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Supplemental Information

**TNF Receptor 1 Promotes Early-Life Immunity
and Protects against Colitis in Mice**

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Figure S1

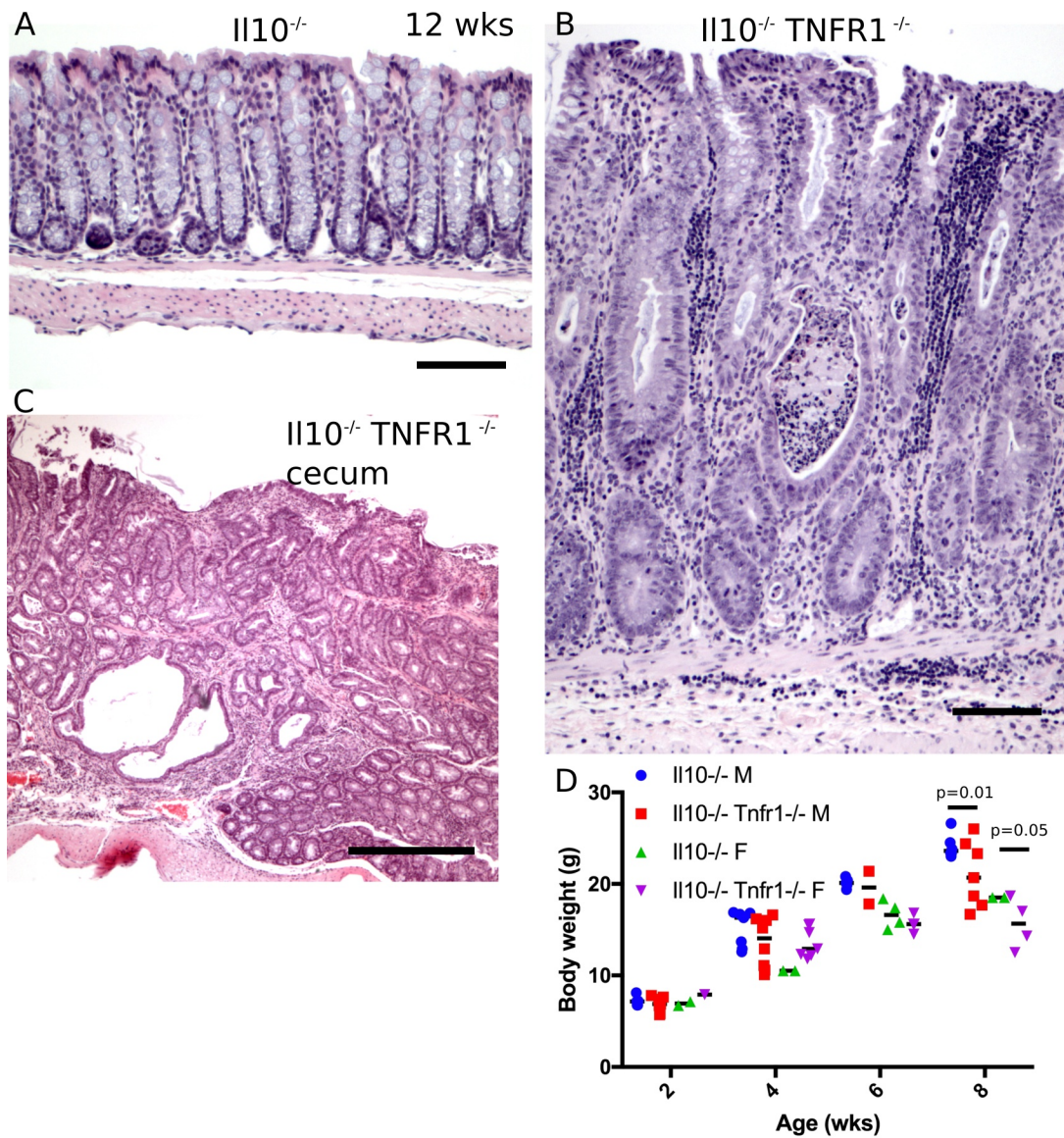


Figure S1. Sequelae of early-onset colitis in *Il10*^{-/-} *Tnfr1*^{-/-} mice, Related to Figure 1.

