Supplementary Information (IAI2856-14)

Figure Legends

Figure S1. Impact of antibody treatment on *V. cholerae* attachment to epithelial surfaces *in vivo*. *V. cholerae* O395 was either (**A**) treated with SyH7 IgG, an IgG isotype control, (**B**) 2D6 IgG, (**C**) Sal4 IgA, an IgA isotype control, or (**D**) untreated motility mutant strain (*V. cholerae* ΔflaA) and injected into ligated ileal loops of BALB/c mice, as described in the Material and Methods. After 30 min, the loops were excised, frozen, cryosectioned, stained with fluorescently labeled *V. cholerae* antiserum (red) or CD11c (blue) and visualized by confocal microscopy. In each case, the panels in the right hand column are zoomed-in images of the panels in the left hand columns. In panels A and C, IgG and IgA isotype control-treated *V. cholerae* were observed in close association with the epithelium and penetrating the intestinal crypts. In panels B, 2D6 IgG treatment resulted in small clumps of agglutinated *V. cholerae*, though some bacteria were all seen associated with the villi as well. In panels D, non-motile *V. cholerae* mutants were observed in close association with the villus epithelium and villus crypts, suggesting bacteria motility is not required for *V. cholerae* attachment in this model. Scale bars: 300 μm (left column), 50 μm (right column). Abbreviations: PP, Peyer's patches; V, villi; L, lumen.

Movie Legends

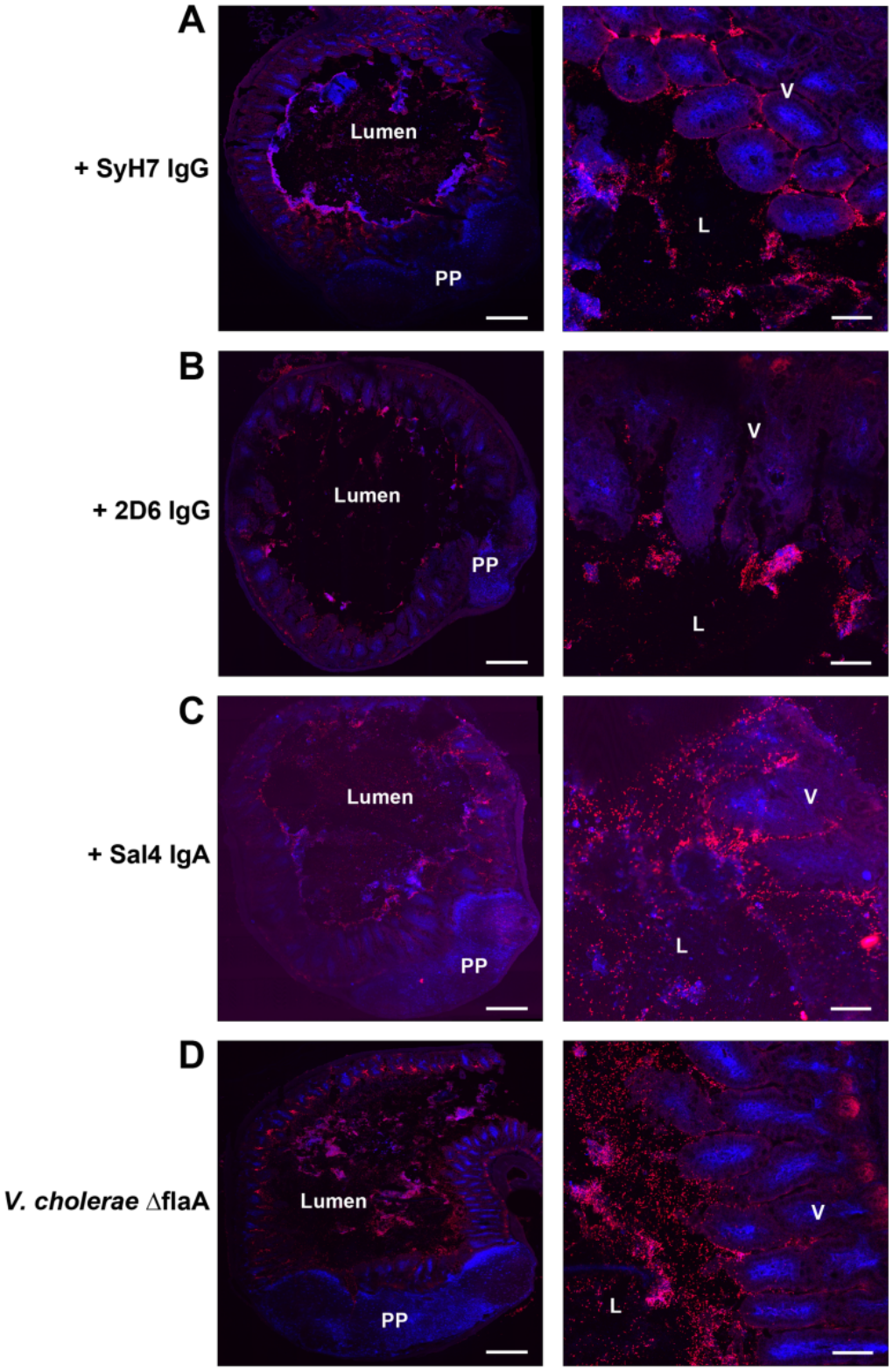
Movie 1. *V. cholerae* is hypermotile in liquid media. A mid-log phase culture of *V. cholerae* strain RT4273 was spotted onto a microscope slide, mounted on Nikon TI inverted microscope equipped with a CoolSnap HQ2® digital camera and imaged for 20 min, as described in the Materials and Methods. *V. cholerae* remained motile for the duration of the filming and no agglutinated bacteria were observed.

