Persistence and toxin production by *Clostridium difficile* within human intestinal organoids results in disruption of epithelial paracellular barrier function.

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Supplemental Information:

Supplemental Table 1 - Antibodies:

Primary Antibodies	**Vendor & Catalog #**	**Application & dilution**
Mouse anti-ZO-1	Life Technologies #33-9100	IF: 1:100
Rabbit anti-Occludin	Life Technologies #71-1500	IF: 1:250
Mouse anti-E-Cadherin	BD Biosciences #610651	IF: 1:500
Mouse anti-Acetylated Tubulin	Sigma-Aldrich #T7451	IF: 1:1,000
Alexa Fluor 546 phalloidin	Life Technologies #A22283	IF: 1:100
Rabbit anti-Cleaved Caspase-3 (Asp175)	Cell Signaling #9664	IF: 1:500
Secondary Antibodies	**Vendor & Cat#**	**Application & dilution**
Donkey Anti-Mouse IgG-Cy3	Jackson ImmunoResearch Lab #715-165-150	IF: 1:1,000
Donkey Anti-Rabbit IgG-Alexa Fluor 488	Jackson ImmunoResearch Lab #711-545-152	IF: 1:1,000
Donkey anti-Mouse IgG-Alexa Fluor 647	Jackson ImmunoResearch Lab #715-605-150	IF: 1:1,000

Supplemental Figure Legends:

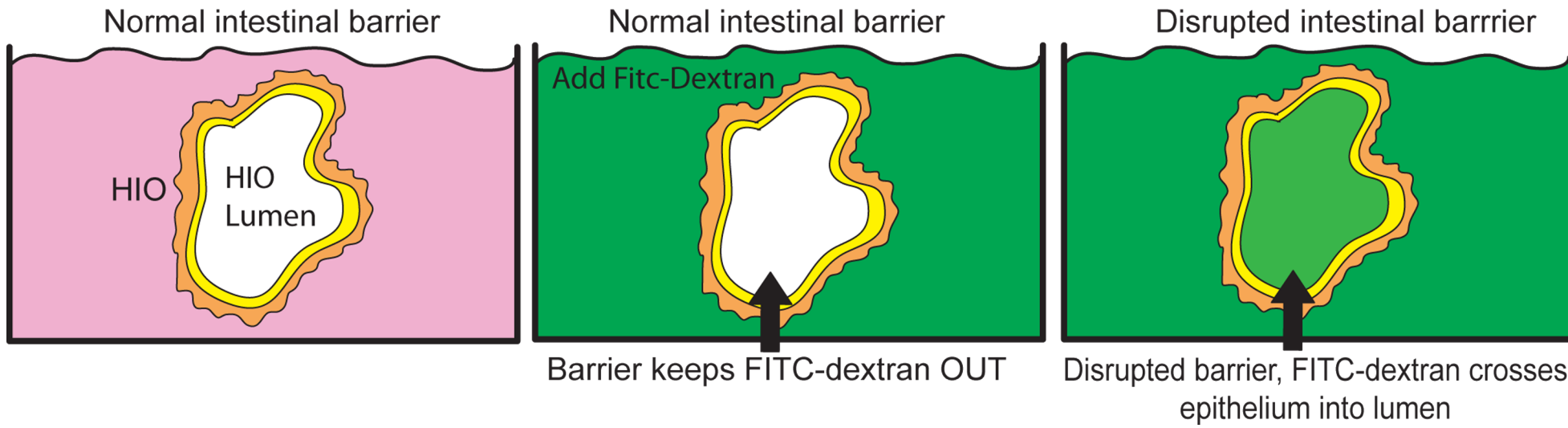
Supplemental Figure 1: "Outside-in" barrier function experiment.
To confirm that HIOs had a paracellular barrier function, FD4 was added to the media outside of the HIOs. A) Schematic of the barrier function assay. Following treatment, FD4 is added to the media containing the HIOs. HIOs with intact epithelium prevent the diffusion of FD4 into the lumen where as those with a disrupted epithelium cannot exclude FD4. B) Brightfield images indicate the location of the HIOs. Untreated HIOs exclude FD4, EGTA HIOs treated lose barrier function and appear green.

Supplemental Figure 2: Toxin activity of filtered supernatants. *C. difficile* culture supernatant was filtered to remove the bacteria and toxin activity was assessed using a Vero cell cytotoxicity assay. The culture filtrate from strain VPI 10463 had detectable cytotoxicity (black bar), however this level was not sufficient to disrupt HIO barrier function when injected into HIOs. Strain F200 (the nontoxigenic strain) had no cytotoxicity. The plot is of the mean with the SD of two independent experiments.

