

Suppl. Figure 1. TNF treatment does not affect organoid budding characteristics. (A) Quantitation of the number of buds per enteroid and the percent of all enteroids that developed buds during TNF treatment. (B) Quantitation of the number of buds per colonoid and the percent of all colonoids that developed buds during TNF treatment. Values represent mean  $\pm$  SEM, \*p<0.05.



Suppl. Figure 2. Time course of anti-CD3 treatment in WT and Tnf-/- mice. (A) Representative H&E sections of WT and Tnf-/- ileum 3, 18, 36 and 96 hours following anti-CD3 administration. (B) mRNA levels of inflammatory cytokines in ileal tissue 3 hours following anti-CD3 treatment. (C) Representative immunofluorescence staining of c-Myc (green) and BrdU (red). DAPI (blue) marks nuclei. (D) Low-power magnification of BAT-GAL and Tnf-/- x BAT-GAL mice shown in Fig 4D. Values represent mean  $\pm$  SEM, \*p<0.05.



## Suppl Figure 3. TNF mediates T cell-induced IEC activation in the small intestine.

Representative survivin staining of control and anti-CD3 mAb-treated WT and *Tnf*<sup>/-</sup> small intestine at 24 hours. Quantification is shown in Figure 5.



Suppl Figure 4. Bone marrow-derived TNF is crucial for maintaining epithelial proliferative responses upon T cell activation. Representative images of H&E, Ki67, survivin, c-Myc, and cleaved caspase 3 staining of colonic tissue of control- and anti-CD3-treated (24 hours) in WT $\rightarrow$ Tnf<sup>/-</sup> and Tnf<sup>/-</sup> $\rightarrow$ WT BMC mice.