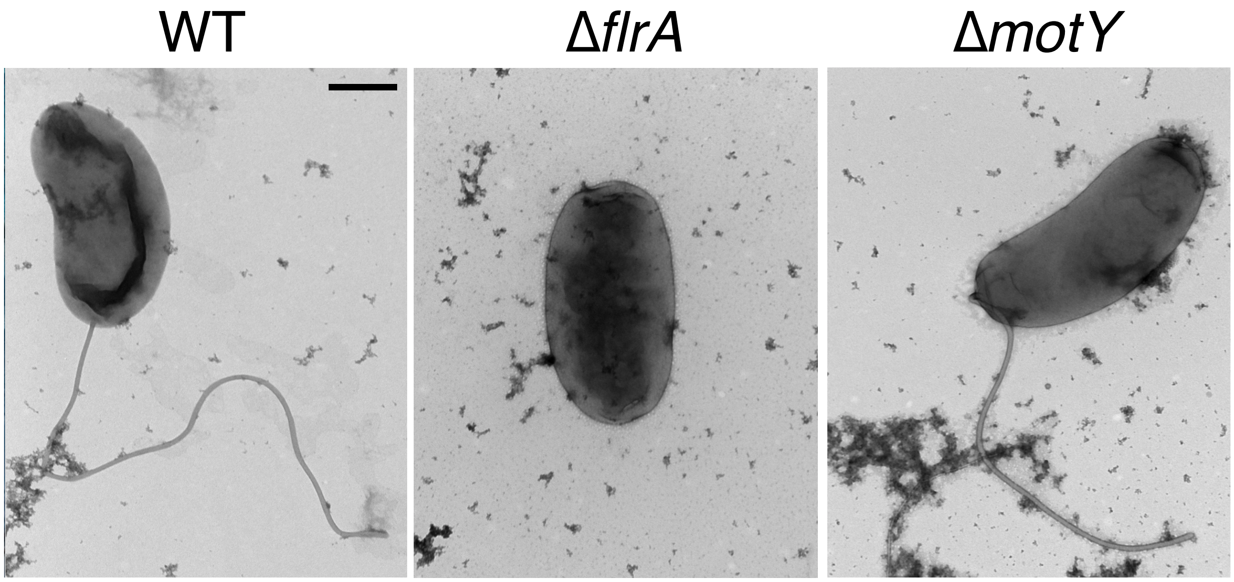
Supplementary Information

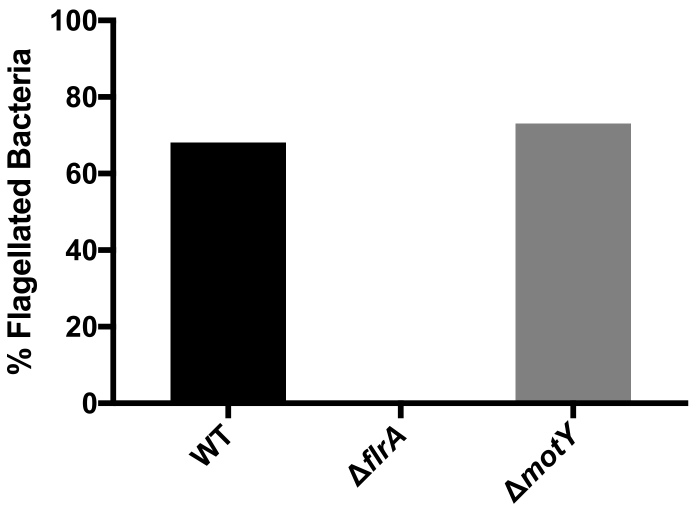
Vibrio cholerae motility exerts drag force to impede attack by the bacterial predator *Bdellovibrio bacteriovorus*