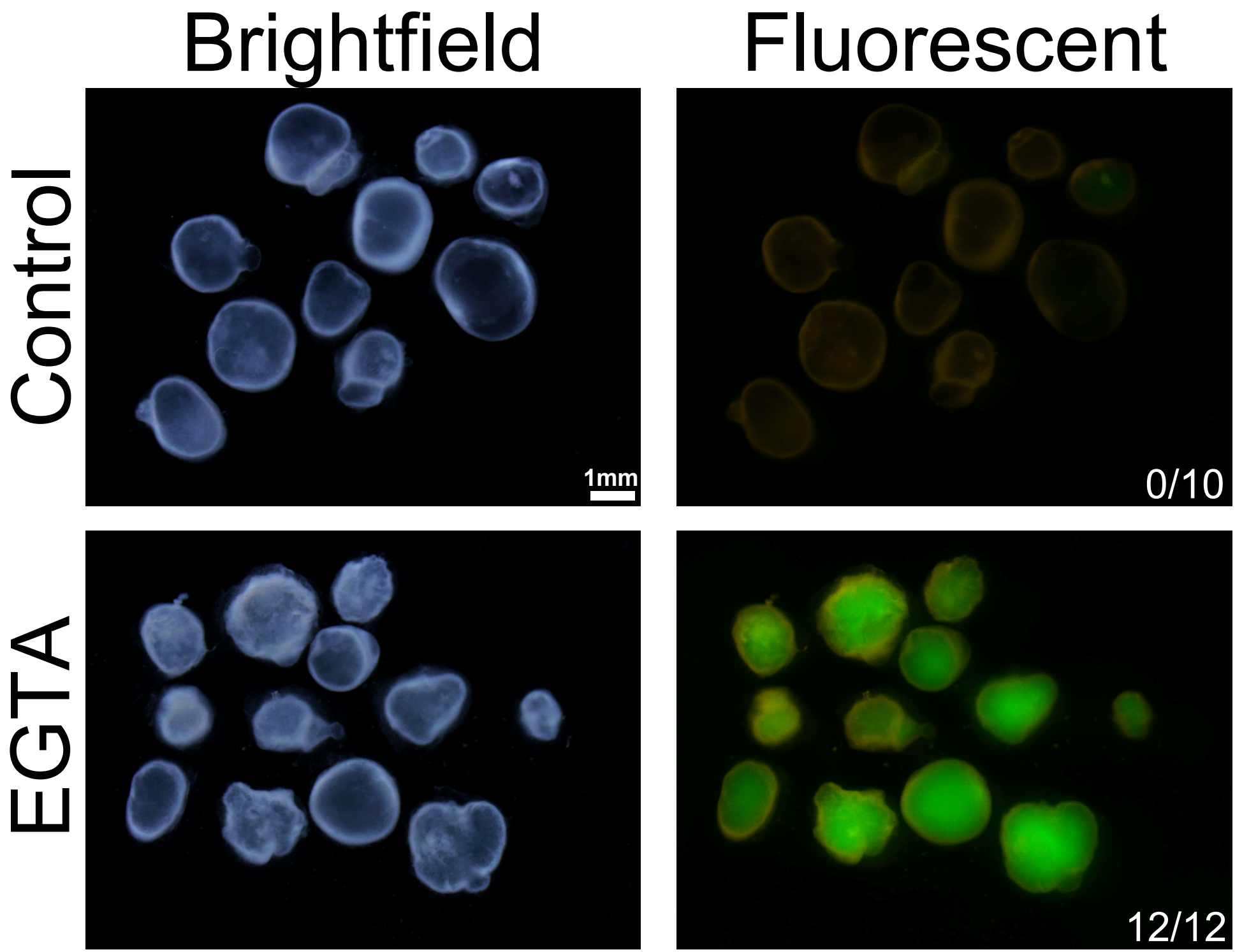
Supplemental Figure 3: Toxin activity of purified TcdA and TcdB used in injections. Purified toxin was tested in cell cytotoxicity assay. At the same concentration, TcdB and TcdA had similar activity on Vero cells.

