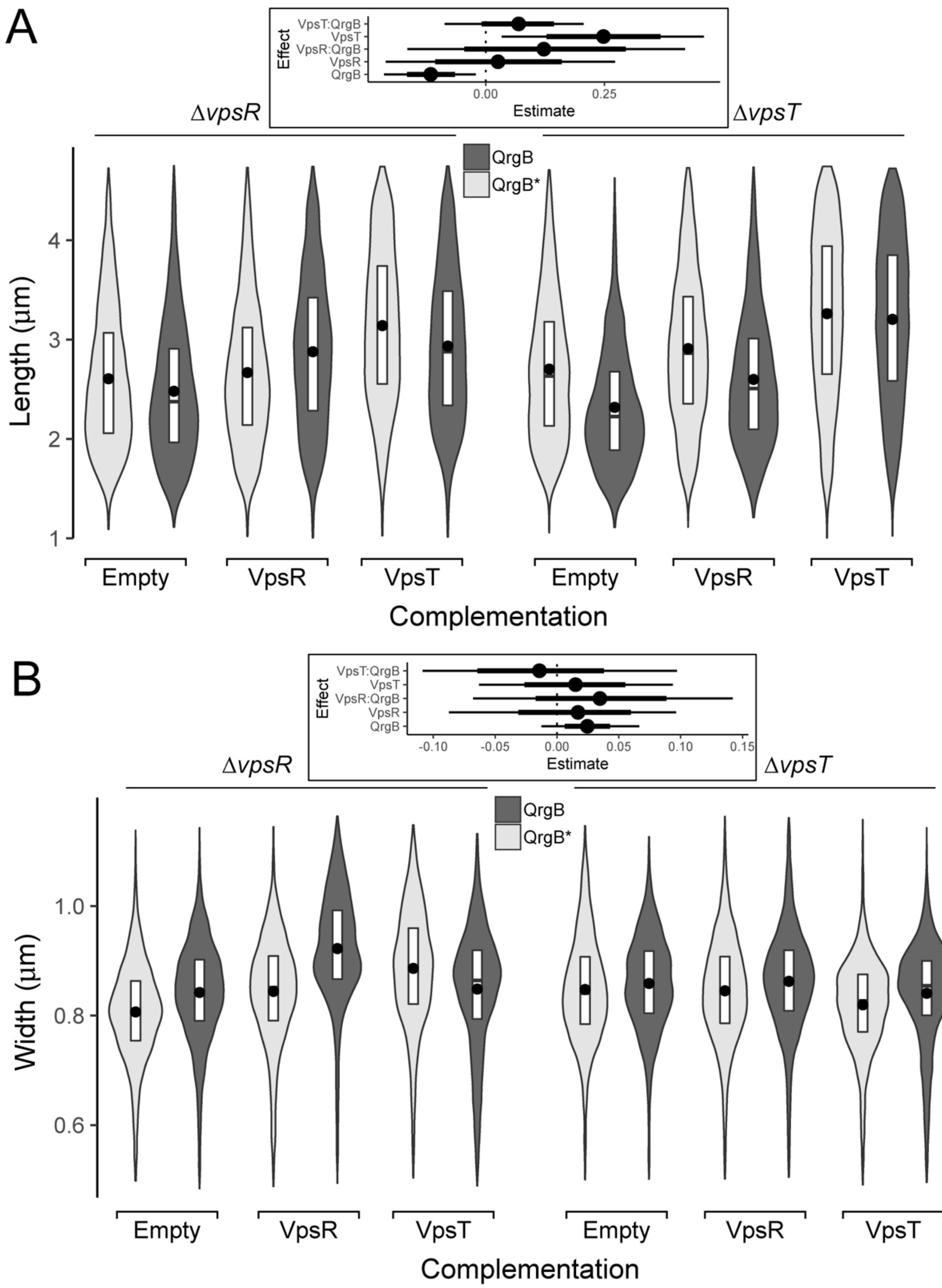
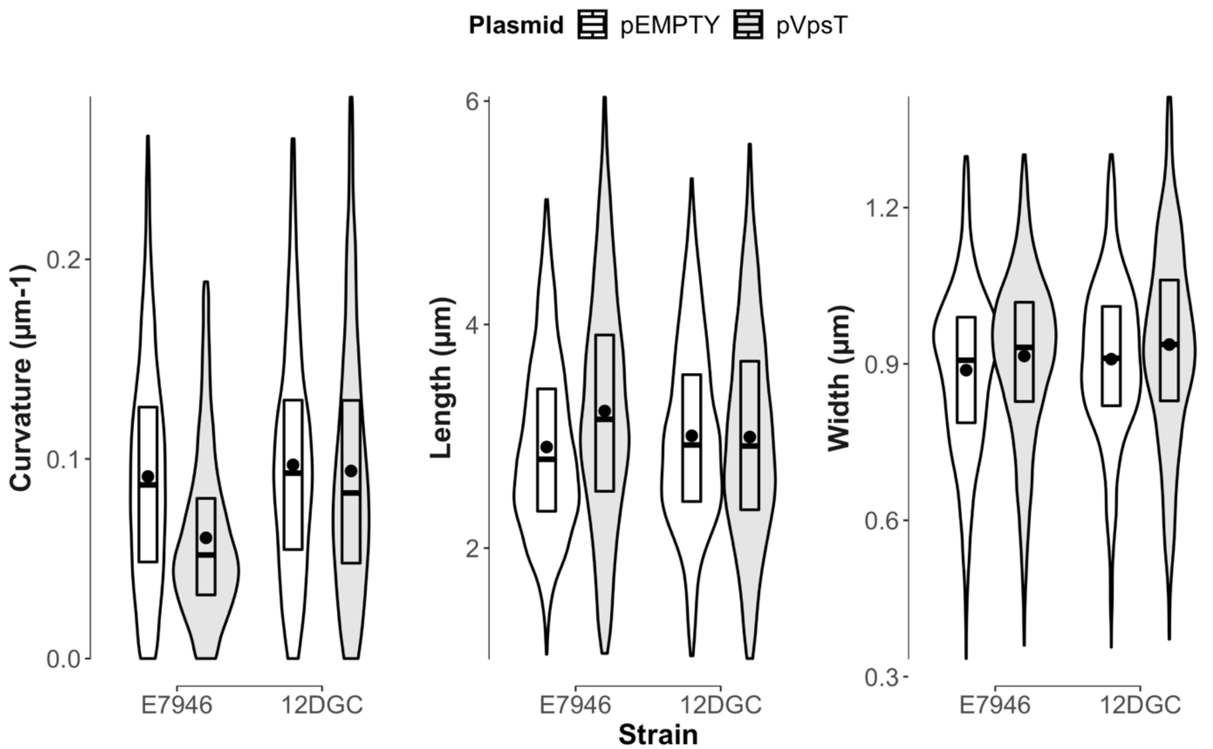
Supplemental Information Appendix 1: Figures and Legends