A-B) While hematoxylin and eosin- (H&E-) stained colonic sections of 12-wk-old *Il10*^{-/-} animals (A) showed minimal changes, sections of age-matched *Il10*^{-/-} *Tnfr1*^{-/-} animals (B) showed severe colitis with mucosal inflammatory infiltration and crypt abscesses. C) H&E-stained sections demonstrated the development of cecal inflammation and sessile adenocarcinoma in *Il10*^{-/-} *Tnfr1*^{-/-} mice. D) Sex-stratified analysis of mouse body weight shows growth restriction in *Il10*^{-/-} *Tnfr1*^{-/-} animals, consistent with worsened colitis. Scale bars: A-B) 100 μ m, C) 500 μ m.

Figure S2

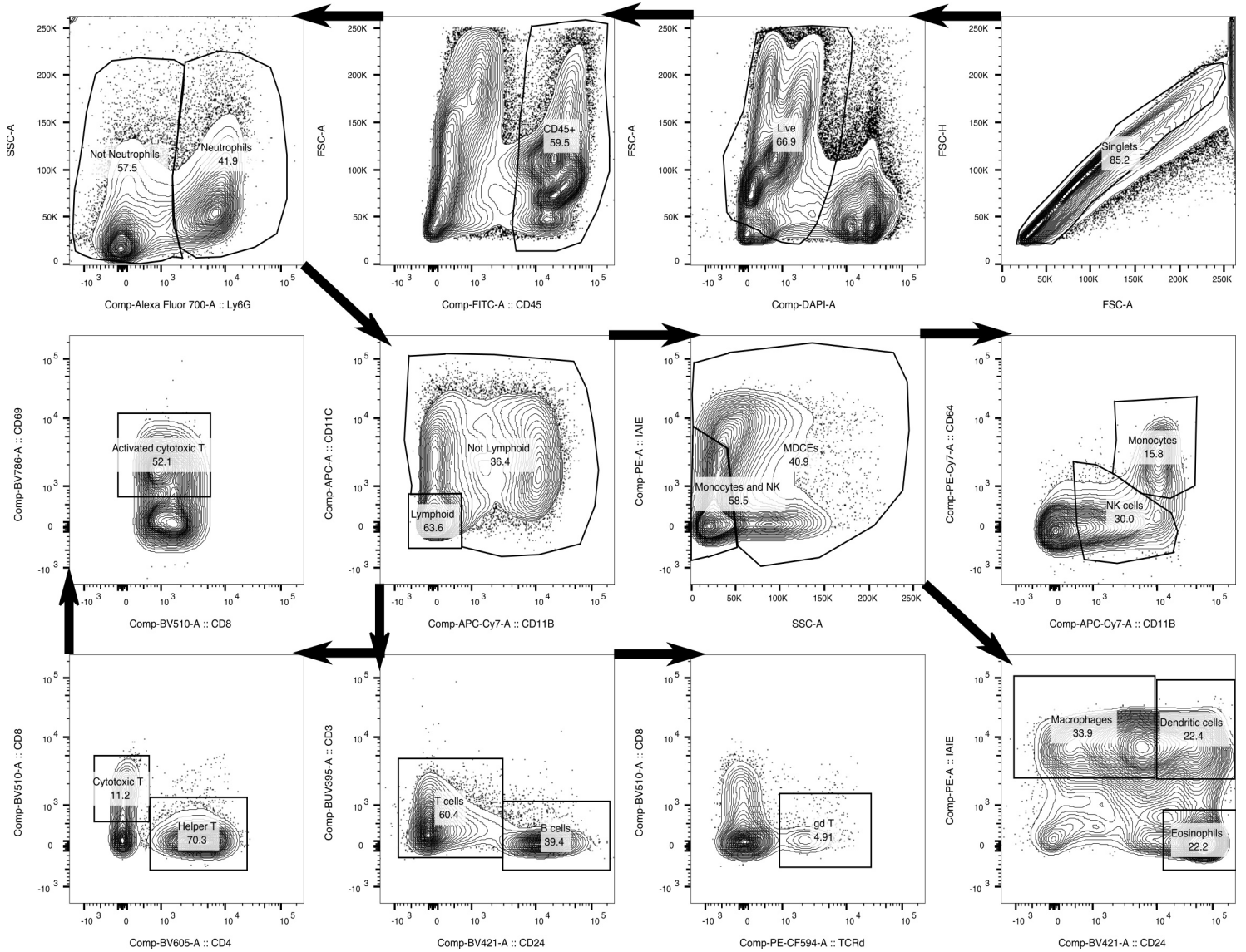


Figure S2. Gating strategy for flow cytometric analysis of colonic immune cell populations, Related to Figure 1.

