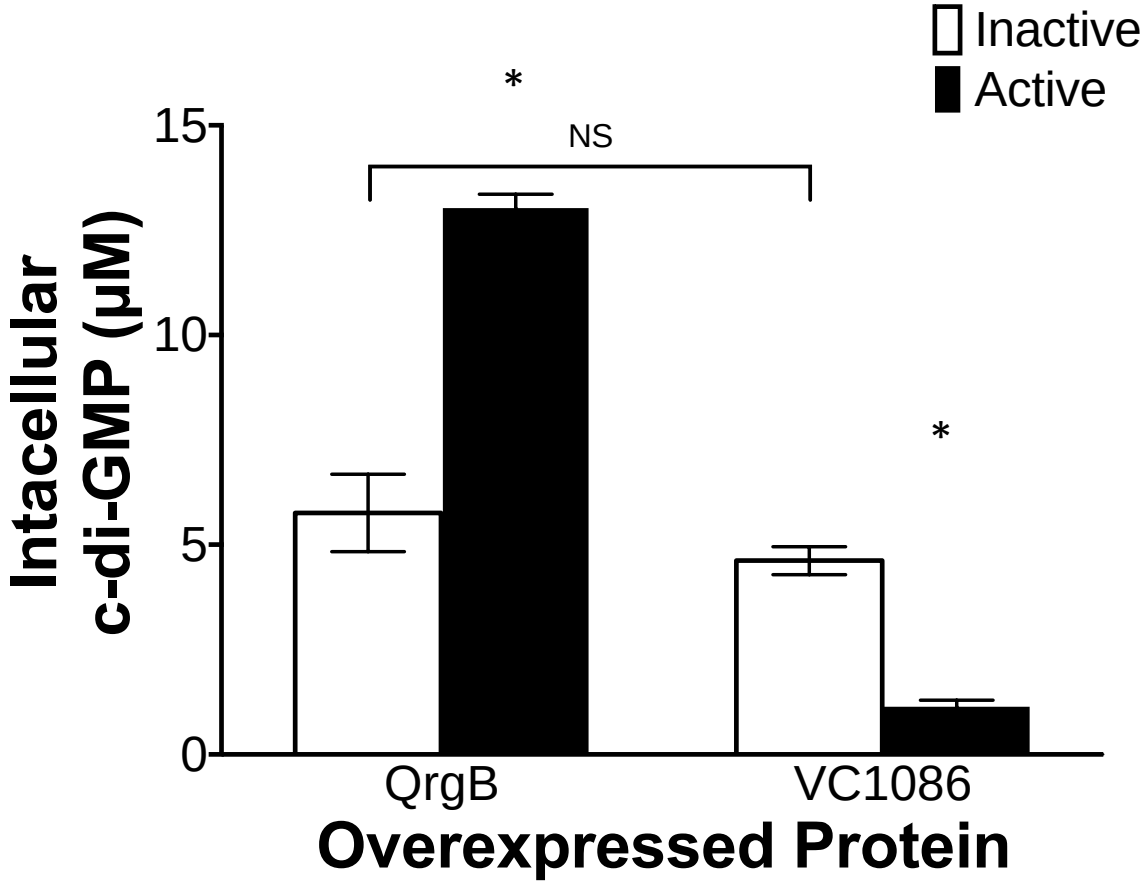


Figure S1: Intracellular concentrations of c-di-GMP generated by QrgB and VC1086. C-di-GMP was extracted from WT *V. cholerae* strains grown to an OD_{595} of 0.2 expressing QrgB, VC1086, or their enzymatically inactive variants and measured by LC-MS/MS. The intracellular concentration of c-di-GMP measured in strains expressing active QrgB and VC1086 enzymes were significantly different (*,$p<0.05$) when compared to their catalytically inactive counterparts.

