

Figure S1 | Related to Figure 1

Volcano plots showing pairwise comparisons of differentially expressed genes in the cell subsets analyzed by mRNA sequencing. Y axis = log₂ fold change; X axis = log₂ mean read count. Red dots represent genes with FDR < 0.01. SI = small intestine; Col = colon; Gall = gall bladder; Thy = thymus; Tr = trachea

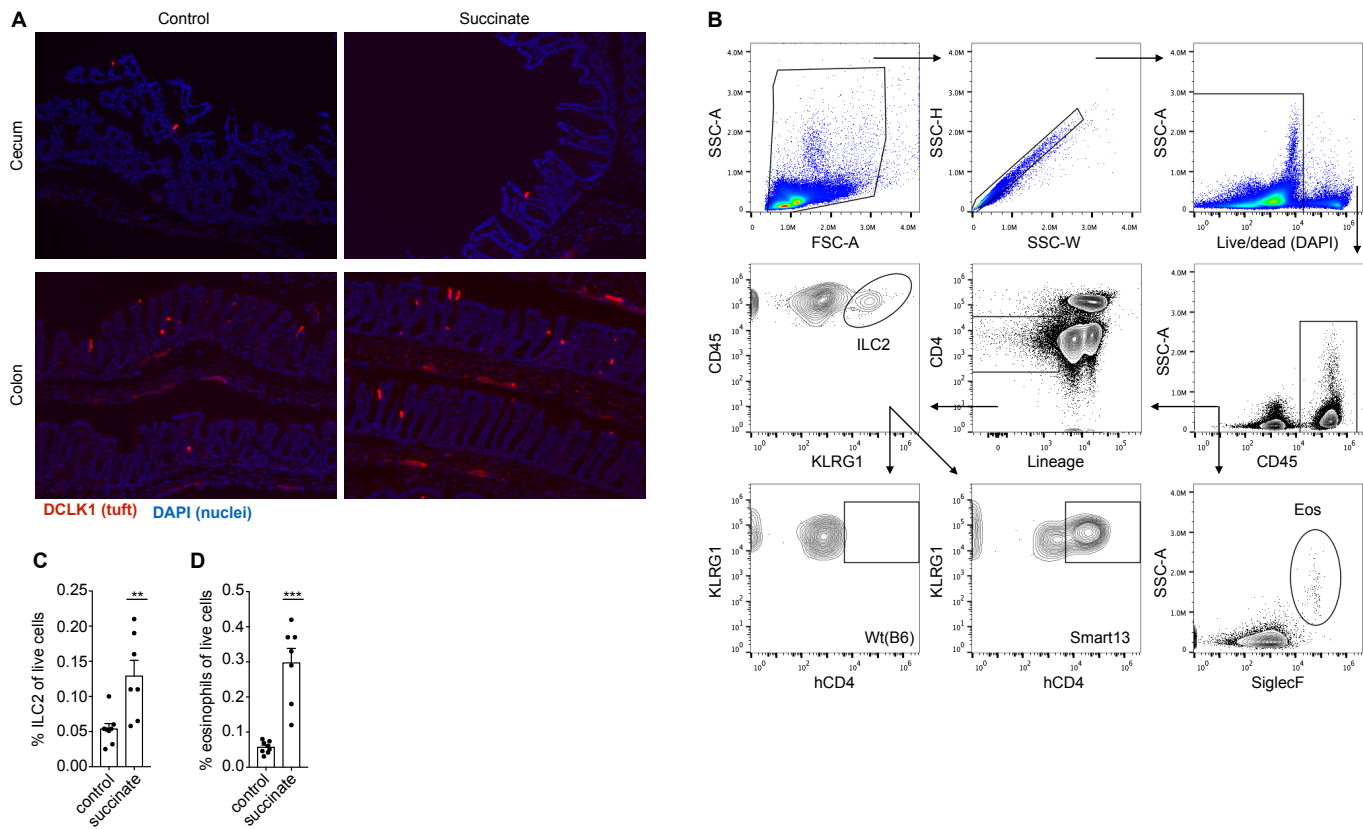


Figure S2 | Related to Figure 2

(A) Indicated tissues from control or succinate-treated (150 mM; 7 days) wild-type mice were stained for tuft cells (anti-DCLK1; red) and nuclei (DAPI; blue). (B) Gating strategy for identification of ILC2s and eosinophils in the mesenteric lymph nodes (MLN). IL-13 production was assessed by staining for human CD4 in Smart13 reporter mice. (C-D) MLN from control or succinate-treated (150 mM; 7 days) wild-type mice were analyzed by flow cytometry to assess the frequency of ILC2s (C) and eosinophils (D) among all live cells. Data in A-B are representative of at least three experiments. Data in C-D are pooled from two experiments and bar graphs depict mean + SEM. Each symbol represents an individual mouse. **, $p < 0.01$; ***, $p < 0.001$ by Mann-Whitney.