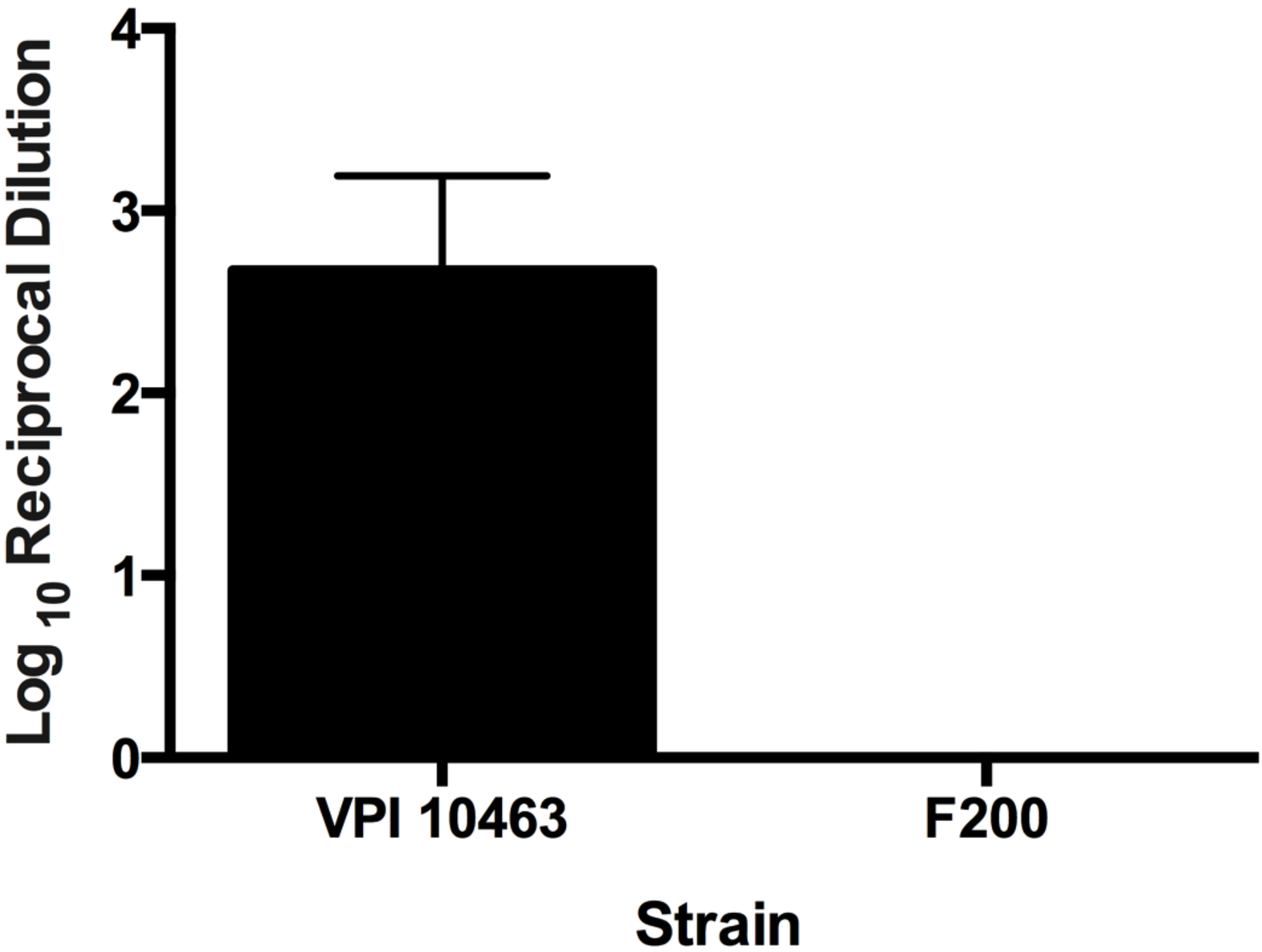
Supplemental Figure 4: Loss of barrier function following treatment with TcdA or TcdB is not due to caspase-3 mediated apoptosis. Controls, EGTA treated, and 400ng/ml of TcdA or TcdB treated HIOs were stained for cleaved caspase-3 an indicator of apoptotic cell death. Treatment with the cytokines TNF-alpha and INF-gamma induces massive cell death and serves as a positive control.