Duncan *et al.*

Supplementary Figure 1a

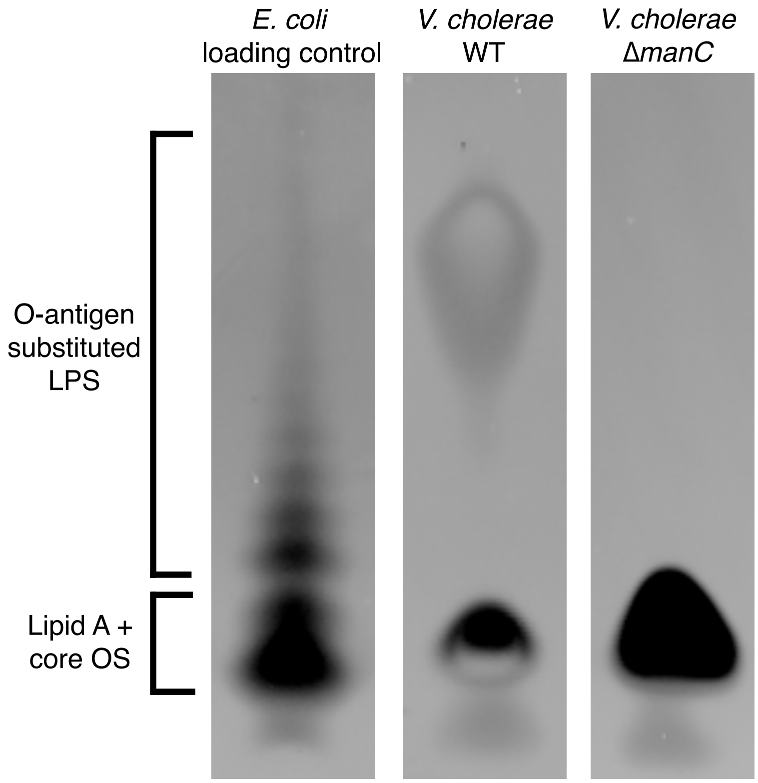


Supplementary Figure 1b



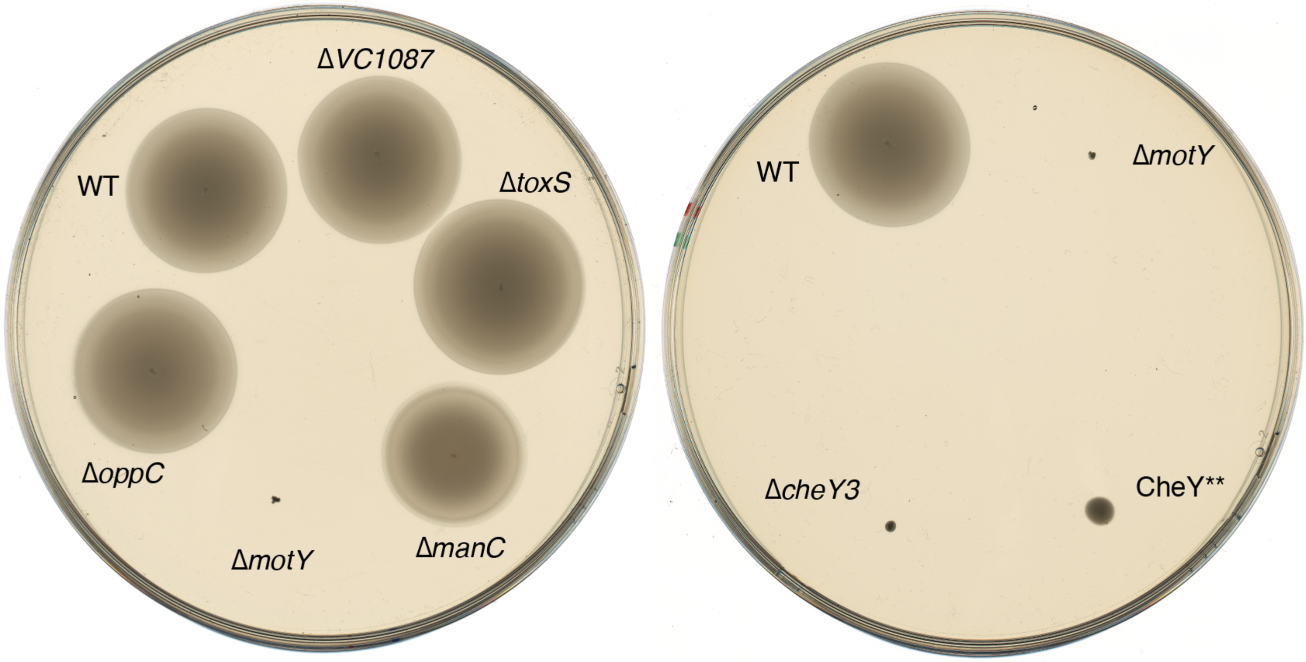
Supplementary Figure 1. The non-motile ΔflrA strain lacks flagella. **a**, Representative transmission electron microscopy images show the Δ*flrA* mutant lacks flagella, while Δ*motY* is also non-motile but retains its flagella. The scale bar indicates 500nm. **b**, The percent of flagellated bacteria for each strain is shown. Only one out of 113 Δ*flrA* bacteria possessed a flagellum.