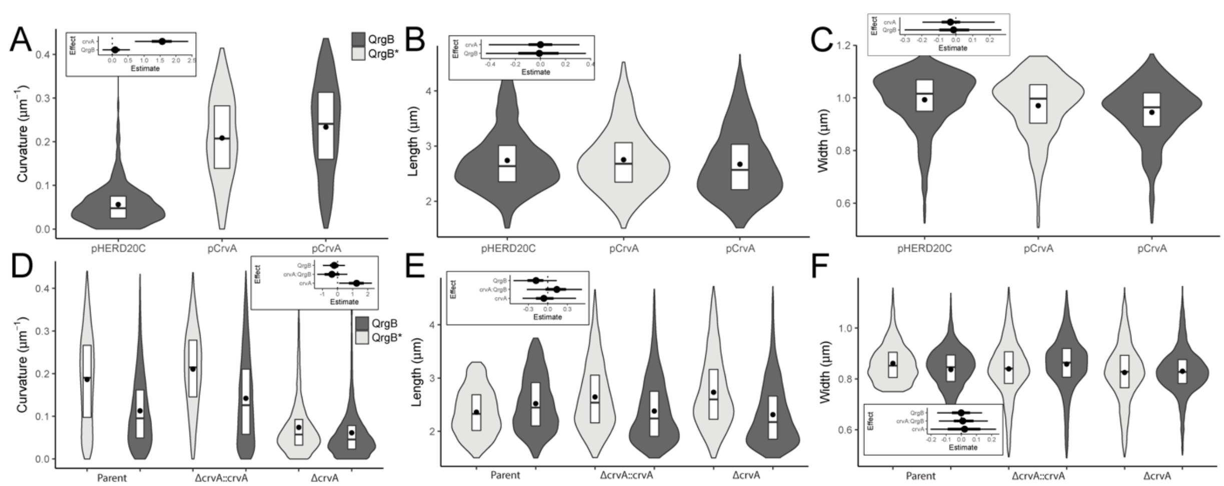
Supplemental Figure 1: C-di-GMP and IPTG Do Not Significantly Alter Cell Length or Width in WT or ∆*vpsL* Backgrounds. Distributions of cell length (A, C) and width (B,D) as a function of IPTG concentration in populations grown to early stationary phase (OD_{600} = 1.3) expressing inactive DGC (QrgB*, light) or active DGC (QrgB, dark). Panels A and B are the ∆*vpsL* strain expressing QrgB* or QrgB while panels C and D are the WT and ∆*vpsL* backgrounds with no vector. The box represents the first, second, and third quartiles. The dot represents the mean. Each distribution represents between 1,000 and 1,200 cells analyzed and pooled from two to three separate experiments. Insert) Estimated effects of QrgB expression and IPTG on the population medians for each morphological parameter. (dot = mean, thick line = 90% CI, thin line = 98% CI).