The workflow starts in the upper right corner.

Figure S3

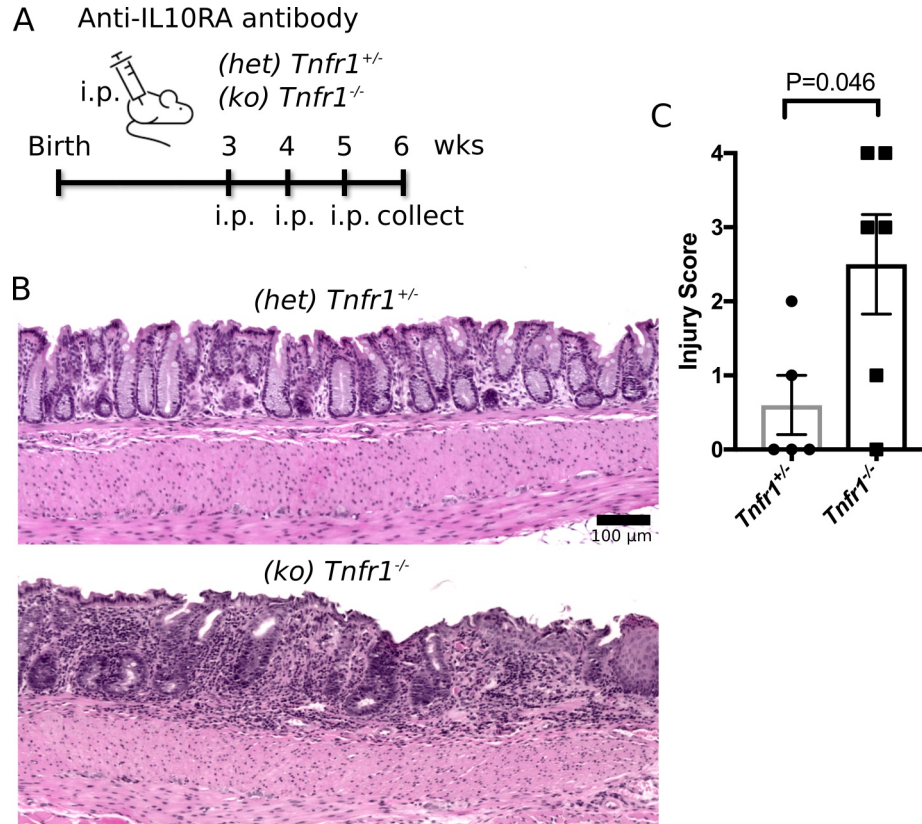


Figure S3. Induction of colitis through antibody-mediated neutralization of the IL-10 receptor in *Tnfr1*^{-/-} mice, Related to Figure 1.

A) The experimental schematic depicts the intraperitoneal injection of 1 mg anti-IL10Ra antibody 1x/wk for 3 wks, beginning at 3 wks of age, into heterozygous *Tnfr1*^{+/-} or knockout *Tnfr1*^{-/-} mice. Dissection and analysis were performed at 6 wks of age. B) H&E-stained sections of antibody-treated *Tnfr1*^{-/-} animals show histologic hallmarks of colitis, including immune cell infiltration and crypt loss. C) Histologic injury scores demonstrate exacerbation of colitis in *Tnfr1*^{-/-} mice in this antibody-mediated IL10Ra depletion model.

