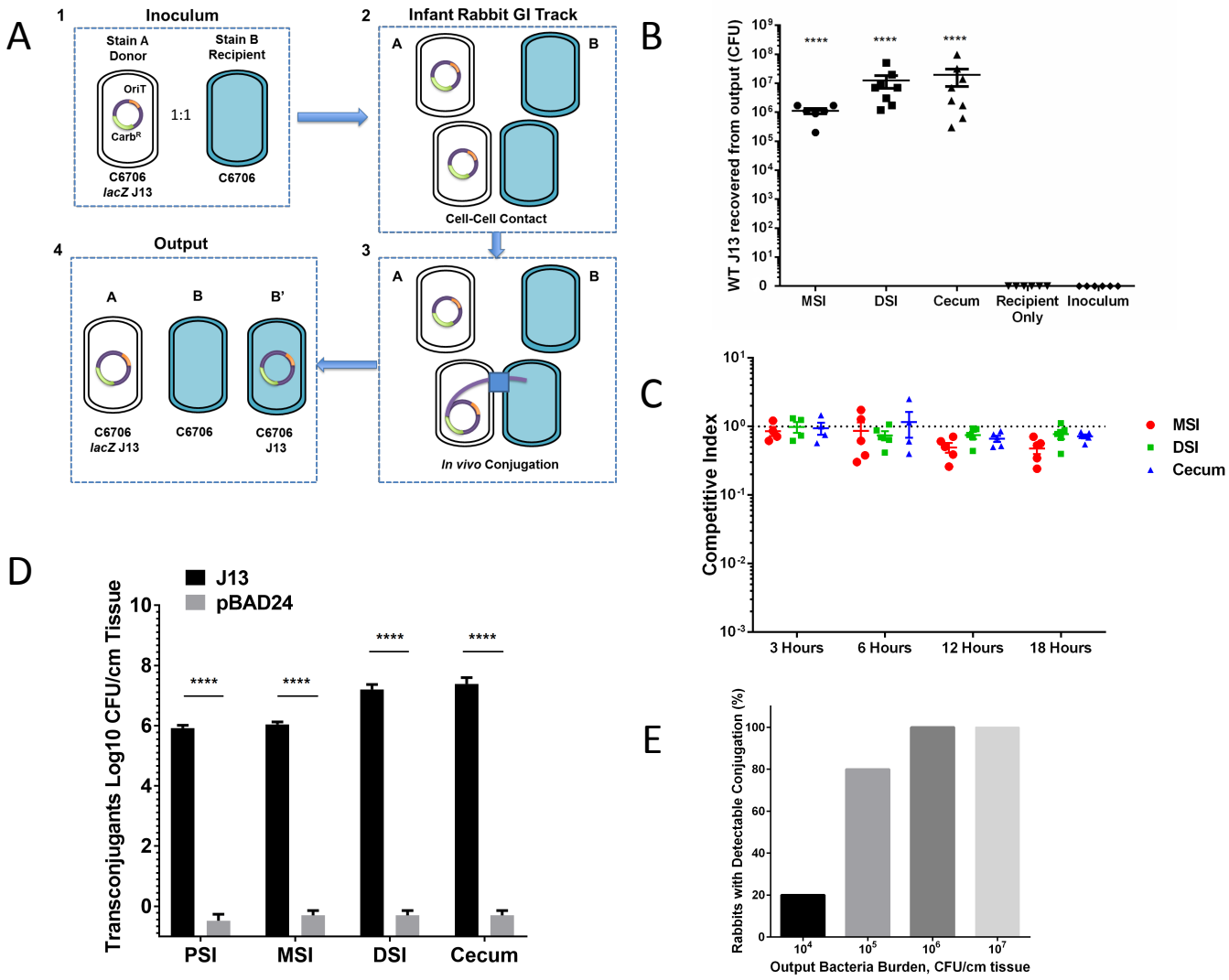

Figure S1. Conjugation assay to tracking bacterial cell-cell contacts in vivo, Related to STAR Methods. **(A)** A representative example of the in vivo cell-cell contact conjugation assay is shown with *V. cholerae* strains, C6706 *lacZ* J13 (Donor, A) and C6706 WT (Recipient, B). In the inoculum, A and B were pre-mixed at a 1:1 ratio (first panel). During colonization of the infant rabbit, cells of each strain contact each other in the GI track (second panel). The conjugative plasmid can transfer from the donor to the recipient cell during cell-cell contacts (third panel). In the output, a new phenotypic recipient cell (B') which acquired the J13 plasmid and its antibiotic resistance can be distinguished from A and B (fourth panel). **(B)** Each point indicates the number of colony forming units (CFU) of WT J13 cells (B') recovered from homogenates of 1 cm of tissue from individual rabbits for the indicated intestinal sections (MSI, DSI and Cecum) 18-20 hr post infection. Control groups of recipient only (rabbits only infected with the WT strain) and inoculum (the inoculum not passaged through the rabbits) are shown in the indicated columns. (C) In vivo competition experiments between C6706 *lacZ* J13 and the wild type C6706 reveal that J13 does not confer competitive advantage during colonization. C.I. relative to the wild type was determined from bacteria recovered from the MSI (Red), DSI (Green) and Cecum (Blue) of each experimental animal at 3, 6, 12 hr, 18-20 hr post infection with the bars indicating mean and standard error. (D) In vivo conjugation assay was performed between donor C6706 *lacZ* pBAD24 or C6706 *lacZ* J13 and recipient wild type C6706. pBAD24 carries resistance to ampicillin but lacks any conjugative machinery and does