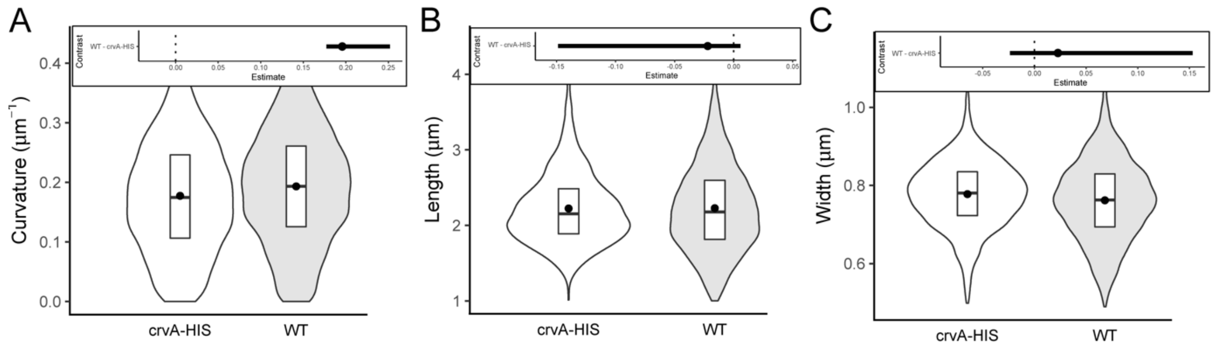
Supplementary Figure 2: Length and Width Measurements from VpsR and VpsR Complementation. Length (A) and width (B) measurements from the same cells as Figure 2. Insert) Estimated effects of QrgB expression and IPTG on the population median for each morphological parameter. (dot = mean, thick line = 90% CI, thin line = 98% CI).