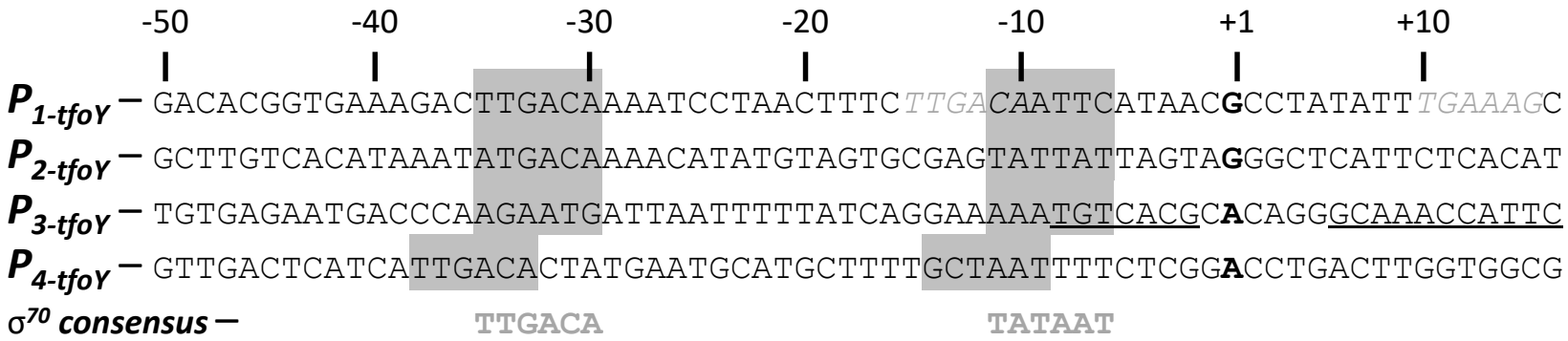


Figure S2: Alignment of P_{tfoY} promoters. -35 and -10 regions of the promoters highlighted in grey; a second possible set of -35 -10 regions near $P_{1\text{-}tfoY}$ is indicated by grey italics. Transcriptional start sites determined by 5'-RACE are indicated in bold. Regions of the $P_{3\text{-}tfoY}$ promoter which overlap with the sequences that form the 5'-sides of the P1 and P2 structures of the Vc2 aptamer are underlined.

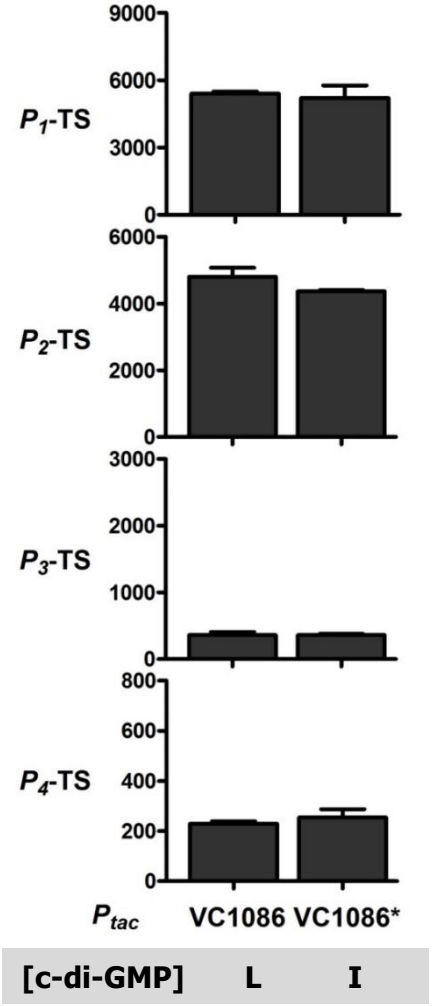


Figure S3: The *tfoY* promoters are not regulated at low c-di-GMP concentrations. Expression of the four promoter transcriptional fusions indicated in Fig. 2 in response to decreasing concentrations of c-di-GMP (P_{tac}-VC1086) or the active site mutant control (P_{tac}-VC1086*) in the parent strain are shown. The data represent fluorescence divided by OD_{595}. Error bars indicate the standard deviation of three biological replicates.