Supplementary Figure 2



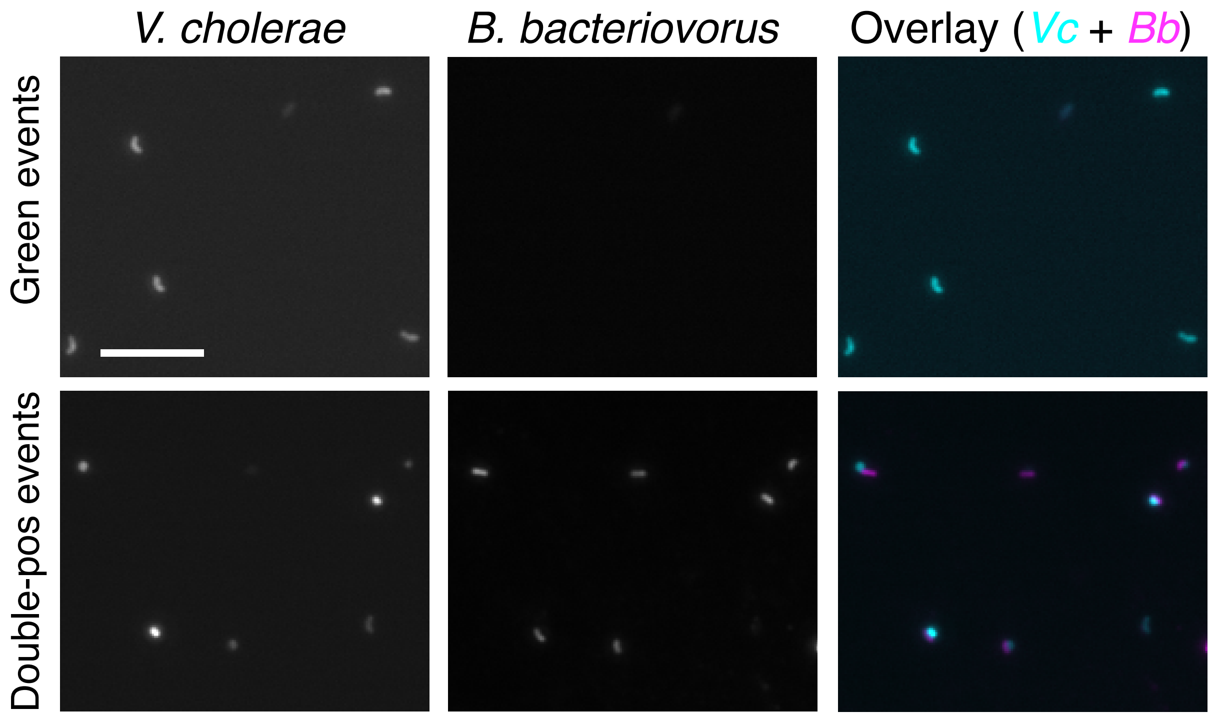
Supplementary Figure 2. The Δ*manC* strain does not produce O-antigen. Silver stain of an SDS-page gel following phenol extraction of LPS. Consistent with previous studies, the Δ*manC* mutant produces no observable O-antigen when compared to WT *V. cholerae*.

