Supporting Information

Nadell and Bassler 10.1073/pnas.1111147108

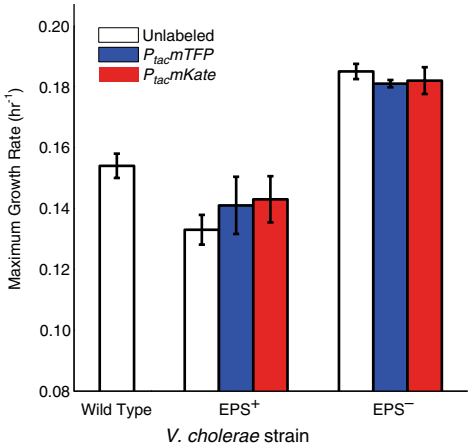

Fig. S1. Maximum growth rates of experimental strains and wild-type *Vibrio cholerae* at 37 °C. Maximum growth rates were measured by calculating the maximum slopes of replicate growth curves performed with shaking at 37 °C. The maximum growth rate of EPS^{+} cells is 25% lower than that of EPS^{-} cells. The maximum growth rates of the fluorescent strains are not significantly different from those of their parental counterparts. Error bars denote SEM (n = 3).

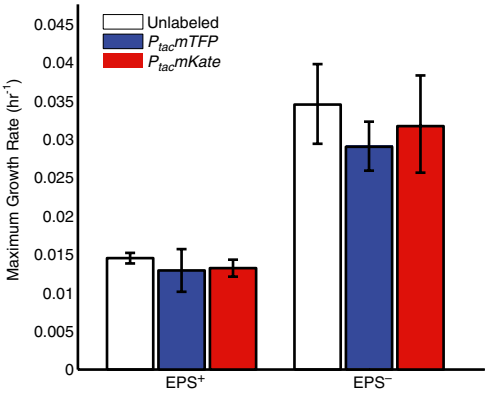

Fig. S2. Maximum growth rates of experimental strains and wild-type *Vibrio cholerae* at room temperature. Maximum growth rates were measured by calculating the maximum slopes of replicate growth curves performed with shaking at room temperature. The maximum growth rate of EPS^{+} cells is 40–50% lower than that of EPS^{-} cells. The maximum growth rates of the fluorescent strains are not significantly different from those of their parental counterparts. Error bars denote SEM (n = 3).

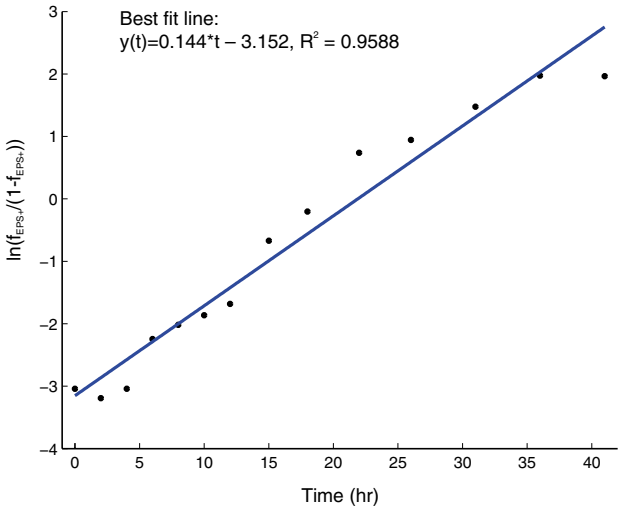

Fig. S3. Calculation of the selection coefficient with respect to EPS$^+$ cells in biofilm coculture with EPS$^-$ cells. The natural logarithm of the mean frequency of EPS$^+$ cells divided by the mean frequency of EPS$^-$ cells is plotted as a function of time during the course of replicate biofilm competition experiments (n = 4). The selection coefficient with respect to the EPS$^+$ strain is defined as the slope of the best-fit line for these data, 0.144 h^{-1}.

