

Supplemental Figures

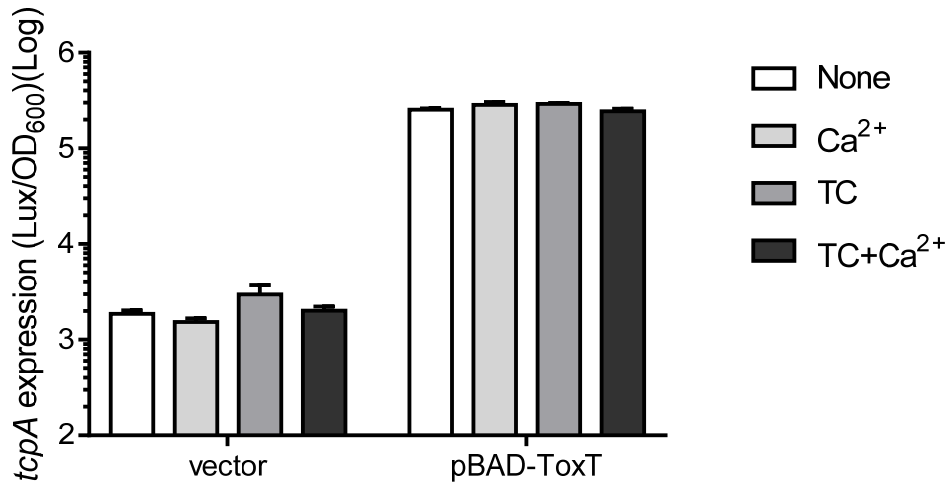


Fig. S1. Contribution of Ca²⁺ to ToxT induction of *tcpA*. *tcpA* expression in $\Delta toxT$ mutant containing P_{*tcpA*}-*luxCDABE* and pBAD-*toxT* plasmids. Cultures were grown in LB containing 0.05% arabinose with 0.1 mM TC, 10mM CaCl₂ or both until mid-log (OD₆₀₀ ~0.2). Luminescence was measured and normalized for growth against the OD₆₀₀. The data shown are from three independent experiments.

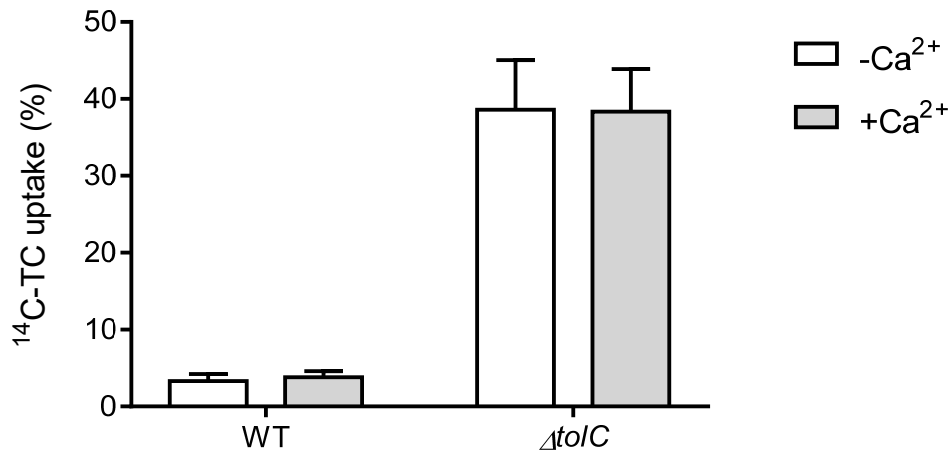


Fig. S2. Accumulation of ¹⁴C-taurocholate in *V. cholerae*. Cultures of WT or efflux deficient ($\Delta tolC$) *V. cholerae* were grown at 37° to mid-log with or without 10mM CaCl₂ and exposed to 200 μ M TC and 20 μ M ¹⁴C-taurocholate. Aliquots of cells were taken at indicated time points, pelleted, and count per minute (CPM) of C¹⁴ in cells was measured. TC uptake is reported as percentage CPM in cells compared to total CPM.

