Supplementary Information for Upregulation of virulence genes promotes *Vibrio cholerae* biofilm hyperinfectivity.

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AUTHOR CONTRIBUTIONS

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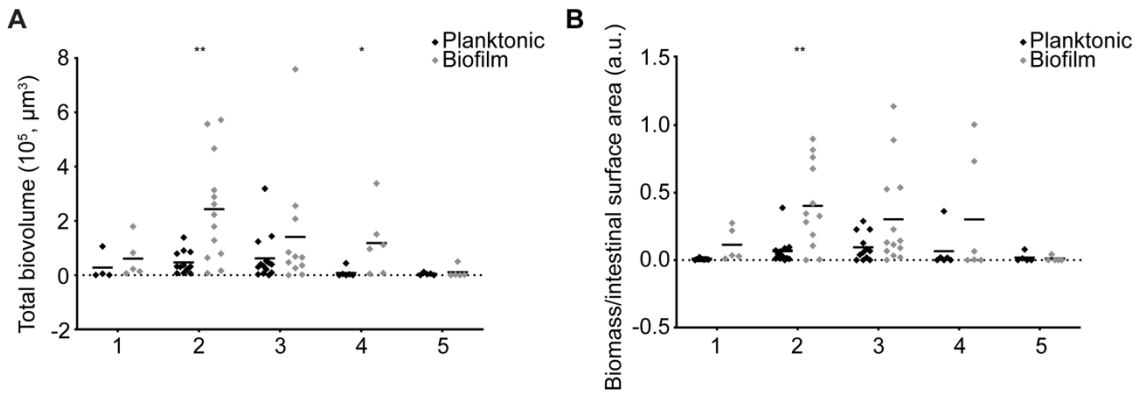
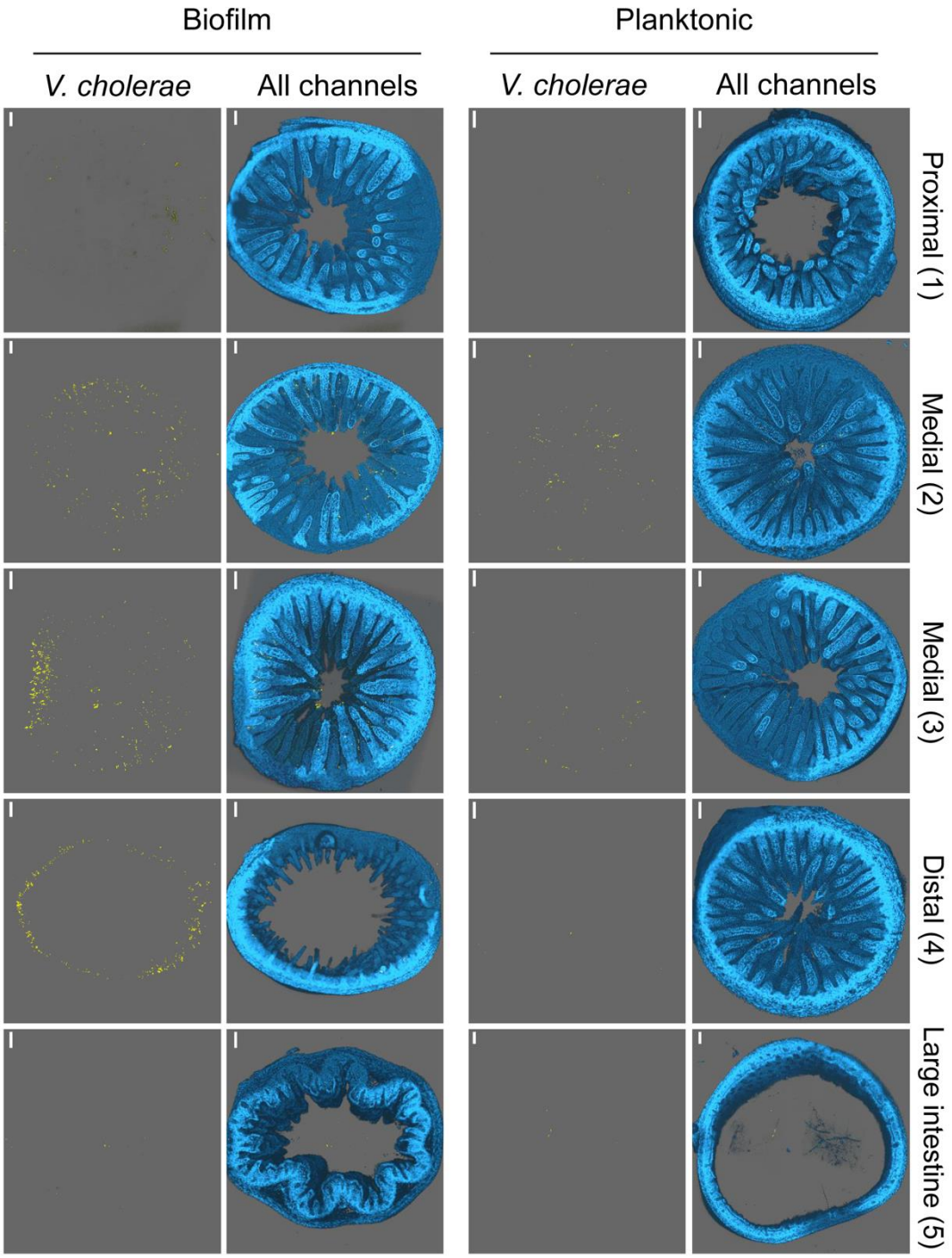
This PDF file includes:

