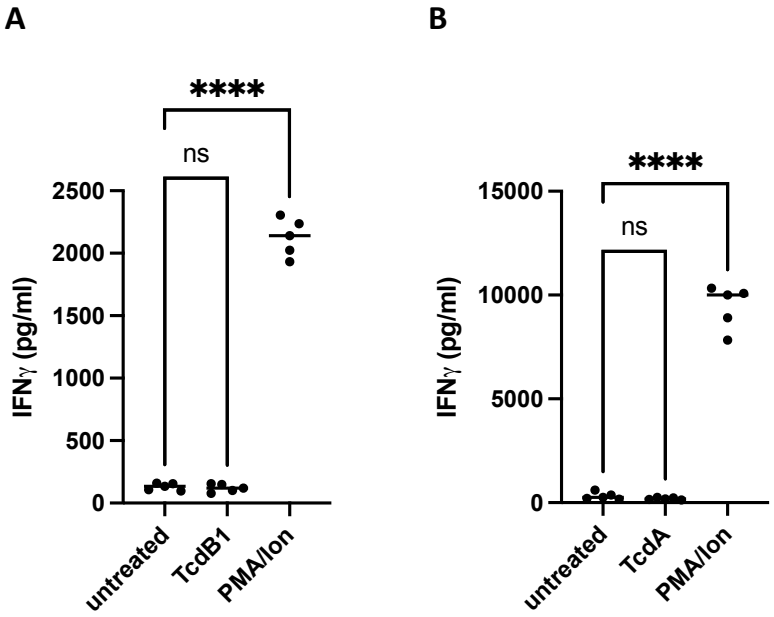
Supplemental Figure 1
Pope *et al.*



Supplemental Figure 1. TcdB1 and TcdA do not induce IFNγ secretion from MNK-1 cells, a mouse ILC1-like cell line.
MNK-1 cells were treated for 18 hrs with (A) 200 ng/ml TcdB1 or (B) 200 ng/ml TcdA, or 5 µg/ml PMA and 0.5 µg/ml ionomycin (PMA/Ion), or left untreated. IFN**γ** in the supernatants was quantitated by ELISA. Each point represents one well, line indicates mean. ns = not significant ($p>0.05$); ****, $p<0.0001$. Similar experiments were performed three times with similar results.