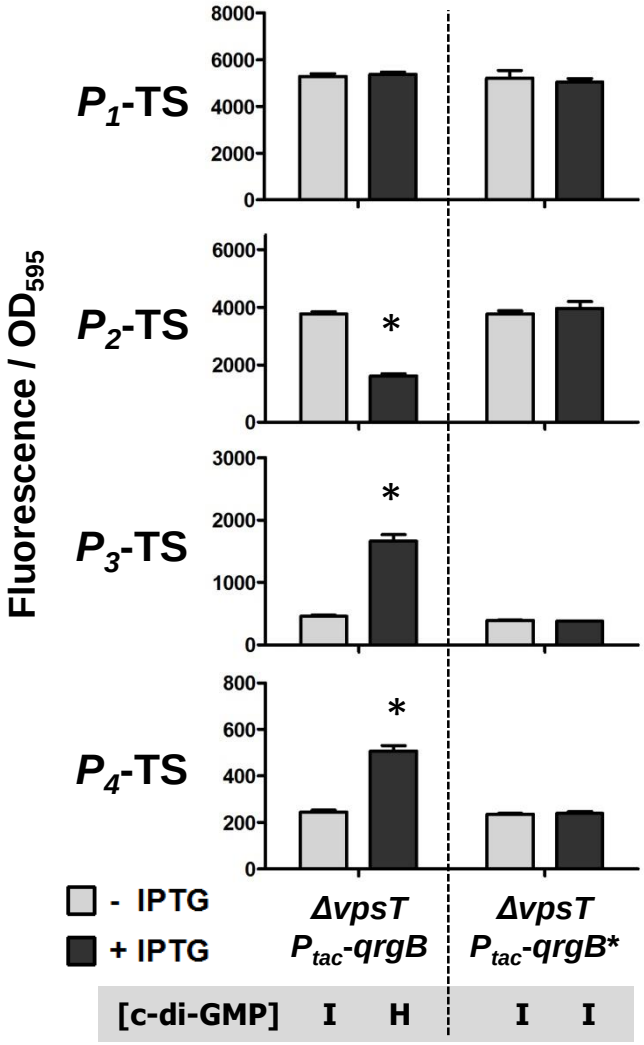
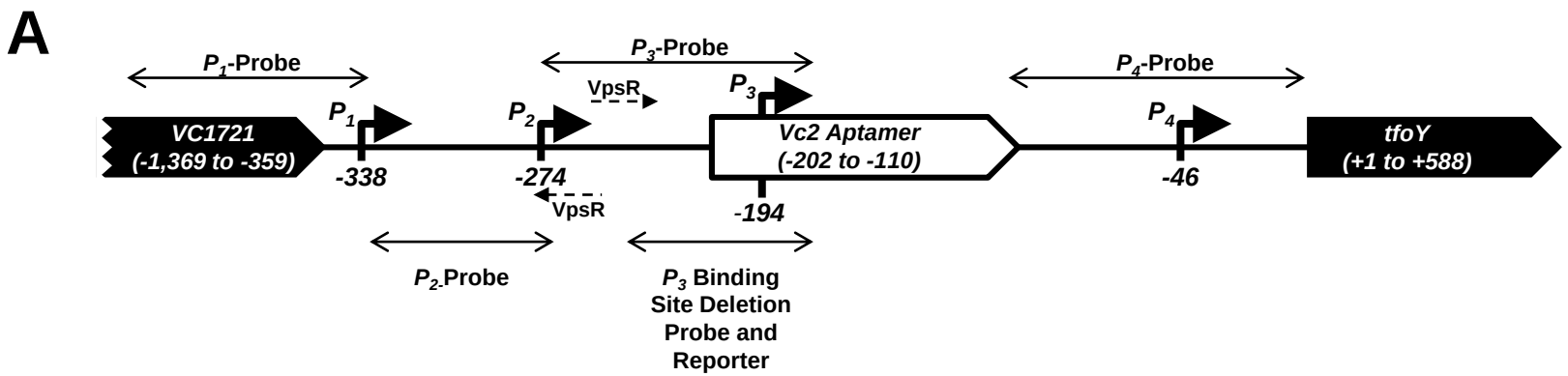
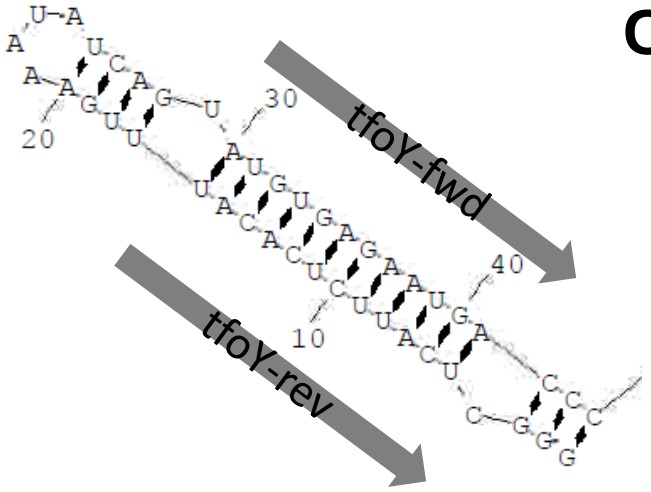