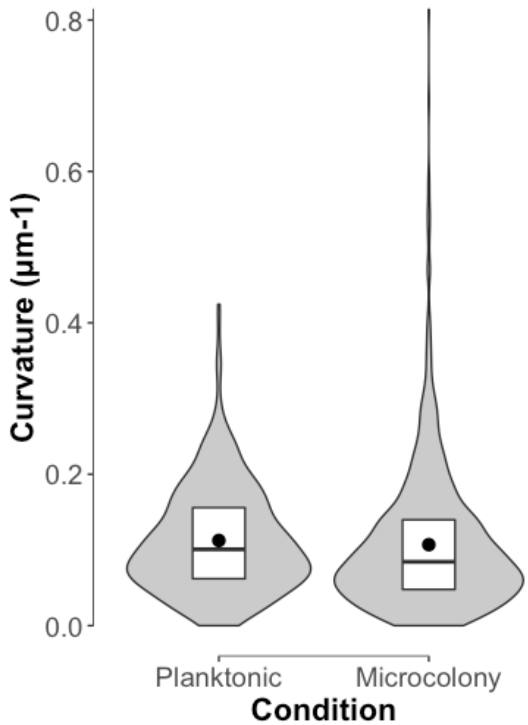
Supplementary Figure 3: VpsT Overexpression in the D12 V. cholerae E7946 DGC Mutant. VpsT (grey) and the empty vector (pEMPTY, white) was overexpressed in the WT E7946 strain and D12 E7946 V. cholerae mutant and curvature (A), length (B), and width (C) were measured. Violin plots represent the distributions from three biological replicates, boxplots show 25th, 50th, and 75th percentile, and dots represent the mean.



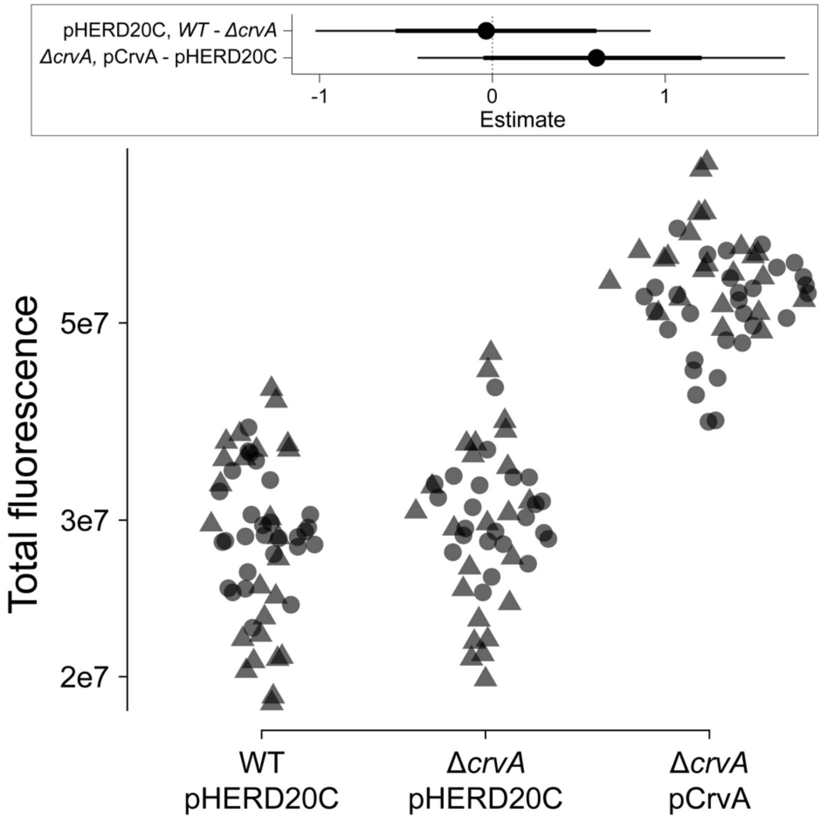
Supplemental Figure 4: Complementation of Δ*crvA* Restores Curvature. ***Plasmid Complementation***. Distributions of cell curvature (A), length (B), and width (C) from Δ*crvA* strains complemented with the control vector (pHERD20C) or CrvA (pCrvA) expressing an inactive DGC (QrgB*, light) or active DGC (QrgB, dark) in early stationary phase (OD_{600}=1.3) populations supplemented with 100 μM. ***Chromosomal Integration Complementation***. Distributions of cell curvature, length, and width of either parent, Δ*crvA*, and Δ*crvA* complemented with a single copy of CrvA driven by its native promoter integrated at the VC1807 locus (Δ*crvA* VC1807::p*crvA*-*crvA*) harboring QrgB* (light) or QrgB (dark). Cultures were grown to early stationary phase (OD_{600} = 1.3) in the presence of 100 μM IPTG. Inserts) Estimated effects of QrgB expression and *crvA* on the population median for each morphological parameter. (dot = mean, thick line = 90% CI, thin line = 98% CI).