Movie 2. 2D6 IgA rapidly arrests *V. cholerae* motility and promotes agglutination. A mid-log phase culture of *V. cholerae* strain RT4273 was treated with 2D6 IgA, spotted onto a microscope slide, mounted on Nikon TI inverted microscope equipped with a CoolSnap HQ2® digital camera and imaged for 20 min, as described in the Materials and Methods. Individual bacteria were observed transitioning from motile to non-motile within minutes of antibody treatment (arrow at 1 min). Clumps of agglutinated bacteria were also observed and increased in size over time. Motile bacteria were seen interacting with agglutinated clumps, becoming "tethered" to them (arrow at 10 min). By 30 min all visible bacteria were non-motile and agglutinated into clumps.

Movie 3. 2D6 IgG reduces *V. cholerae* motility and promotes low levels of agglutination. A mid-log phase culture of *V. cholerae* strain RT4273 was treated with 2D6 IgG, spotted onto a microscope slide, mounted on Nikon TI inverted microscope equipped with a CoolSnap HQ2® digital camera and imaged for 20 min, as described in the Materials and Methods. Clumps of 2-3 bacteria were observed between 10-20 min post-antibody treatment. IgG antibody-mediated cross-linking and motility arrest were present but less pronounced than what was observed with IgA antibody treatment.

Movie 4. 2D6 Fab fragments arrest *V. cholerae* motility in the absence of agglutination. A mid-log phase culture of *V. cholerae* strain RT4273 was treated with 2D6 Fab fragments, spotted onto a microscope slide, mounted on Nikon TI inverted microscope equipped with a CoolSnap HQ2® digital camera and imaged for 20 min, as described in the Materials and Methods. Non-motile bacteria were observed within minutes of Fab treatment, and by 20 min more than half of the visible bacteria were no longer motile. No agglutination was observed, suggesting antibody binding can inhibit *V. cholerae* motility separate from its ability to cross-link.

Figure S1


A
+ SyH7 IgG
Lumen
PP
V
L
B
+ 2D6 IgG
Lumen
PP
V
L
C
+ Sal4 IgA
Lumen
PP
V
L
D
V. cholerae ΔflaA
Lumen
PP
V
L