Figure S4: C-di-GMP regulation of *tfoY* promoters is VpsT-independent. Expression of the indicated transcription reporters shown in Fig. 2 was assessed in a Δ*vpsT V. cholerae* mutant with the DGC QrgB or its active site mutant derivative QrgB*. Error bars indicate the standard deviation among three biological replicates. *, $p<0.05$ when comparing the absence and addition of IPTG.





C

```
VC0930    GTCTCATTAC-TGAGGCGT
vpsT      TTCTTAATAT-TGAGAAGG
vpsL      GTCTTAGAAT-TGATGCAG
aphA      TTTTCATTAT-TGAGAATA
tfoY-rev  ATTTCAA-ATGTGAGAATGAGCCC
tfoY-fwd  ATATCAGTATGTGAGAATGA-CCC
```

Figure S5: VpsR binding sites are located between $P_{3\text{-}tfoY}$ and $P_{4\text{-}tfoY}$ promoters. (A) Genetic map indicates the regions of sequence used in the construction of probes for the EMSA, in bracketed arrows, shown in Figure 5. Dashed arrows indicate the putative VpsR binding sites downstream of P_2. **(B)** The sequence of the VpsR binding sites at the *tfoY* locus form an inverted repeat that was previously predicted to serve as a rho-independent transcriptional terminator for the upstream gene *VC1721*. **(C)** An alignment of the two VpsR binding sites with other experimentally verified VpsR binding sites from Lin et al., 2007. The alignment uses the anti-sense strand bases, relative to the coding sequence of *tfoY*, of the upstream putative binding site ("vpsR-rev"), and the sense strand bases of the downstream putative binding site ("vpsR-fwd"). Highlighted bases indicate positions of shared sequence identity.