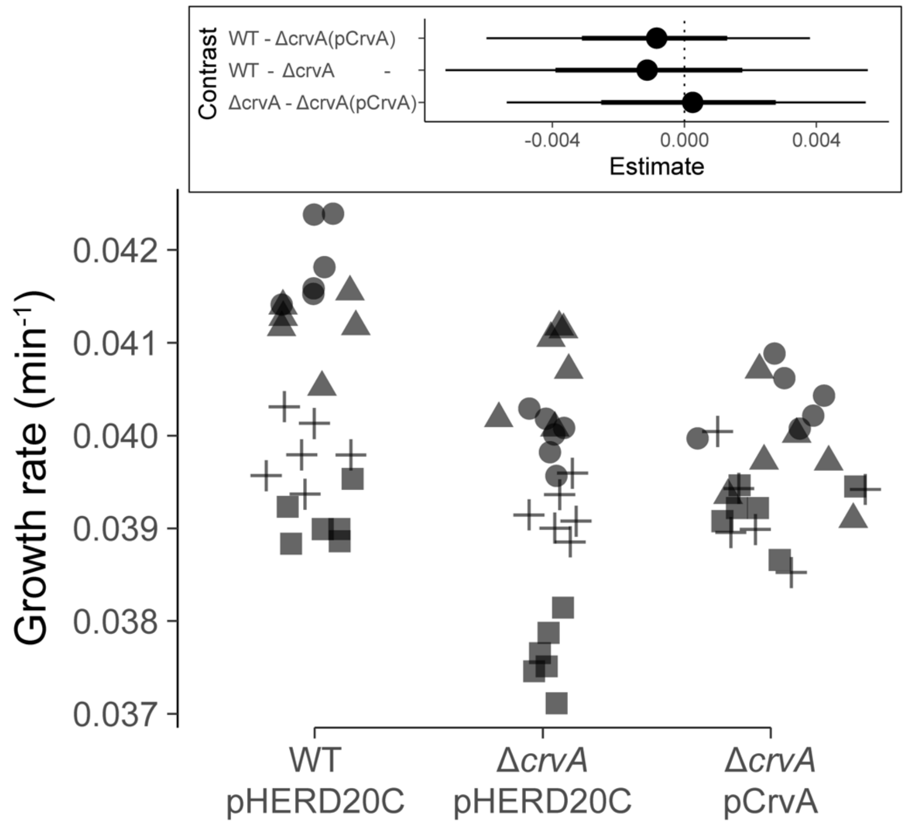
Supplemental Figure 5: Cell Shape Parameters for WT and CrvA-HIS backgrounds. Curvature (A), length (B), and width (C) were measured in WT and CrvA-HIS backgrounds. Cultures were grown to early stationary phase (OD_{600} ~ 1.3) and imaged by phase microscopy. Insert) Estimated differences in the population medians of each morphological parameters between mutant strains. (dot = mean, thick line = 90% CI, thin line = 98% CI).