Supplementary Figure 3



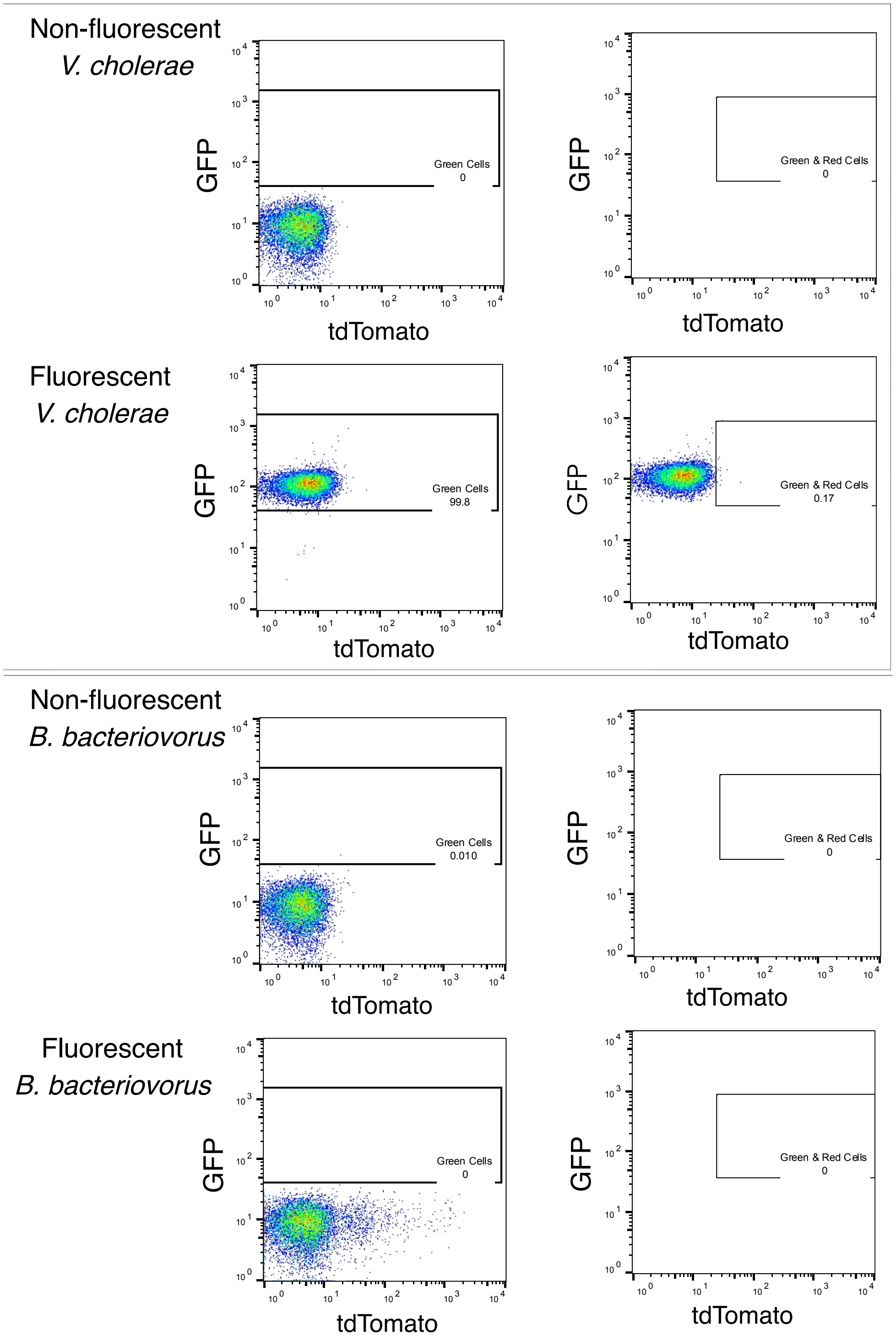
Supplementary Figure 3. Soft agar motility assay. Each mutant strain shows a similar swimming diameter compared to WT *V. cholerae* apart from the non-motile Δ*motY* strain and the chemotaxis mutant Δ*cheY3* and CheY** strains.

Supplementary Figure 4.