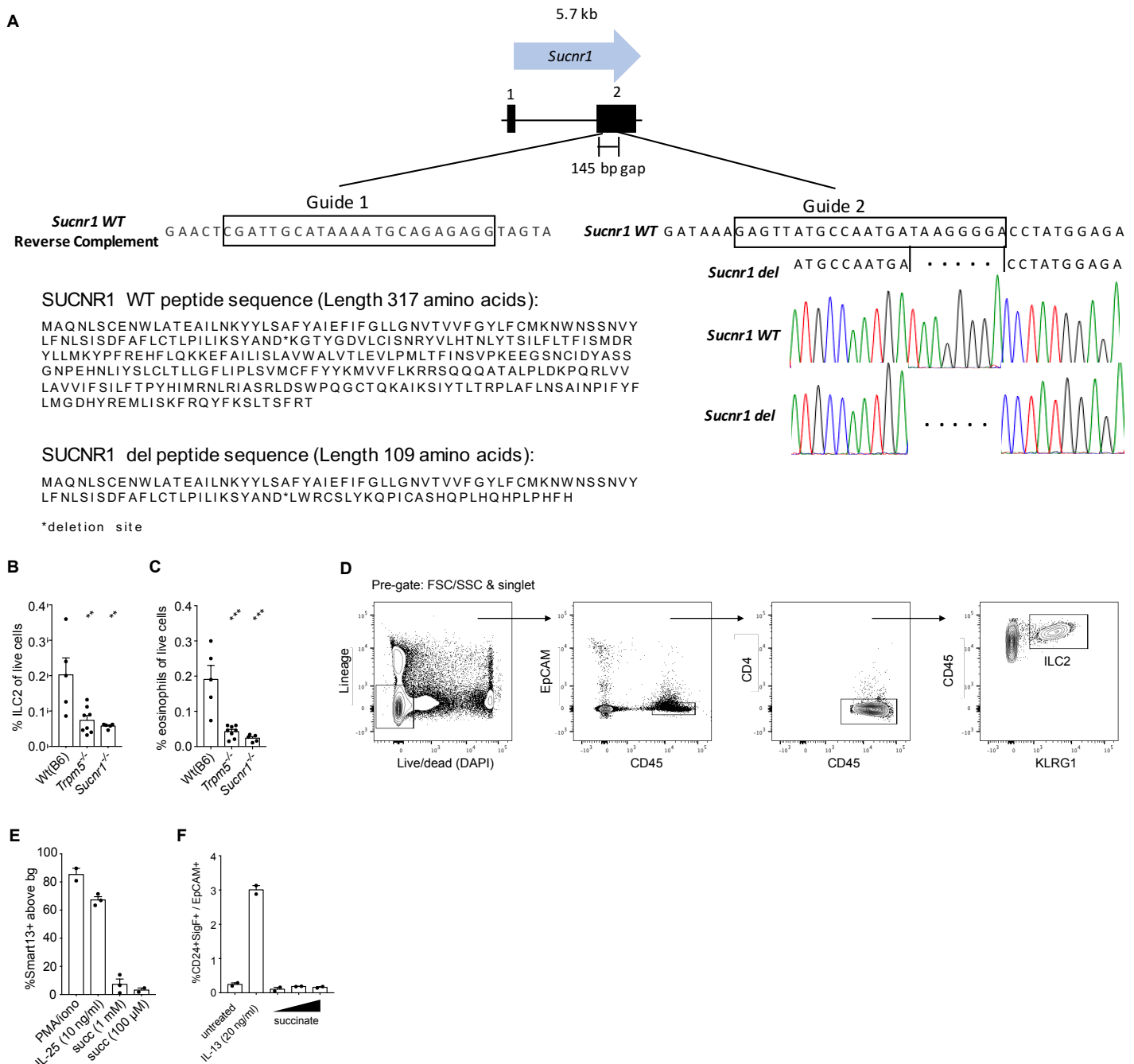


Figure S3 | Related to Figure 3

(A) Diagram depicting the location of guide RNAs used to target *Sucnr1* for disruption by CRISPR/Cas9. Guide 1 failed to induce any mutations. In the founder used to establish the *Sucnr1*^{-/-} line reported in this study, an 8 base-pair deletion was detected at the binding site for guide 2. The resulting frame shift inserts a premature stop codon that truncates the C-terminal 208 amino acids of SUCNR1. (B-C) Mice of indicated genotypes were treated with 150 mM succinate for 7 days and mesenteric lymph nodes were harvested to quantify the frequency of ILC2s (B) and eosinophils (C) among all live cells by flow cytometry. (D) Gating strategy to identify ILC2s in the lamina propria of the small intestine. (E) ILC2s were stimulated in vitro as indicated for 6 hours and IL-13 production was quantified by flow cytometry using Smart13 reporter expression. (F) Epithelial organoids from murine small intestine were cultured for 7 days under indicated conditions. Succinate concentrations = 100 μM, 1mM, and 10 mM. Tuft cell frequency (CD24+SigF+EpCAM+ cells) was quantified by flow cytometry. Data are representative of one (E) or two (D, F) experiments or are pooled from three experiments (B-C). Bar graphs depict mean ± SEM. Each symbol represents an individual mouse (B-C) or technical replicate (E-F). *, p < 0.05 ** , p < 0.01; *** , p < 0.001 by one-way ANOVA with comparison to Wt(B6).