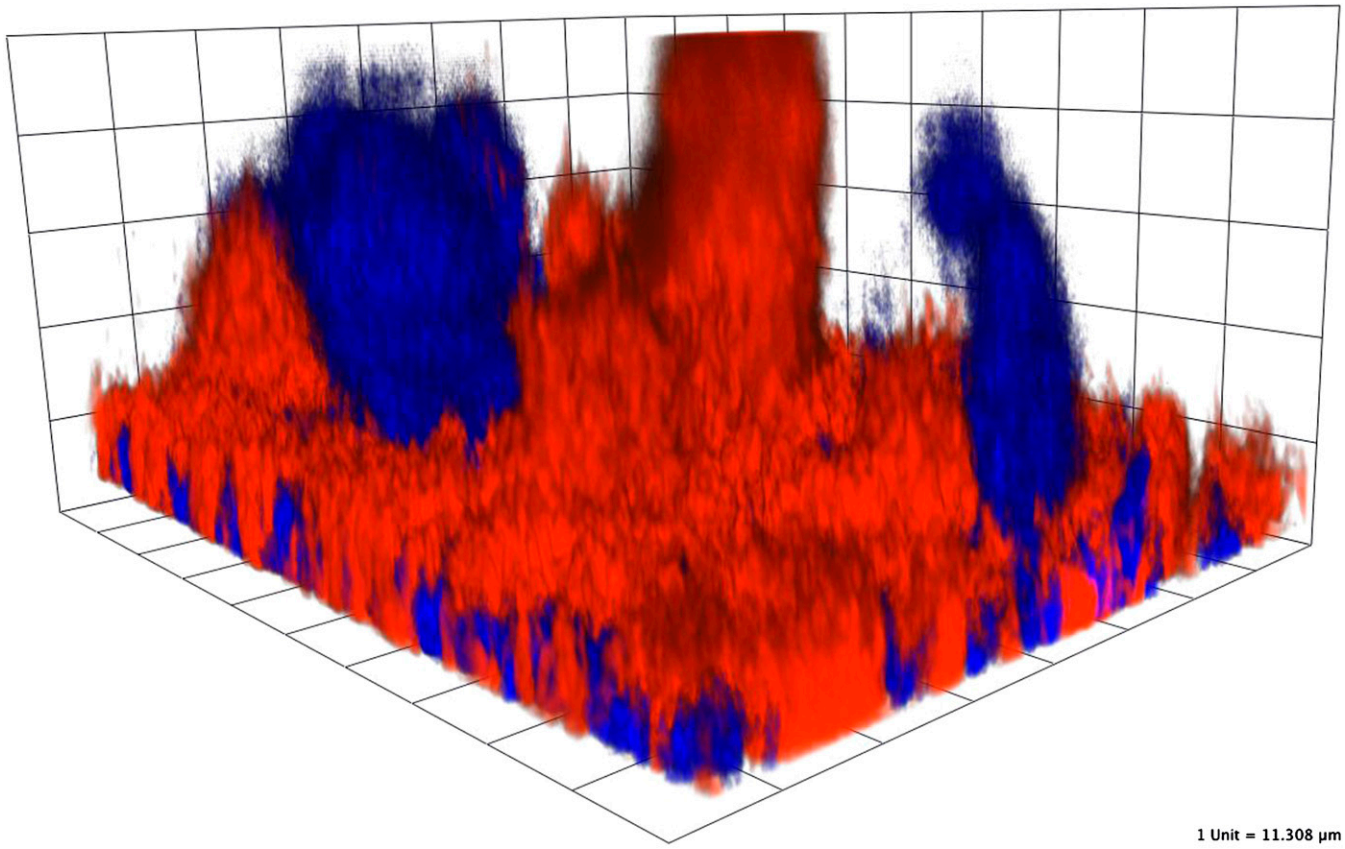

Fig. S4. When EPS$^+$ cells, half expressing *mTFP1* (blue) and half expressing *mKate* (red), are inoculated together at a 1:1 initial ratio, each tower structure produced is composed of primarily one color of cells. This result indicates that biofilm structures generated by the EPS$^+$ strain are largely derived from single cell lineages.

Table S1. Strains and plasmids used in this study

Strain/plasmid	Relevant features	Source/reference
Vibrio cholerae strains		
C6706str2	El Tor wild type	1
MM194	C6706str2 Δ*hapR*	2, 3
CN001	C6706str2 Δ*hapR*Δ*flaA* (EPS^+)	This study
CN002	C6706str2 Δ*hapR*Δ*flaA*Δ*vpsL* (EPS^-)	This study
CN003	CN001 *lacZ*: P_{tac}*mTFP1*: *lacZ*	This study
CN004	CN001 *lacZ*: P_{tac}*mKate*: *lacZ*	This study
CN005	CN002 *lacZ*: P_{tac}*mTFP1*: *lacZ*	This study
CN006	CN002 *lacZ*: P_{tac}*mKate*: *lacZ*	This study
Plasmids		
pKAS32	Suicide plasmid for allelic exchange	4
pBH050	pKAS32 Δ*flaA*	B. H. Hammer (Georgia Tech, Atlanta); this study
pCMW112	pKAS32 Δ*vpsL*	C. M. Waters (Michigan State University, East Lansing, MI); this study
pCN003	pKAS32 *lacZ*: P_{tac}*mTFP1*: *lacZ*	This study
pCN004	pKAS32 *lacZ*: P_{tac}*mKate*: *lacZ*	This study

1. Thelin KH, Taylor RK (1996) Toxin-coregulated pilus, but not mannose-sensitive hemagglutinin, is required for colonization by Vibrio cholerae O1 El Tor and O139 strains. *Infect Immun* 64:2853–2856.
2. Miller MB, Skorupski K, Lenz DH, Taylor RK, Bassler BL (2002) Parallel quorum sensing circuits converge to regulate virulence in *Vibrio cholerae*. *Cell* 110:303–313.
3. Zhu J, et al. (2002) Quorum-sensing regulators control virulence gene expression in *Vibrio cholerae*. *Proc Natl Acad Sci USA* 99:3129–3134.
4. Skorupski K, Taylor RK (1996) Positive selection vectors for allelic exchange. *Gene* 169:47–52.