not transfer between cells. (E) Conjugation assays were performed in infant rabbits at different bacterial density. Bars indicate the percentage of the animals with detectable levels of in vivo conjugation based on the output *V. cholerae* bacteria burden (CFU/cm tissue) indicated along the X-axis (10^4, 10^5 ,10^6, 10^7 CFU/cm tissue). Each group represents data from 5 to 8 rabbits.

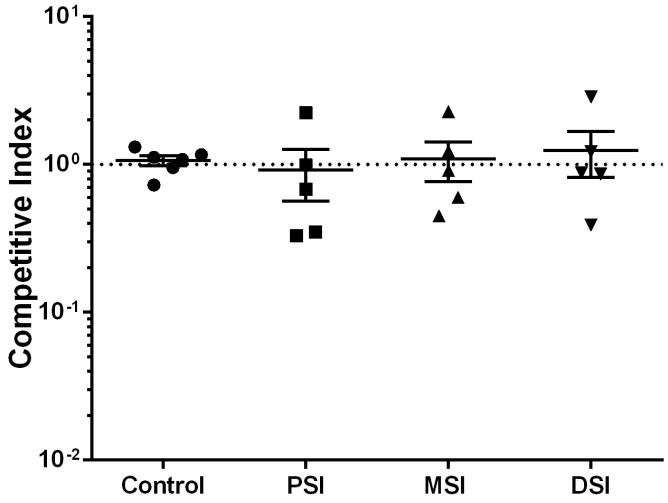

Figure S2. Eukaryotic effector VgrG-1 is not required for T6SS competitive advantage in the MSI, Related to Figure 3. Competition assays were performed between C6706 *vgrG*1and its wild type parent. Bacteria were recovered from the PSI, MSI and DSI of each experimental animal at 18 hr post infection. The control group indicates the C.I of two wild type strains at the DSI at 18 hr post infection. Data points are the C.I. relative to the wild type strain for each experimental animal with bars indicating mean with standard error.

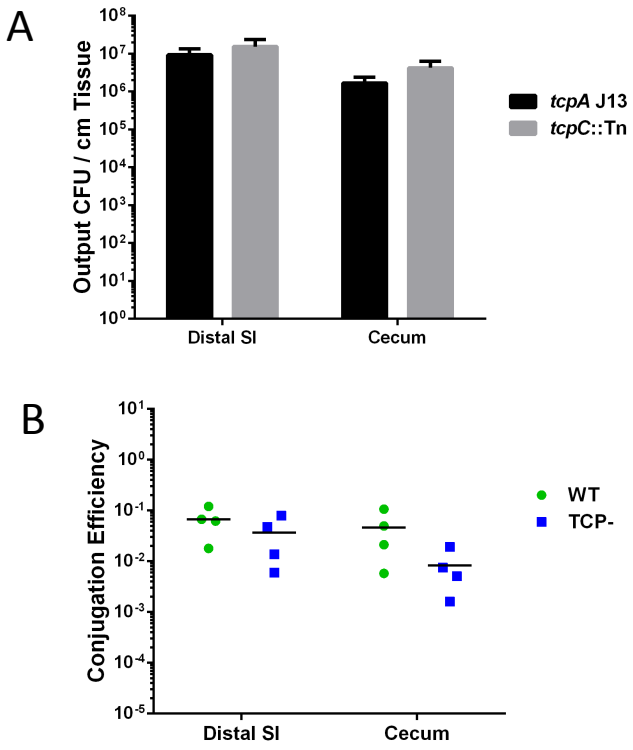

Figure S3. TCP is not required for *V. cholerae* cell-cell interactions, Related to Figure 4. (A) Bacterial burden of C6706 *tcpA* J13 (Donor, shown in black) and C6706 *tcpC*::Tn (Recipient, shown in grey) recovered from two different tissue sections (DSI, Cecum) 3 hr after co-inoculation. Columns represented mean of 4 animals with error bars indicating standard error. (B) Conjugation efficiency of J13 from C6706 *tcpA* J13 to C6706 *tcpC*::Tn (TCP$^-$, Blue) and from C6706 *lacZ* J13 to wild type C6706 (WT, Green) in the DSI and Cecum after 3 hr. Data points are the conjugation efficiency in a different experimental animal with the bar representing the mean. Mean value of the TCP$^-$ group is slightly lower than the WT group in the cecum, but the difference was not statistically significant.