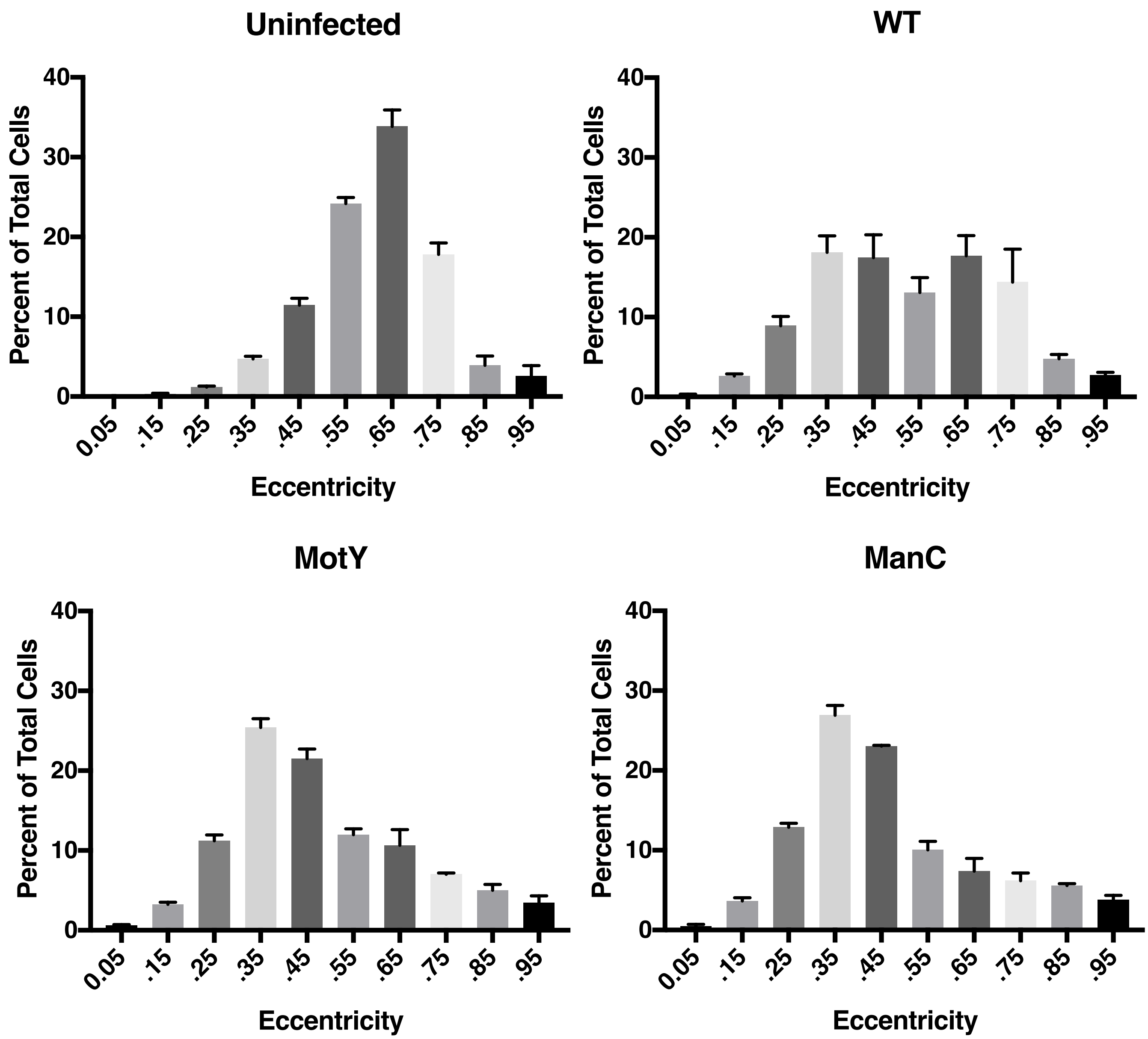
Supplementary Figure 4. Sorting and fluorescence microscopy to confirm flow cytometry distinguishes green-only and double-positive events. We sorted the two relevant populations into separate tubes and observed the cells by fluorescence microscopy. The gating strategy correctly identified green-only (*V. cholerae*) and double-positive (*V.* cholerae + *B. bacteriovorus*) events. In the overlay panels, *V. cholerae* are cyan and *B. bacteriovorus* are magenta.

Supplementary Figure 5



Supplementary Figure 5 Gating strategy for flow cytometry analysis of *B. bacteriovorus* interaction with *V. cholerae*. 10,000 events were collected for each sample. The gating strategy was designed to limit false-positive interactions.

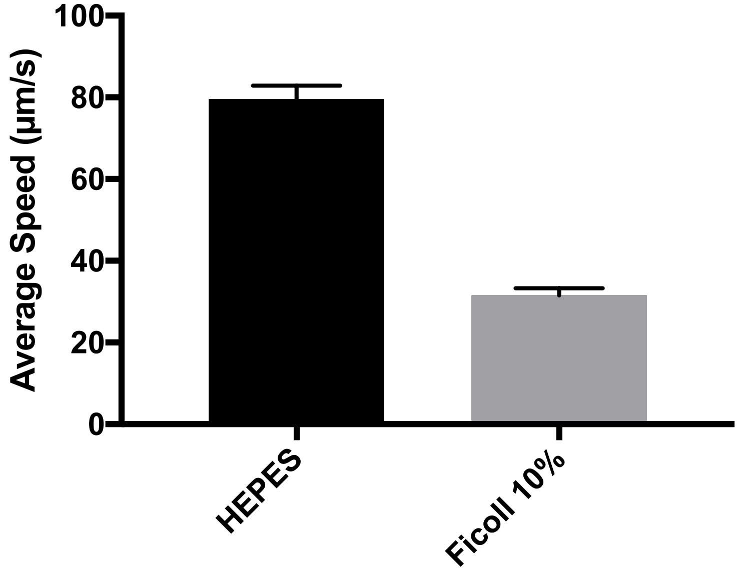
Supplementary Figure 6



Supplementary Figure 6. Histograms of eccentricity values used in Fig 4b.