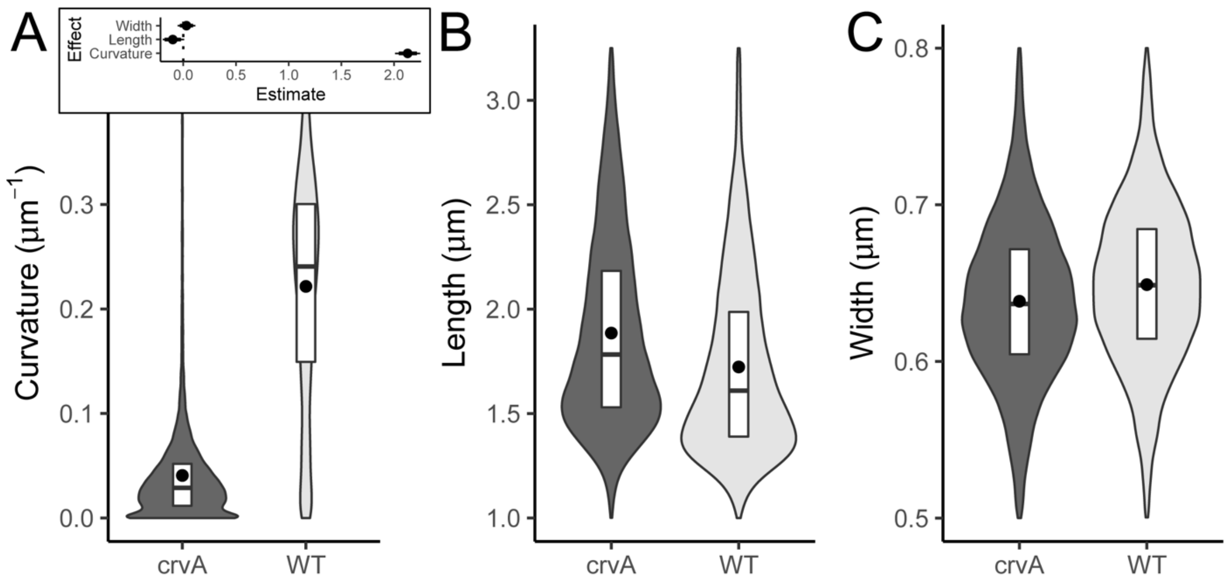
Supplemental Figure 6: Cell Shape Curvature of Surface Attached and Planktonic Cells. Curvature of in the planktonic phase or cells attached to the surface (Microcolony) was measured for the WT strain at 8 hours following the conditions described for Fig. 5. Violin plots represent the curvature distributions for two to three replicates, boxplots represent 25th, 50th, and 75th percentiles, and dots represent the mean curvature.



Supplemental Figure 7: Effect of Curvature on Surface Coverage. Total fluorescence intensities from stained cells (FM4-64) attached to a glass surface for different strains. Each dot represents one field of view. Dot shapes represents independent replicates. Insert) Estimate of the differences in the means explained by *crvA* and pCrvA (dot = mean, thick line = 90% CI, thin line = 98% CI).



Supplemental Figure 8: WT, Straight, and Curved Populations Have Indistinguishable Growth Rates. Each shape represents an independent trial with six biological replicates. Insert) Estimate of the differences in the means between WT-pHERD20C and Δ*crvA*-pCrvA, WT-pHERD20C and Δ*crvA*-pHERD20C, and Δ*crvA*-pHERD20C and Δ*crvA*-pCrvA (dot = mean, thick line = 90% CI, thin line = 98% CI).



Supplemental Figure 9: Cell shape Parameters of WT and Δ*crvA* (*crvA*) cells. Curvature (A), length (B), and width (C) measurements of cells grown to early stationary phase in M9 pyruvate. Insert) Estimated effect of crvA deletion on populations medians for each morphological parameter. (dot = mean, thick line = 90% CI, thin line = 98% CI).

Supplemental Information Appendix 2: Strains, Plasmids, and Oligonucleotides

Supplemental Table 1: Strains Used in this Study

Strain	Description	Reference
	Escherichia coli	
S17-λpir	Tpr Smr *recA thi pro hsdR17*($r_K^- m_K^+$) *RP4::2-Tc*::Mu Km Tn7 λpir	Lab stock
	Vibrio cholerae	
C6706	Wild Type	Lab Stock
CW2034	C6706Δ*vpsL*	(11)
JC1195	CW2034Δ*vpsT*	(14)
WN310	CW2034Δ*vpsR*	(14)
NF6	CW2034Δ*crvA*	This study
NF7	Δ*crvA*	This study
NF8	CW2034Δ*crvA* VC1807::P_{crvA}-*crvA*	This study
NF9	CW2034 *crvA::crvA-HIS*	This study
E7946 Sm^R	Wild Type	(30)
Δ12DGC	ΔVCA0956, ΔVC1599, ΔVC2454, ΔVC1104, ΔVCA0939, ΔVCA0074, ΔVC2224, ΔVC1376, ΔVC1067, ΔVC1216, ΔVCA0697, ΔVC2285, P*tac*-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Tm^R	(30)