Figure S4

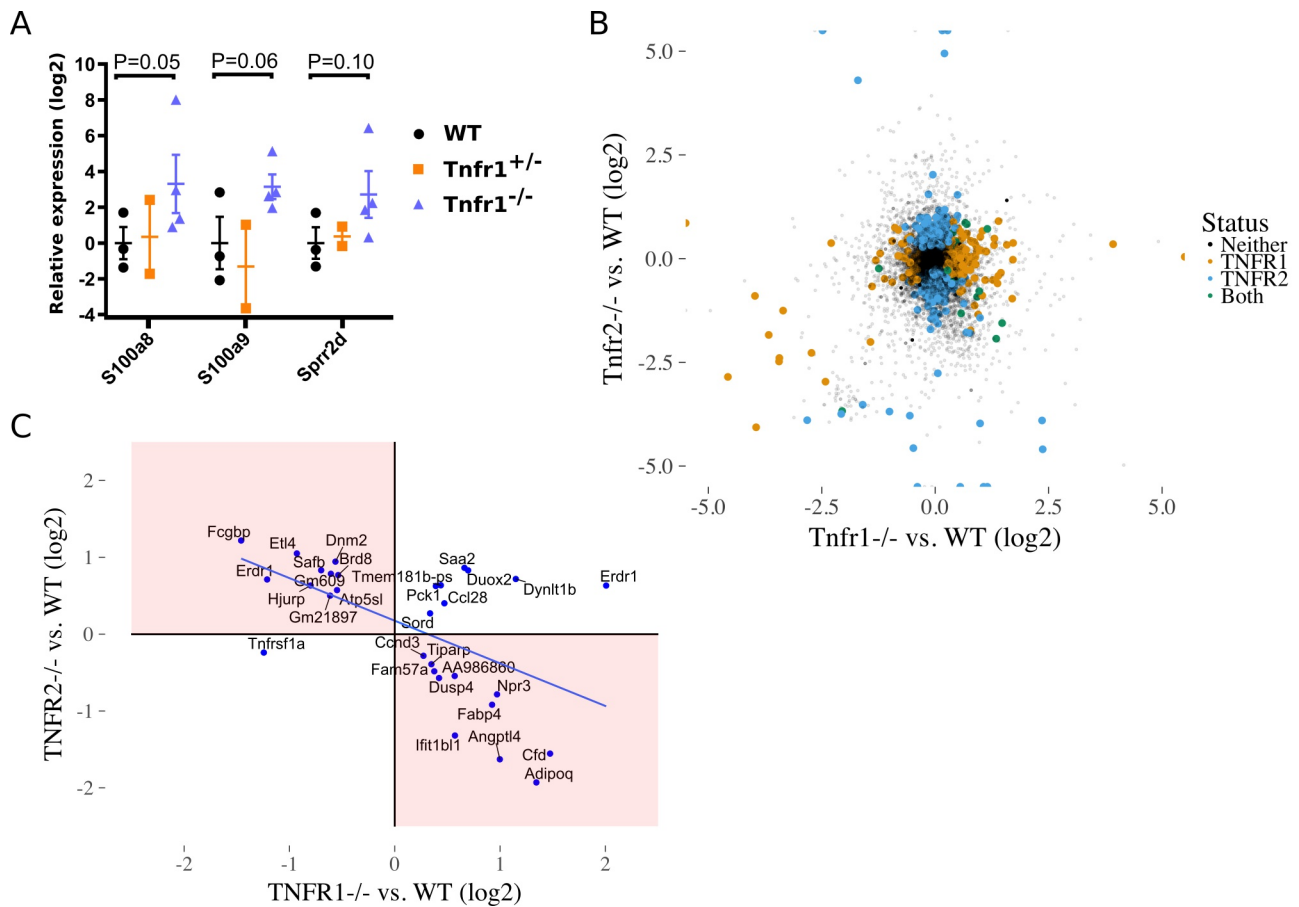


Figure S4. Expanded analysis of RNA-Seq results, Related to Figure 4.

A) RT-qPCR assessment of colonic calprotectin (*S100a8*, *S100a9*) expression in an independent cohort replicates increased levels in adult *Tnfr1*^{-/-} mice and reduced levels in heterozygous *Tnfr1*^{+/-} mice. B) Gene expression profiles of *Tnfr1*^{-/-} colons were compared with *Tnfr2*^{-/-} colons reanalyzed using the kallisto pipeline. Differentially expressed transcripts (versus wildtype) of the two knockout strains are displayed in a scatterplot (i.e., each point represents a gene) based on the magnitude of differential regulation. Common transcripts are color-coded in green. Most differentially expressed transcripts are unique to either *Tnfr1*^{-/-} or *Tnfr2*^{-/-} animals. C) The scatterplot demonstrates that TNFR1 and TNFR2 antagonistically regulated the 30 shared transcripts. The correlation coefficient defining the co-regulation between these two genotypes was -0.55.