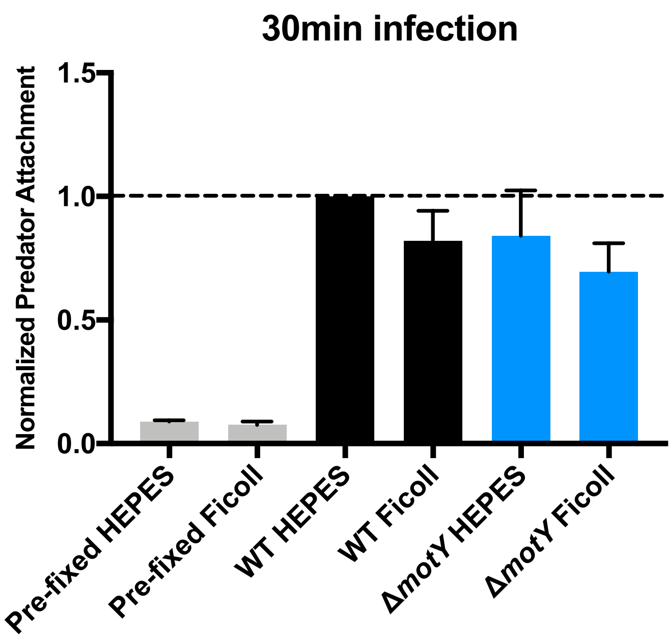
Eccentricity values closer to zero indicate more rounded cells. Each bar represents cell values within 0.05 of the marked value. The average is shown for three biological replicates. Error bars represent standard error of the mean.

Supplementary Figure 7

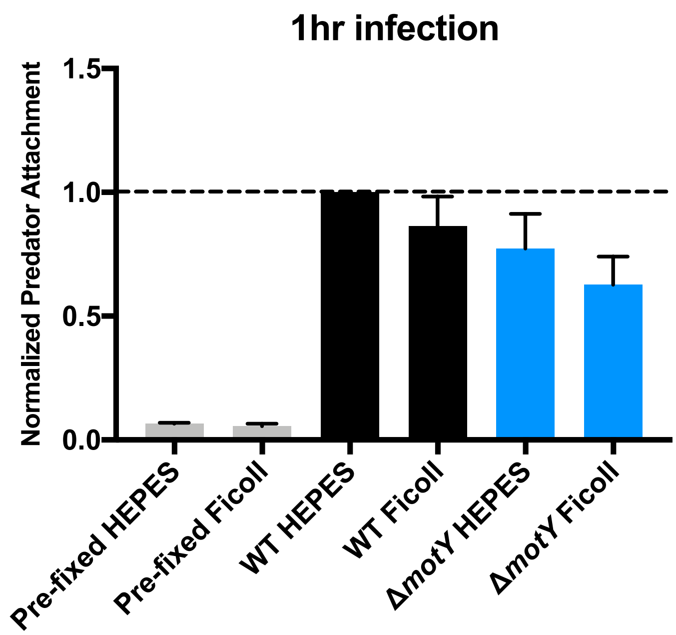


Supplementary Figure 7. 10% ficoll decreases *V. cholerae* swimming speed 2.5-fold. A 10% ficoll solution in HEPES reduces *V. cholerae* swimming speed from 80*µ*m/s to 32*µ*m/s. This 2.5-fold decrease in speed is less than the 6.5-fold increase in viscosity from adding ficoll. Individual bacteria were manually tracked using time-lapse microscopy. Error bars represent standard error of the mean. Each bar represents the average of three biological replicates.