Other supplementary materials for this manuscript include the following:

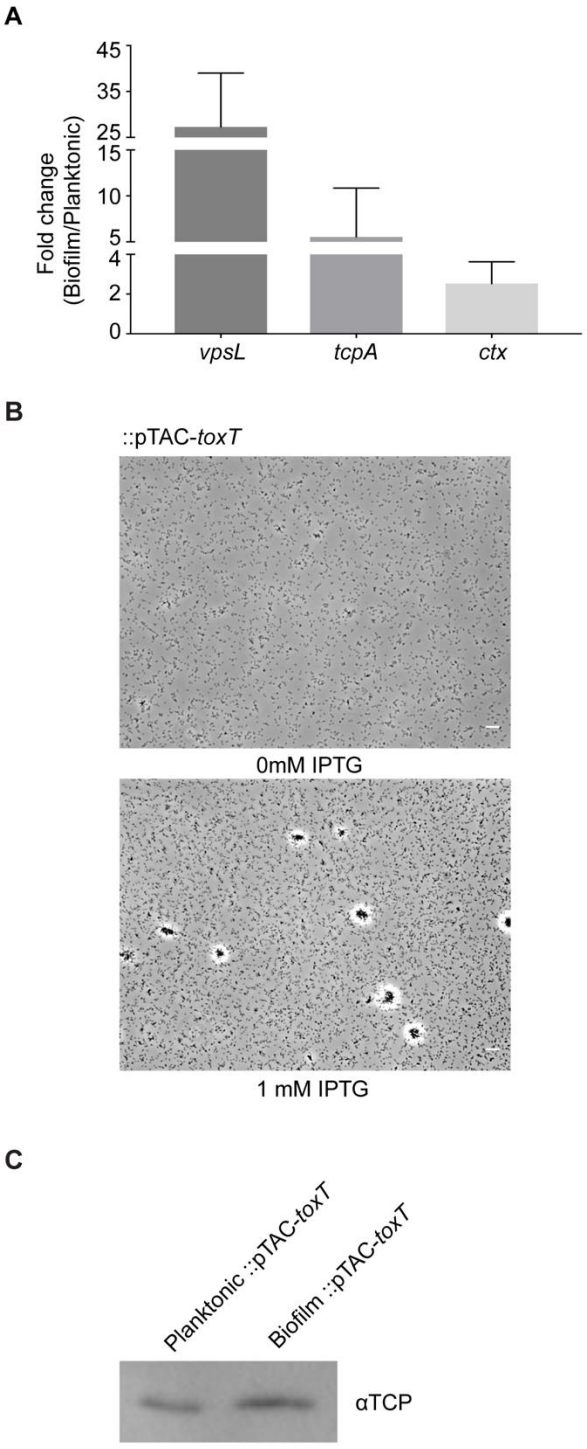


Supplementary Figure 1. Colonization patterns of planktonic and biofilm-grown cells along intestines reveal differences in bacterial abundance. A) Total biovolume of cells found in sections 1, 2, 3, 4, and 5 of the intestines infected with planktonic or biofilm-grown cells ($n \geq 4$). Statistical analysis was carried out using an unpaired two-sample t-test. (*, $p<0.05$, **, $p<0.005$). B) Bacterial biomass volume normalized by the intestinal surface area in sections 1, 2, 3, 4, and 5 of the intestines infected with planktonic or biofilm-grown cells ($n \geq 4$). Statistical analysis was carried out using an unpaired two-sample t-test. (**, $p<0.005$).