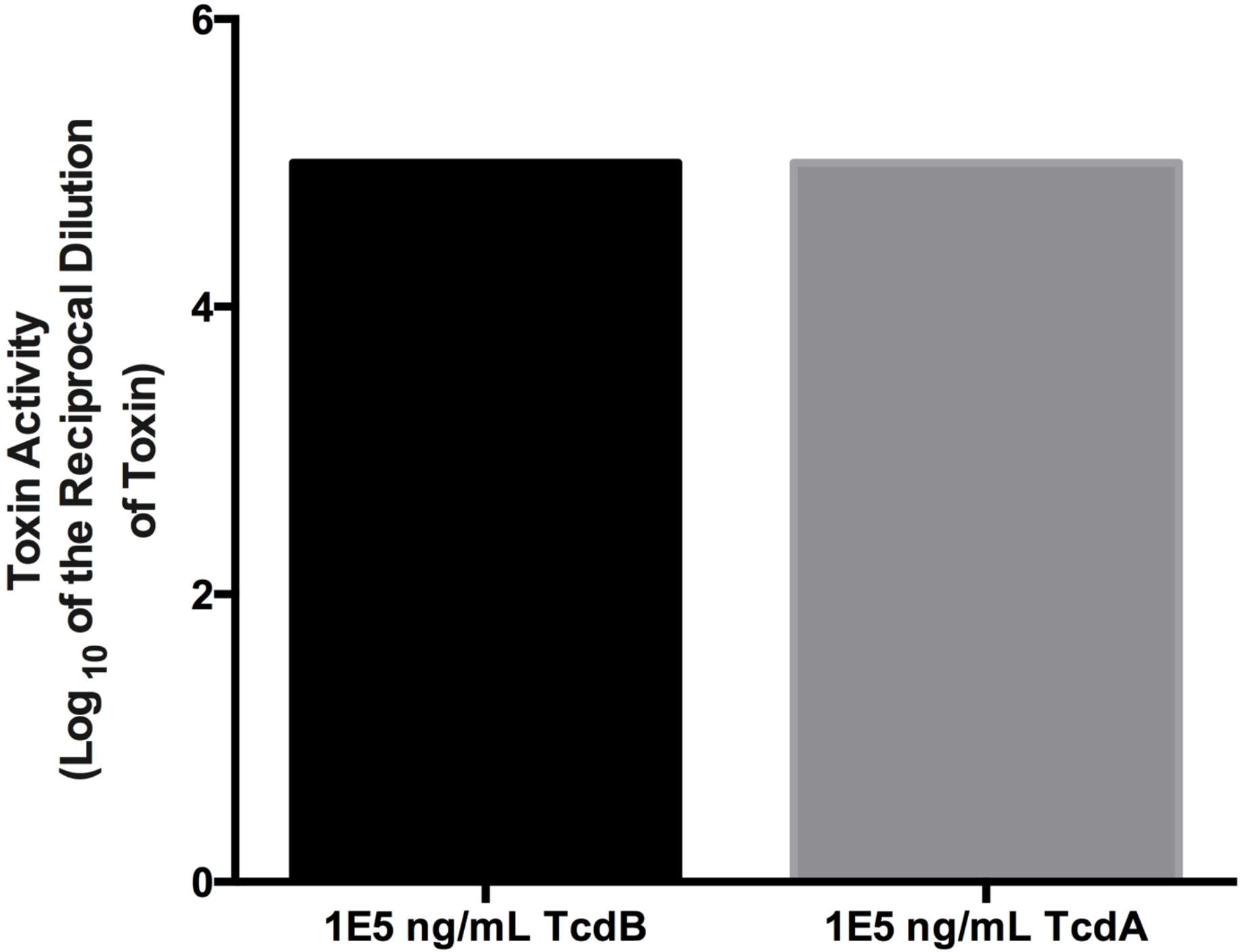
A

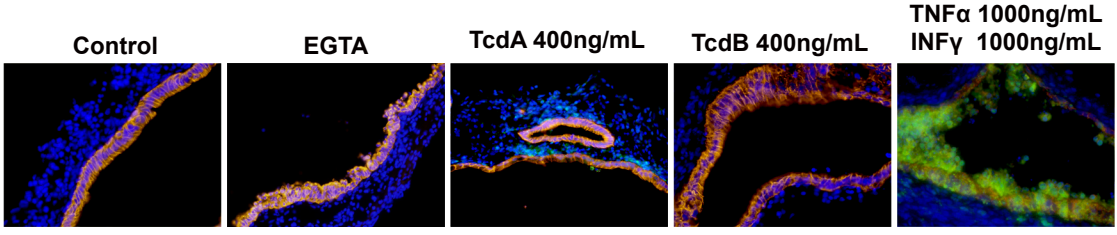

Normal intestinal barrier
Normal intestinal barrier
Disrupted intestinal barrrier
HIO
HIO
Lumen
Add Fitc-Dextran
Barrier keeps FITC-dextran OUT
Disrupted barrier, FITC-dextran crosses epithelium into lumen

B


Brightfield
Fluorescent
Control
EGTA
1mm
0/10
12/12


Log 10 Reciprocal Dilution
4
3
2
1
0
VPI 10463
F200
Strain


Toxin Activity
(Log 10 of the Reciprocal Dilution
of Toxin)
6
4
2
0
1E5 ng/mL TcdB
1E5 ng/mL TcdA


Control
EGTA
TcdA 400ng/mL
TcdB 400ng/mL
TNFα 1000ng/mL
INFγ 1000ng/mL