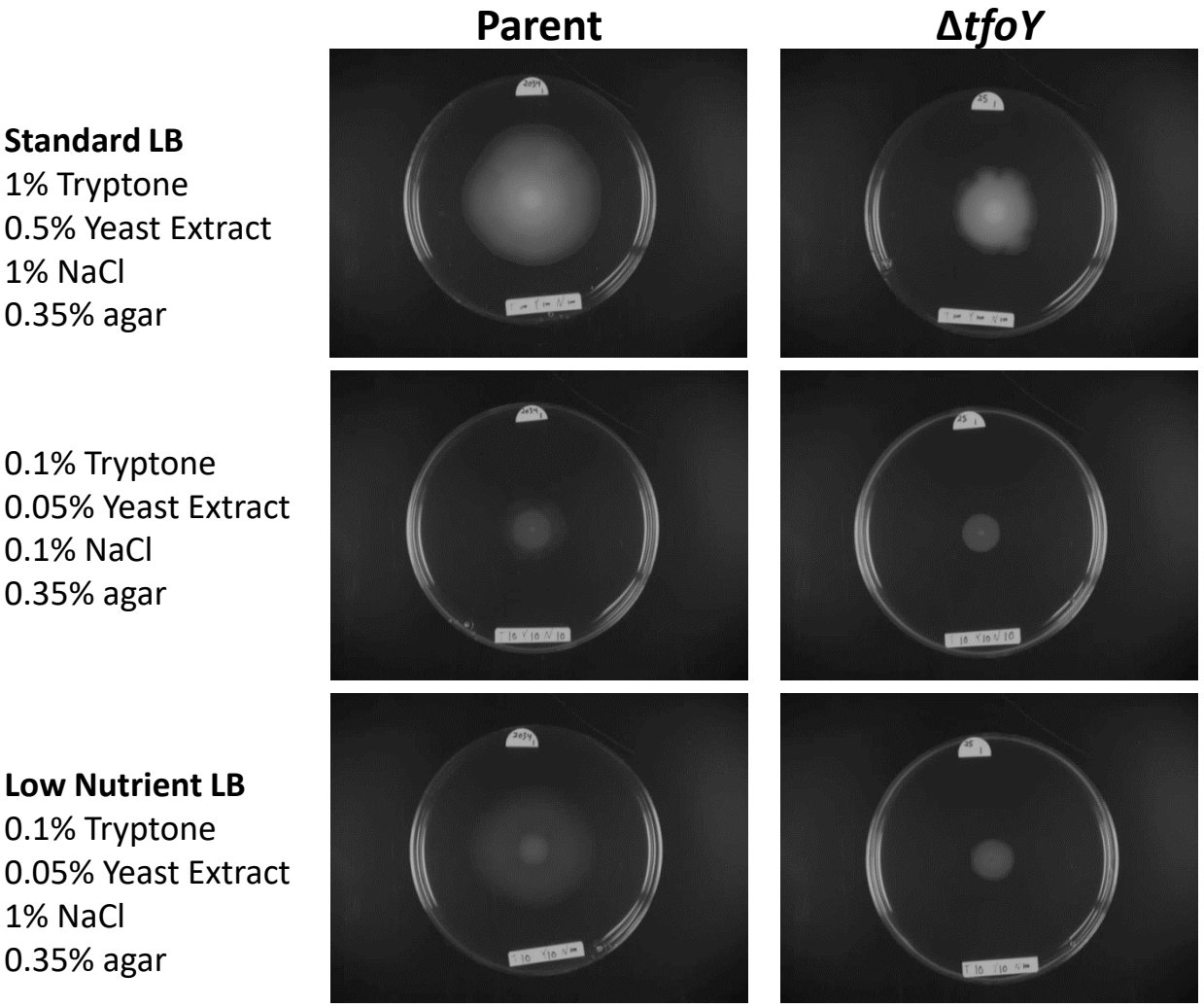