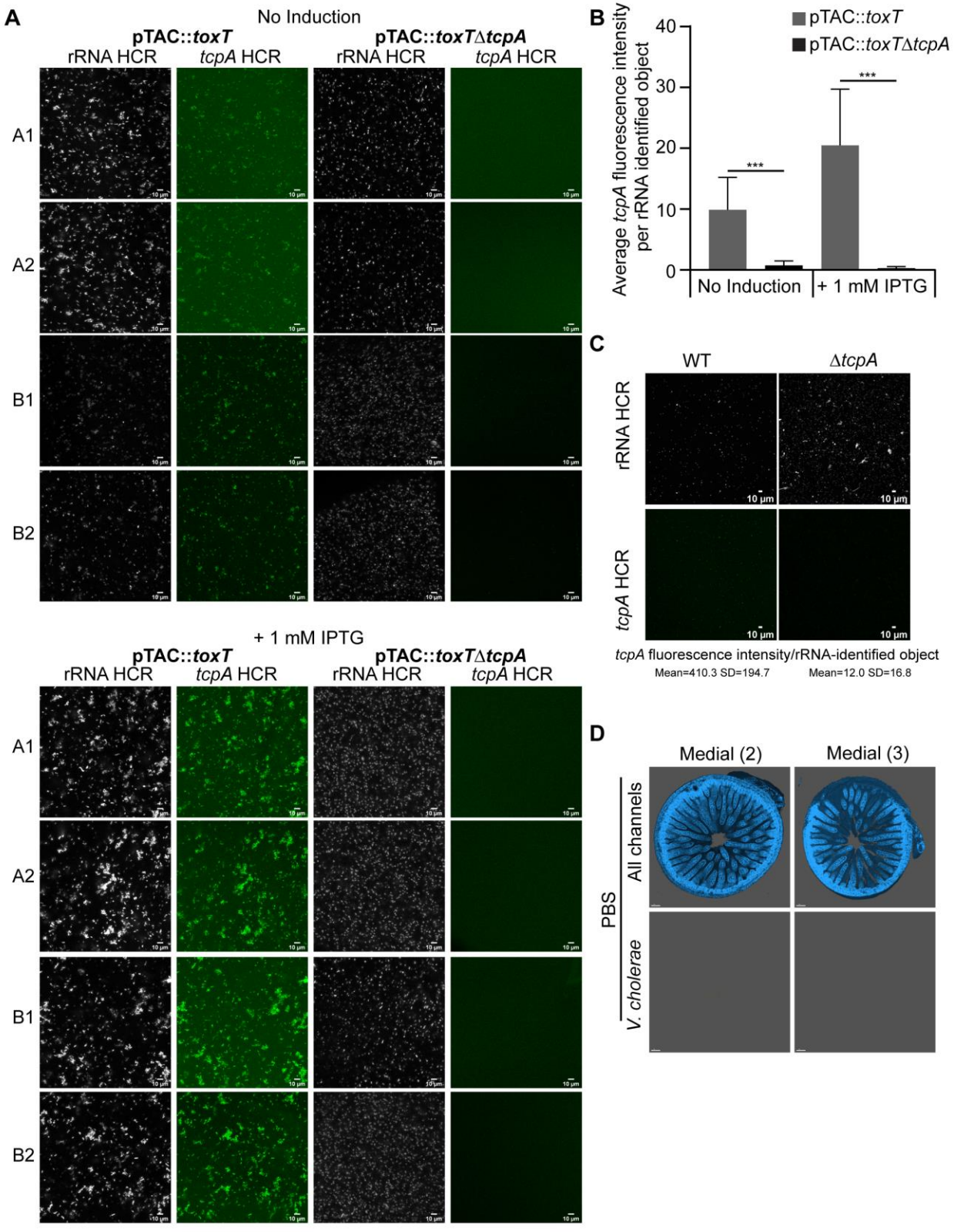
Supplementary Figure 2. MiPACT images of infected infant mouse intestines reveals differences in abundance and spatial patterns of colonization between planktonic and biofilm-grown cells. Images of biofilm and planktonic-grown cells

colonizing the intestine (showing representative images from $n \geq 3$). Projections of infected intestine show bacteria in yellow and intestines in blue. Scale bar is 100 μm.



Supplementary Figure 3. Biofilm-grown cells have enhanced expression of biofilm and virulence genes. A) Quantitative PCR was used to demonstrate fold-change in *vpsL*, *tcpA,* and *ctx* gene expression in biofilm-grown cells compared to planktonic-grown

cells (*n* = 3). B) TCP bunding upon induction of *toxT* using an inducible promoter was visualized using light microscopy (*n* = 2). C) Production of the TCP pilus upon induction of *toxT* in planktonic-grown and biofilm-grown cells was visualized using western blot (*n* = 2).


