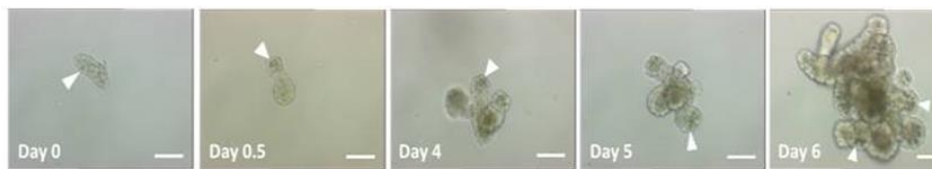


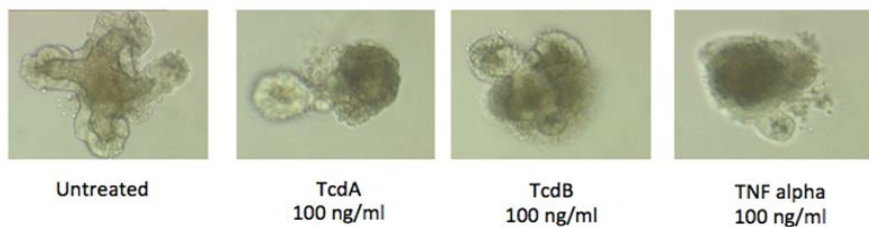
Supplementary materials:

Figure S4. Treatment of murine intestinal organoids with *Clostridioides difficile* toxins A (TcdA) and B (TcdB) induce epithelial cytotoxicity. (A) Growth in culture of stem cell derived 3-D intestinal organoids from C57BL/6J mice. Representative brightfield images showing a time course of the growth from an isolated crypt grown in Matrigel and cultured for 6 days. White arrow heads indicate Paneth cells. Scale bar = 100 μ m. Organoids were passaged at day 7 and re-grown for 4d, then treated for 24 h with *C. difficile* TcdA and TcdB. (B) Images showing morphological changes induced by 100 ng/mL TcdA and TcdB similar to that seen following treatment with 100 ng/mL tumour necrosis factor alpha (TNF α), a positive control for induction of organoid rounding, indicative of epithelial cytotoxicity. (C) Dose-dependent induction of cytotoxicity following treatment with 1 to 100 ng/mL TcdA and TcdB was quantified, with organoids assessed for increased circularity (a value of 1.0 equating to a full circle) determined using Image J software (N=4). Significant differences compared to untreated controls, ** $P < 0.01$, *** $P < 0.001$; one-way ANOVA, with Dunnett's post-hoc comparisons of treatment means.

A



B



C