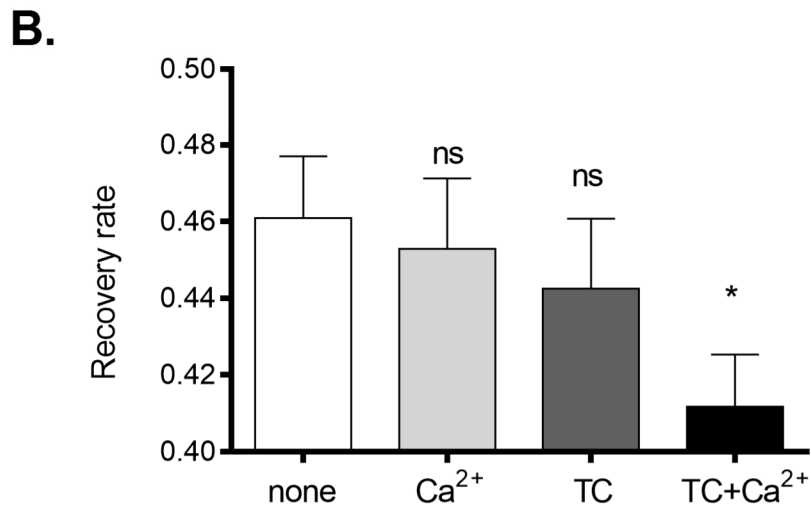
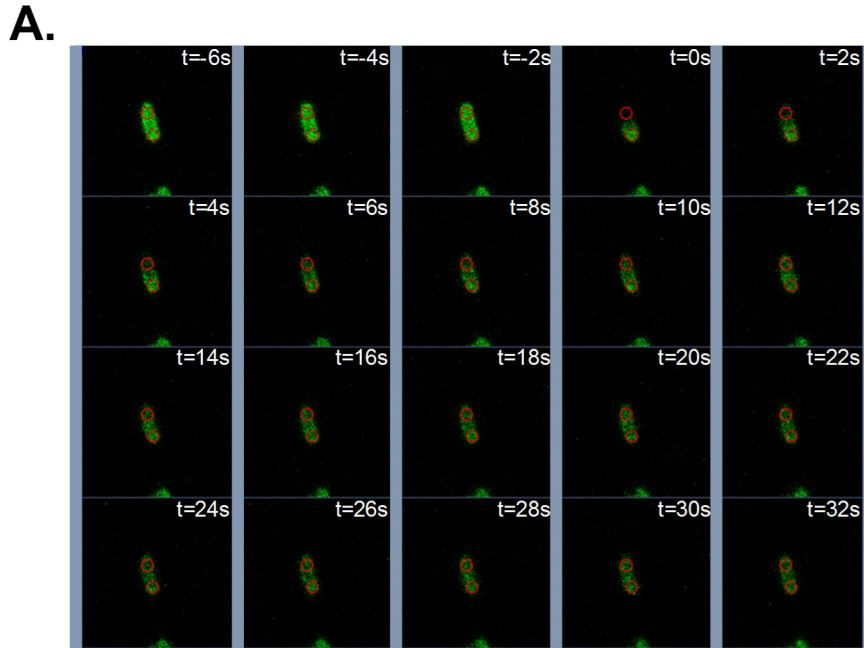


Fig. S3. Fluorescence recovery after photobleaching (FRAP). Cells expressing fluorescently labeled TcpP (pBAD-*gfp-tcpPH*) were grown to mid-log phase in the presence of arabinose before microscopy. Following near complete photobleaching with an argon laser, fluorescence intensity was recorded within two equally sized circular regions, one in the middle of the bleached compartment $A_1(t)$ and the other in the middle of the unbleached compartment $A_2(t)$ for a given time, t . **A.** Representative timecourse images of fluorescence recovery of fluorescently labeled TcpP. Images were captured on ZEN 2012 software at 2s intervals preceding and immediately following bleaching. For this representative cell, $A_1(t)$ corresponds to the upper circle in the bleached compartment and $A_2(t)$ corresponds to the lower circle in the unbleached compartment. **B. Recovery of** fluorescently labeled TcpP. Cells were grown in the presence of arabinose with 1mM TC and 10mM CaCl₂ where indicated. Following near complete photobleaching, recovery data was normalized to the total remaining fluorescence. The data

shown are from three independent experiments and over 10 individual cells. *: Student t-test, $P < 0.05$. ns: not significant.

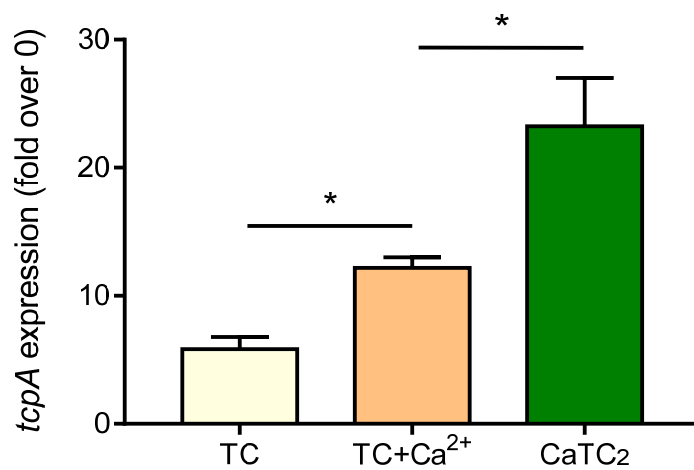


Fig. S4. Effect of CaTC₂ on the expression of virulence gene *tcpA*. *V. cholerae* induction of TcpA when incubated with synthesized CaTC₂ compared to TC, or TC+Ca²⁺. *V. cholerae* containing P_{tcpA}-*luxCDABE* plasmids were grown in LB containing 0.1mM TC, 0.1mM TC with 10mM CaCl₂, or 0.1mM CaTC₂. Cultures were grown microaerobically until mid-log (OD₆₀₀ ~0.2). Luminescence was measured normalized for growth against the OD₆₀₀. The data shown are from three independent experiments. *: Student t-test, $P < 0.05$.