Supplementary Figure 8a

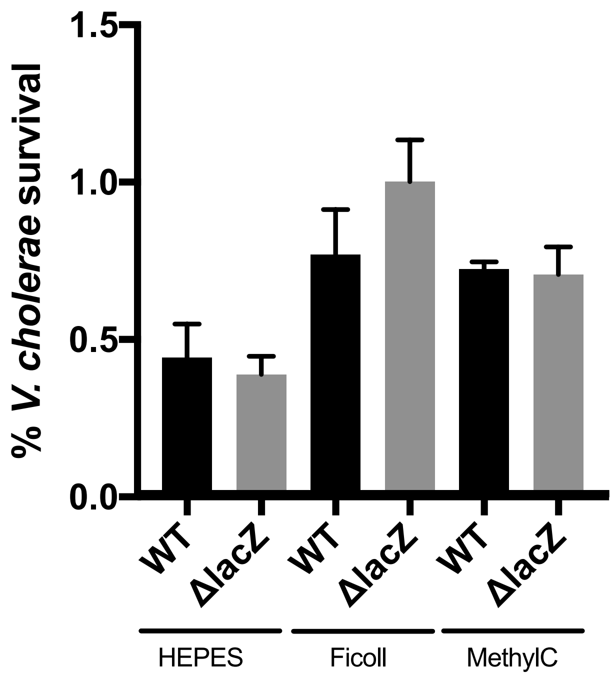


Supplementary Figure 8b

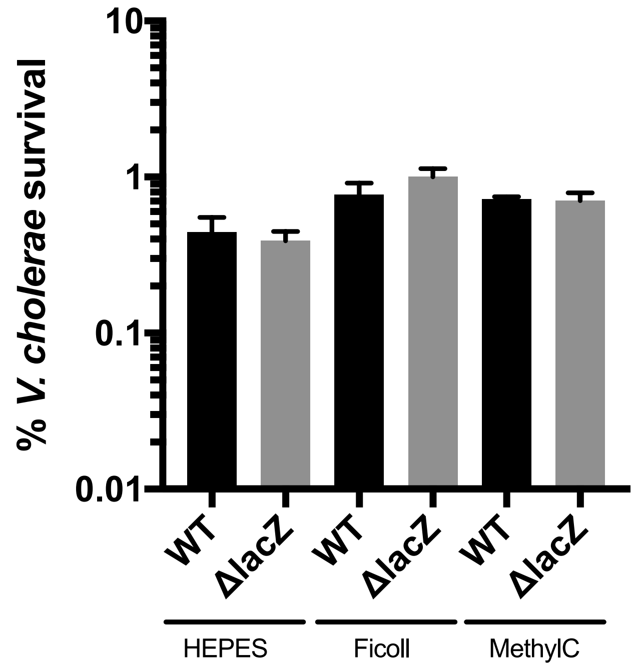


Supplementary Figure 8. Increased viscosity does not significantly reduce predator-prey attachment. Attachment was measured by flow cytometry at 30 minutes (**a**) or one hour (**b**) post infection in HEPES or 10% ficoll media. While both WT and Δ*motY V. cholerae* showed a slight decrease in attachment, only the motile WT strain demonstrates improved survival in the high viscosity medium. Each bar shows the average of three biological replicates. Error bars represent standard error of the mean.