A
No Induction
pTAC::toxT
pTAC::toxTΔtcpA
rRNA HCR
tcpA HCR
A1
A2
B1
B2
+ 1 mM IPTG
B
pTAC::toxT
pTAC::toxTΔtcpA
Average tcpA fluorescence intensity per rRNA identified object
40
30
20
10
0
***
***
No Induction
+ 1 mM IPTG
C
WT
ΔtcpA
rRNA HCR
tcpA HCR
10 µm
tcpA fluorescence intensity/rRNA-identified object
Mean=410.3 SD=194.7
Mean=12.0 SD=16.8
D
Medial (2)
Medial (3)
PBS
All channels
V. cholerae

Supplementary Figure 4. HCR controls. (A) pTAC::*toxT* and pTAC::*toxT* Δ*tcpA* strains were grown at 30ºC in the presence or absence of 1mM IPTG until they reached an OD_{600} of approximately 1.0 before being spun down, resuspended in 1X phosphate-buffered saline (PBS) by pipetting, fixed, embedded and stained with HCR probes against rRNA (white, left panels) and *tcpA* mRNA (green, right panels). Maximum intensity projections of all Z-stacks from technical (1 vs. 2) and biological (A vs. B) replicates. Images were acquired in 8 bit mode (fluorescence intensity range of 0-255). (B) Using the 3D Objects Counter plugin in Fiji, the average fluorescence intensity of the *tcpA* channel within each rRNA identified object was calculated. Background signal for each Z-stack was determined by inverting the gray values of the rRNA channel and calculating the average *tcpA* fluorescence intensity within all non-object voxels. This Z-stack-specific background value was subtracted from the *tcpA* signal for each object in that respective Z-stack. Each bar represents the average of all objects in all four Z-stacks for each condition. Error bars represent standard deviation. Statistical analysis was carried out using an unpaired two-sample t-test. (***, $p<0.0001$). (C) Wild type or Δ*tcpA V. cholerae* biofilm-grown cells were prepared by scraping into 1X phosphate-buffered saline (PBS) and resuspended by pipetting, fixed, embedded and stained with HCR probes against rRNA (white, top row) and *tcpA* mRNA (green, bottom row). Images show maximum intensity projections of Z-stacks. Images were acquired in 12 bit mode (fluorescence intensity range of 0-4095). The 3D Objects Counter plugin in Fiji was used to quantify the average fluorescence intensity of the *tcpA* signal in each rRNA probe identified object, and background was subtracted as described in B. (D) Images of intestine infected with PBS control (showing representative images from $n \geq 2$) and treated with specific HCR *V. cholerae* specific

rRNA probe. Projections of infected intestine show intestines in blue and any non-specific binding of the HCR probe in yellow. Scale bar is 30 μm.

Supplementary Movie 1 (separate file). Three-dimensional rendering of the medial sections of the small intestine (blue) infected with planktonic-grown cells (yellow).

Supplementary Movie 2 (separate file). Three-dimensional rendering of the medial sections of the small intestine (blue) infected with biofilm-grown cells (yellow).

Table S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant genotype	Source
E. coli strains		
CC118λ*pir*	Δ(*ara-leu*) *araD* Δ*lacX74 galE galK phoA20 thi-1 rpsE rpoB argE*(*Am*) *recA1* λ*pir*	(1)
S17-1λ*pir*	Tp^r Sm^r *recA thi pro* r_K^- m_K^+ RP4::2-Tc::MuKm Tn*7* λ*pir*	(2)
V. cholerae strains		
FY_VC_0001	*Vibrio cholerae* O1 El Tor A1552, wild type, Rif^r	(3)
FY_VC_ 12506	*Vibrio cholerae* O1 El Tor A1552 Δ*tcpA*	This study
FY_VC_ 14975	*Vibrio cholerae* O1 El Tor A1552 ΔVC1464	This study
FY_VC_ 14629	*Vibrio cholerae* O1 El Tor A1552 pTAC::*toxT*, Rif^r,	This study
FY_VC_ 14685	*Vibrio cholerae* O1 El Tor A1552 pTAC::*toxT* Δ*lacZ*, Rif^r,	This study
FY_VC_ 15844	*Vibrio cholerae* O1 El Tor A1552 pTAC::*toxT* Δ*tcpA*, Rif^r,	This study

References

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