Supplemental Table 2: Plasmids Used in this Study

Plasmid	Description	Reference
pBBRlux	*luxABCDE* containing promoter-less plasmid, Cam^R	Lab collection
pEVS143	*pTac* overexpression vector, Kan^R	(66)
pKAS32	Sucide vector for mutant construction, Amp^R	(53)
pBRP333	Control expression vector, Kan^R	(20)
pBRP1	*qrgB** (inactive DGC) cloned into expression vector pMMB67Eh, Amp^R	(20)
pBRP2	*qrgB* (active DGC) cloned into expression vector, pMMB67Eh, Amp^R	(20)
pCMW131	*vpsR* in pEVS143, Kan^R	(23)
pCMW132	*vpsT* in pEVS143, Kan^R	(23)
pHERD20T	P_{BAD} Expression vector	(67)
pNF057	pHERD20T $_{p}$BAD expression vector with Cam^R replacing Amp^R	(24)
pNF069	358 bp upstream of ATG of *crvA* (P_{crvA}) in pBBRlux	This study
pNF070	Deletion construct for *crvA* in pKAS32, Amp^R	This study
pNF071	VC1807 knock-in construct, Amp^R	This study
pNF072	VC1807 knock-In construct with CrvA driven by native promoter P_{crvA}, Amp^R	This study
pNF073 (pCrvA)	CrvA from C6706 gDNA into pNF057	This study

Supplemental Table 3: Oligonucleotides Used in this Study

Oligo Name	5' Overhang	Gene Specific
Vector Construction		
pNF069_For[1]	cggtggcggccgctctagaa	GGTATCTCAAATTGCTTCAAAAC
pNF069_Rev[2]	ccattttgcggccgcaactagag	AAAGTGGGAAAGACAAAC
pNF070_US_For[3]	tgcgcatgctagctatagtt	CAGTAGTAGCGGATCATC
pNF070_US_Rev	ttttgtgcgg	AAAGTGGGAAAGACAAAC
pNF070_DS_For[4]	ttcccacttt	CCGCACAAAAATCCAACG
pNF070_DS_Rev	gtggaattcccgggagagct	TGGTGAAGCTGACTTTTTG
pNF071_US_For	tgcgcatgctagctatagtt	ATTTTTCAGTTGGCCTAC
pNF071_US_Rev	ttttgtgcgg	TAGTCACCTCTATTGTTAACTTG
pNF071_DS_For	ttcccacttt	TAGTCGAAAATAAAAAAAAGAGG
pNF071_DS_Rev	gtggaattcccgggagagct	CGATGAGGATAAAAAACAC
pNF072_For	acaatagaggtgactaggta	GGTATCTCAAATTGCTTCAAAAC
pNF072_Rev	cgactagagctcaccggtac	CTAGCTGTCTTTGTTTGG
pNF073_For	atctgataagaattcgagct	ATGTGGCTAAACATAAATATGTTG
pNF073_Rev	tctagaggatccccgggtac	CTAGCTGTCTTTGTTTGG
pNF074_US_For	tgcgcatgctagctatagtt	ATCGATGAGAAATTGAGTAAC
pNF074_US_Rev	-	GCTGTCTTTGTTTGGTCTG
pNF074_DS_For	tcagaccaaacaaagacagccatcaccatcaccatcac	TAGCCGCACAAAAATCCAAC
pNF074_DS_Rev	gtggaattcccgggagagct	GCGAAACAGTACTGCGAG
qRT-PCR		
gyrA-For	-	AAATTCATCTTCATGGCAAC
gyrA-Rev	-	GCGATGTTTTCTTCACAG
crvA-For	-	GTACTCCCGTTGAACTCGTATT
crvA-Rev	-	CCGTAATCACACGATTGTAGGT

[1] **For = Forward**
[2] **Rev = Reverse**
[3] **US - Amplifies Upstream Fragment**
[4] **DS - Amplifies Downstream Fragment**