Supplemental Figure 2
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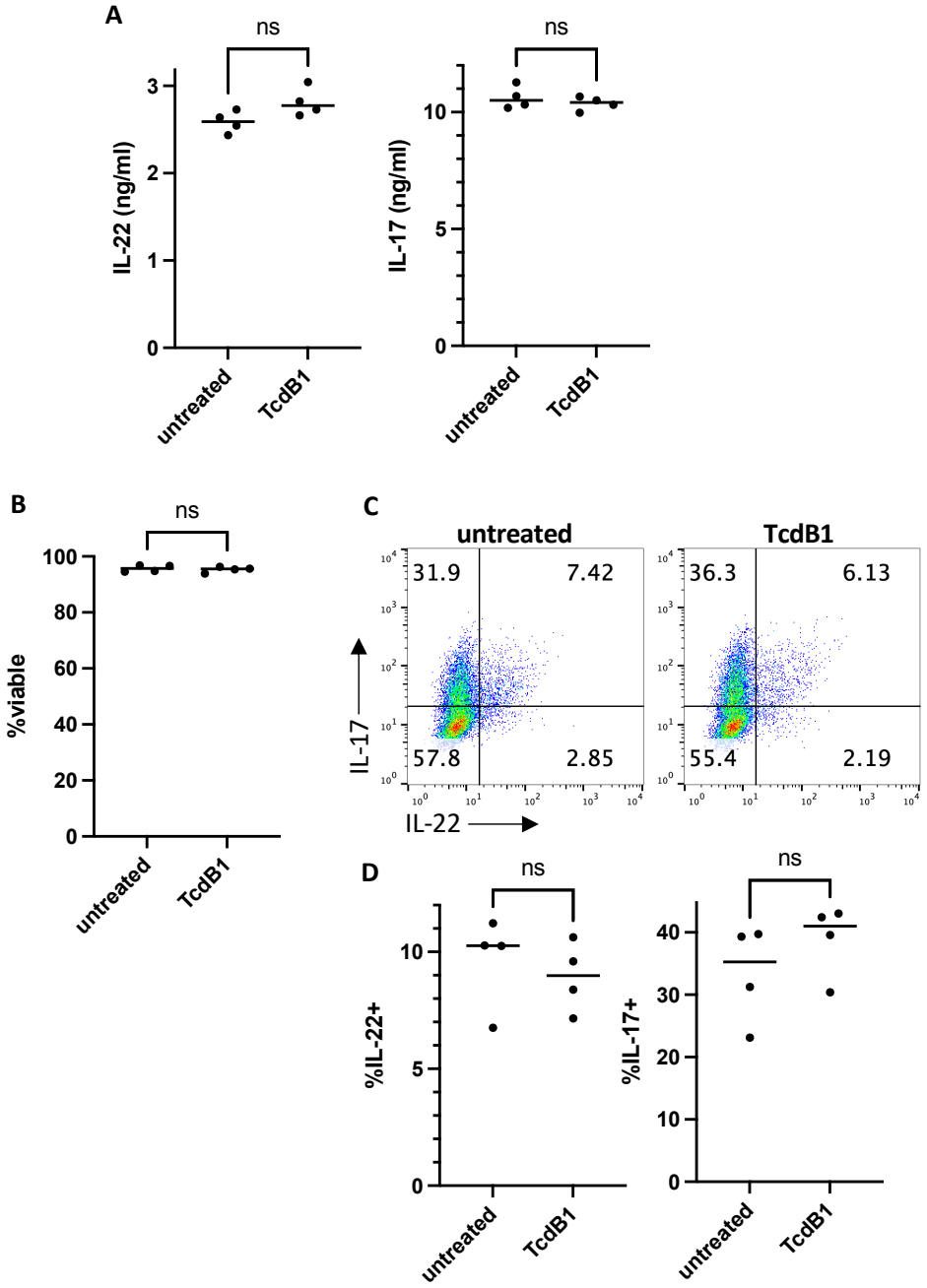

Supplemental Figure 2. TcdB1 has no detectable effects on *in vitro* Th22 differentiation.
Naïve CD4 T cells isolated from C57BL/6 mice were for 3 days activated *in vitro* with plate-bound anti-CD3 Ab and anti-CD28 Ab in the presence of Th22-differentiation factors (10 µg/ml anti-IFN**γ** Ab, 10 µg/ml anti-IL-4 Ab, 50 ng/ml recombinant mouse IL-23, 20 ng/ml recombinant mouse IL-6, 20 ng/ml recombinant IL-1β, and 200 nM 6-formylindolo(3,2-b)carbazole (FICZ). Cells were differentiated in the absence (untreated) or presence (TcdB1) of 200 ng/ml TcdB1. (A) IL-22 (left) and IL-17 (right) levels in the cell supernatant after 3 days of culture as measured by ELISA. (B-D) After 3 days of culture cells were restimulated with 5 µg/ml PMA and 0.5 µg/ml ionomycin in the presence of brefeldin A (BFA) for 5 hrs. Cells were stained with a fixable viability dye, intracellularly stained for IL-22 and IL-17 and analyzed by FACS. (B) Percent viable (viability dye-) cells. (C) Representative FACS plots of viable cells. Number indicates percent of cells within a quadrant. (D) Summary data for percent IL-22+ or IL-17+ cells. Each dot represents one well, line indicates mean. ns = not significant ($p>0.05$). We obtained similar results from more than three independent experiments. Reference for Th22 cultures: Budda *et al. J. Immunol.* 197(7): 2646-52.