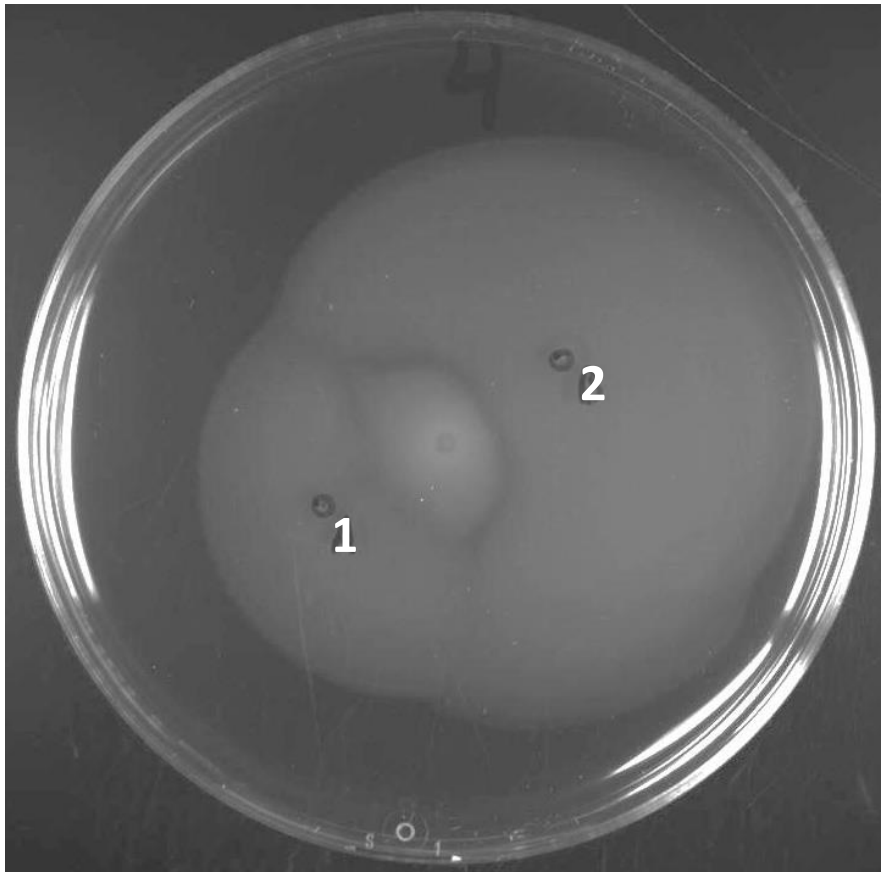