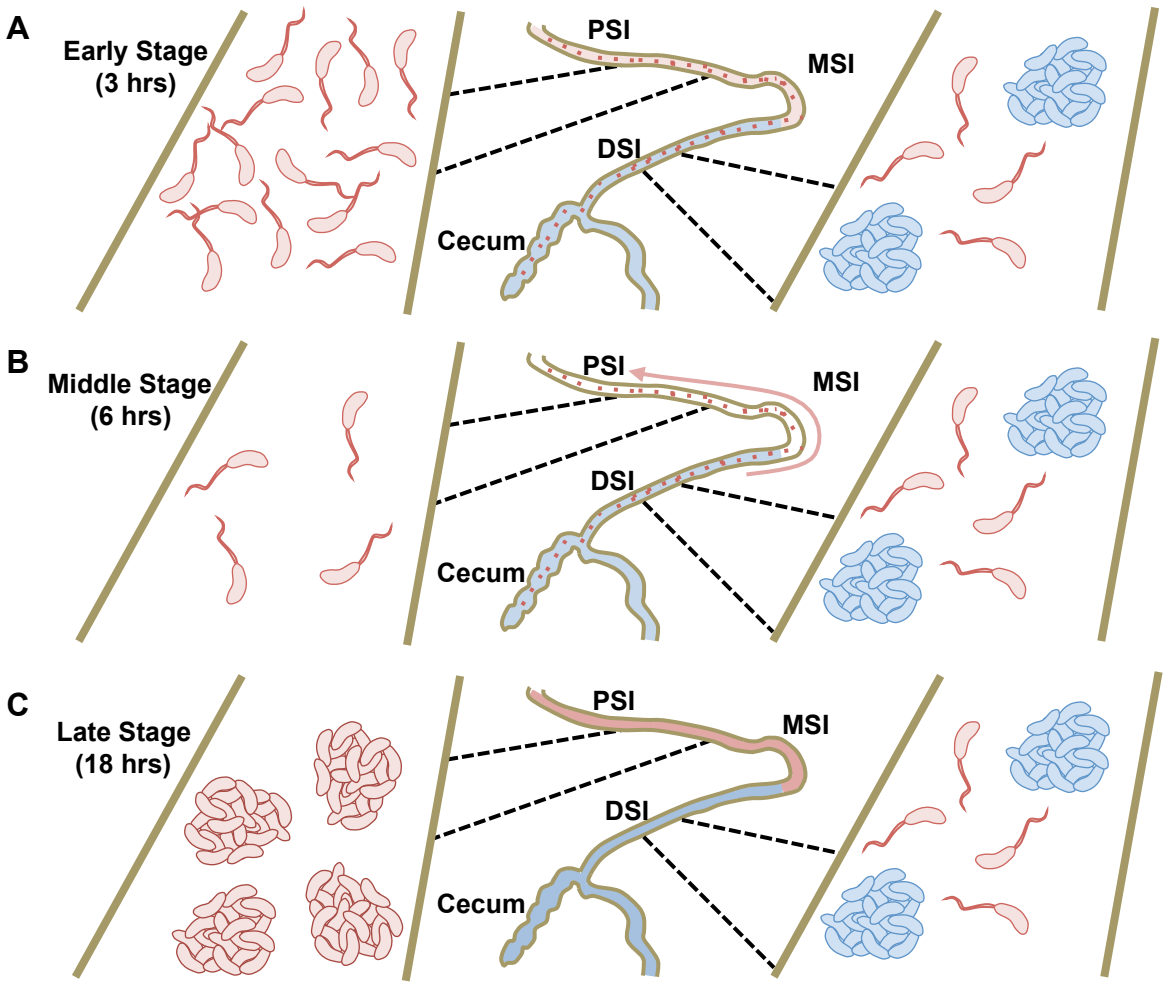

Figure S4. *V. cholerae* population dynamics during infection, Related to Figures 1, 3, and 4. (A) At the early stages of infection, there is an even distribution of bacteria throughout the proximal, middle, and distal small intestine (PSI, MSI, DSI, respectively), and the cecum. The blue population represents the subpopulation that attaches to each other and stays in the DSI and cecum. The free-floating red cells stay separate from each other and are present throughout the intestine. (B) During the middle stage of infection, the population in the MSI and PSI is restricted, resulting in elimination of a majority of the bacteria. (C) By the late stage of infection, single cells eventually expand into microcolonies. The free-floating subset of the DSI/cecum population is able to travel retrograde through the intestine to colonize the PSI (red arrow in B), joining the restricted population in the PSI. In retrograde colonization of the PSI, most of these bacteria do not contact engage in direct contact with each other.