Supplementary Figure 9a

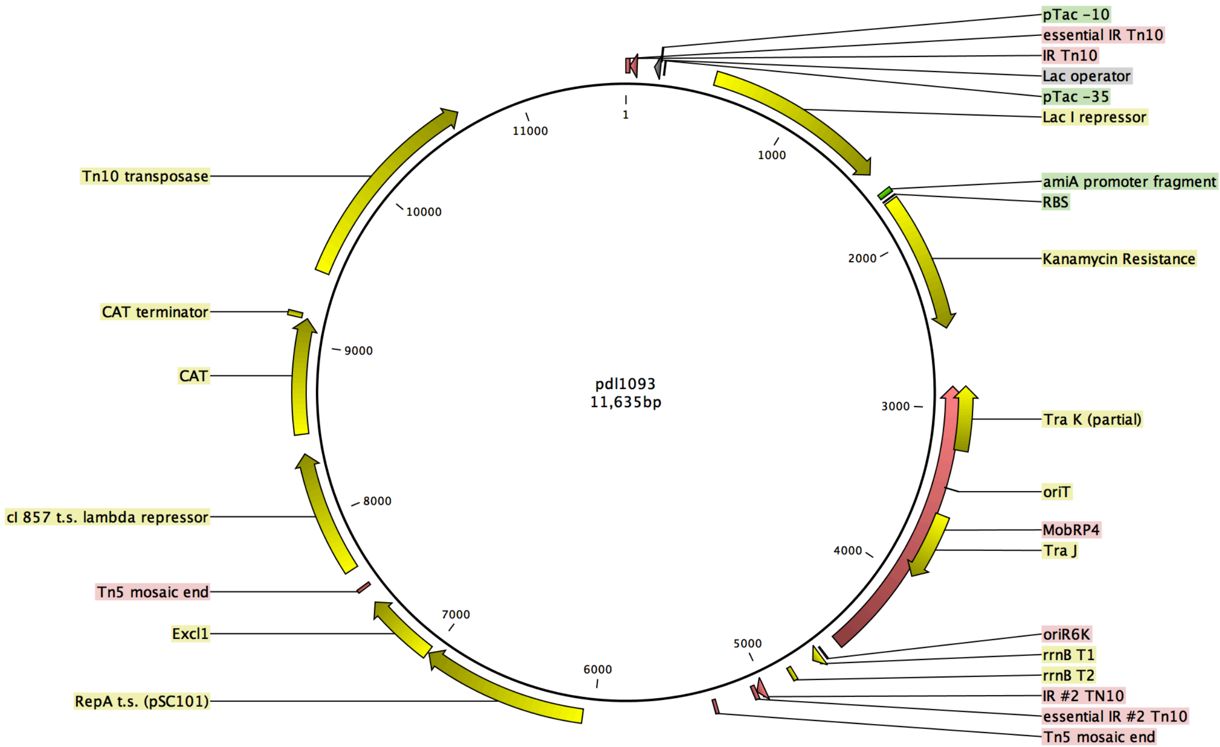


Supplementary Figure 9b



Supplementary Figure 9. Increased viscosity does not alter the competitive index between WT *V. cholerae* and an isogenic ΔlacZ strain. a-b, Survival percentages are shown for the WT vs. Δ*lacZ* controls from Fig. 4a and 4b. The survival rates are shown on a linear scale (a) and log scale (b) to allow comparison to the results in Figure 4a-b. Each bar shows the average of three biological replicates. Error bars represent standard error of the mean.

Supplementary Figure 10



Supplementary Figure 10. pDL1093, a temperature-sensitive mTn*10* delivery vector.

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Supplementary Methods

Transmission electron microscopy (TEM). TEM was performed as previously described[1] using overnight cultures of cells grown in LB broth, washed with PBS, spotted on formvar coated grids, strained with uranyl acetate (1%) and viewed with a JEM-1210 electron microscope. At least 10 individual bacteria were imaged for each of the *V. cholerae* strains.

Phenol extraction of LPS. LPS was extracted and analyzed as previously described[2]. Briefly, phenol extraction was performed using phase-lock gel light tubes (Eppendorf). Extracts were centrifuged at 75,000× g for 60 min, the pellet was washed with TM buffer and centrifuged as before. Purified LPS was separated on a 4–12% NuPage Bis-Tris gel (Invitrogen) and visualized by silver-staining (SilverQuest, Invitrogen). The concentration of *V. cholerae* LPS was determined by comparison to a standard curve of *E. coli* O26:B6 LPS (Sigma) using a Fujifilm FLA-900 scanner.

***V. cholerae* speed determination.** Bacterial speed was determined as previously described[3]. Briefly, overnight *V. cholerae* cultures were resuspended in HEPES buffer or HEPES buffer with 10% Ficoll 400. Samples were imaged using dark-field microscopy at 40x magnification using a Nikon Eclipse 80i fluorescence microscope. Time-lapse images were taken every 0.4 seconds. At least 40 individual bacteria were manually tracked in the movies using ImageJ, and these distances were compared to the number of frames observed to calculate *V. cholerae* speed in μm/ second.

Movies of *V. cholerae* dragging *B. bacteriovorus*. We infected *V. cholerae* at MOI 10 with *B. bacteriovorus* in HEPES buffer as done for previous experiments. After 20 minutes of infection, we diluted the samples 1:10 into HEPES buffer and observed the predator-prey interactions using a Revolve microscope (Echo) at 90x magnification. We used iMovie software (Apple) to slow the movies to 0.25x full speed and insert annotation.

Supplementary References

1 Kalivoda, E. J., Brothers, K. M., Stella, N. A., Schmitt, M. J. & Shanks, R. M. Bacterial cyclic AMP-phosphodiesterase activity coordinates biofilm formation. *PLoS One* **8**, e71267, doi:10.1371/journal.pone.0071267 (2013).

2 Seed, K. D. *et al.* Phase variable O antigen biosynthetic genes control expression of the major protective antigen and bacteriophage receptor in *Vibrio cholerae* O1. *PLoS Pathog* **8**, e1002917, doi:10.1371/journal.ppat.1002917 (2012).

3 Butler, S. M. & Camilli, A. Both chemotaxis and net motility greatly influence the infectivity of *Vibrio cholerae*. *Proc Natl Acad Sci U S A* **101**, 5018-5023, doi:10.1073/pnas.0308052101 (2004).