Figure S6: Low nutrient LB agar amplified bi-phasic motility of *V. cholerae* in a *tfoY*-dependent manner. Motility of the parent and Δ*tfoY V. cholerae* in the indicated nutrient (wt/vol) conditions at 20 hours is shown. Motility is dependent on NaCl due to the sodium driven flagellum of *V. cholerae,* but the impact of *tfoY* on dispersive motility is most evident in low nutrient LB that contains the standard LB concentration of NaCl but 1/10 the standard concentration of both tryptone and yeast extract.

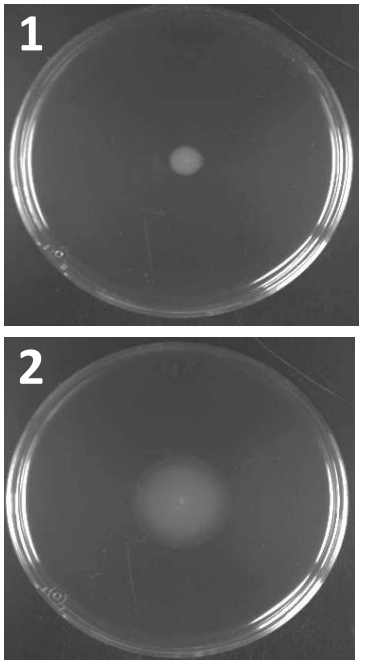
A

Original parent Motility Plate at 25 Hours



B

Motility of Isolated single colonies at 10 hours



C

Motility of Isolate 1 at 10 hours

control vector ***P_{tac}-tfoY***

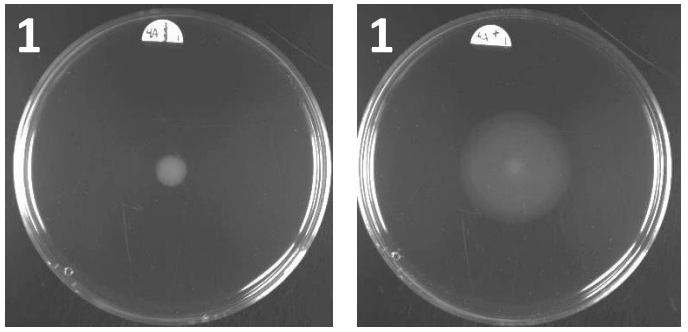
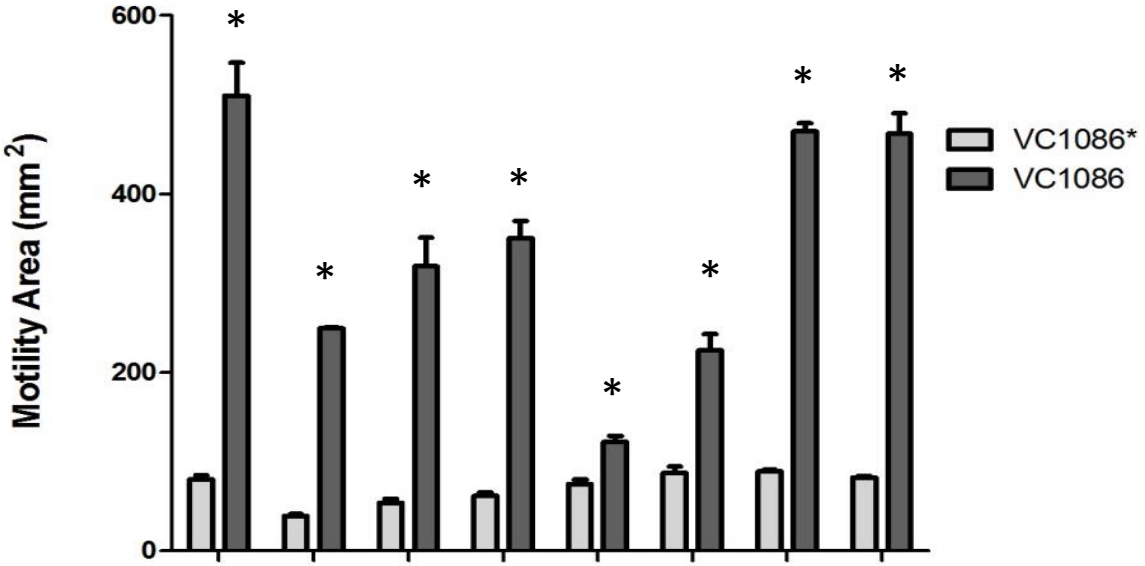


Figure S7: Dispersive motility is not caused by genetic suppressors. (A) Bacteria were recovered after 25 hours from the dispersive motility region of a low nutrient 0.35% agar motility plate which had been inoculated with the *V. cholerae* parent strain in the normal dispersive zone (1) or a hyper-motile mutant (2). **(B)** The recovered bacteria were streaked on a new plate and motility of a single isolated colony was examined at 10 hours. **(C)** TfoY overexpression vector was mated into the isolated colony from region 1 shown in B and motility was assayed at 10 hours in plates containing IPTG. This assay was replicated many times with different biological samples.



FlrA	WT	WT	WT	R176H	R176H	R176H	R176H	R176H
tfoY	WT	*Δ*	WT	WT	*Δ*	WT	*Δ*	WT
Vc2	WT	WT	G20U, C92U	WT	WT	G20U, C92U	WT	G20U, C92U
lacZ	WT	WT	WT	WT	WT	WT	::*tfoY*	::*tfoY*

Figure S8: Inhibition of FlrA and induction of TfoY both promote enhanced motility at low c-di-GMP. Motility of the mutants indicated on the x-axis in low nutrient LB 0.35% agar plates is indicated at 10 hours. Dark bars represent the low c-di-GMP state while light bars are the intermediate c-di-GMP state. All indicated mutations are located on the chromosome. Error bars indicate the standard deviation. *, $p<0.05$ when comparing VC1086 versus VC1086*.