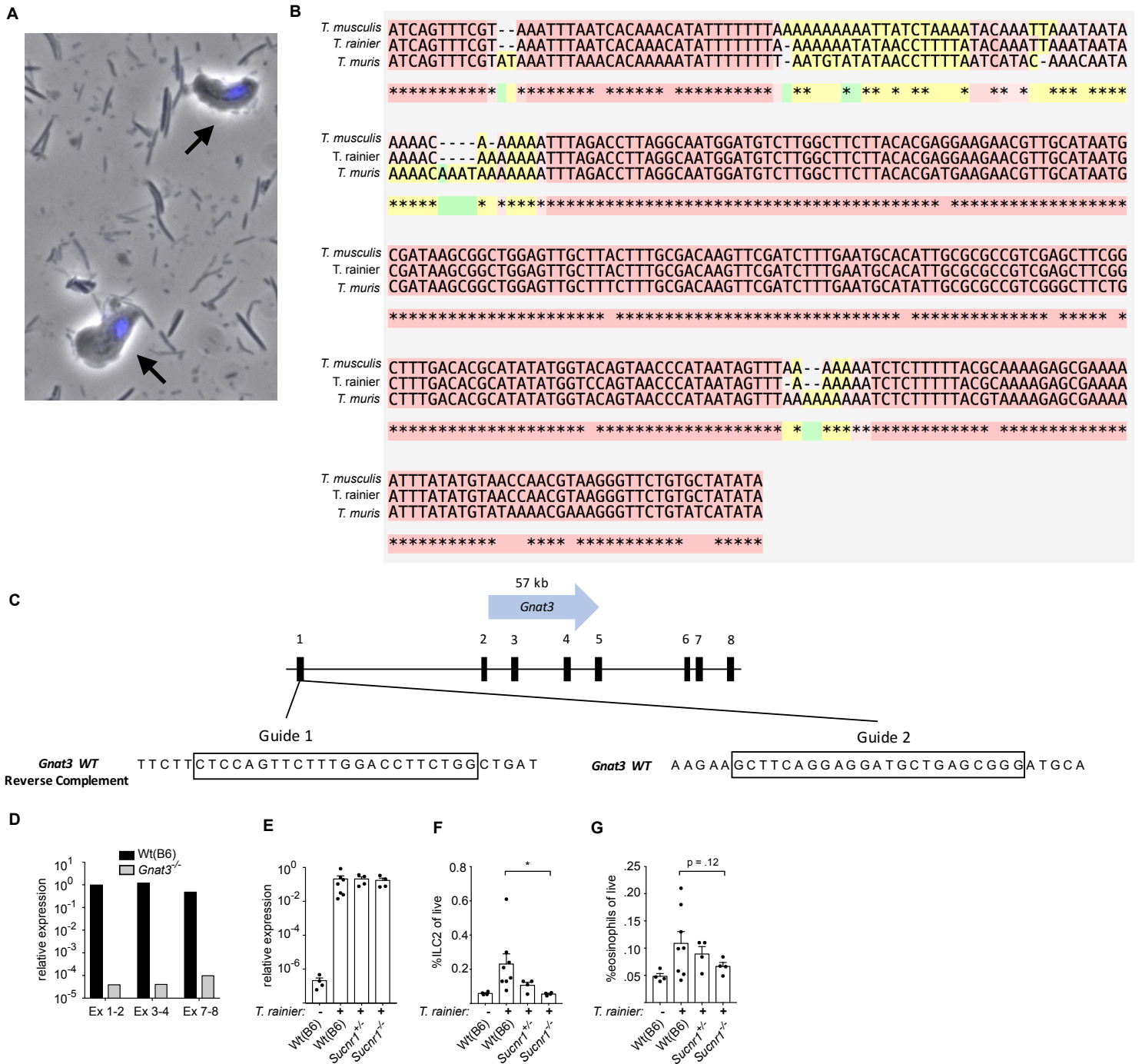


Figure S4 | Related to Figure 4

(A) Representative images of *T. rainier* (arrows) with DAPI (blue) staining of nuclei. **(B)** Alignment of internal transcribed spacer sequence of *T. rainier* with published sequences for *T. muris* and *T. musculus*. **(C)** Diagram depicting the location of guide RNAs used to target *Gnat3* for disruption by CRISPR/Cas9. In the founder used to establish the *Gnat3*^{-/-} line reported in this study, the region between guide 1 and guide 2 was deleted. **(D)** Functional deletion of *Gnat3* was confirmed by qPCR using primer pairs spaced across the entire mRNA. **(E)** *Trichomonas* colonization of indicated mice was quantified by qPCR using DNA generated from cecal contents. **(F-G)** Mice of indicated genotypes were colonized with *T. rainier* for 7 days and mesenteric lymph nodes were harvested and the frequency of ILC2s **(F)** and eosinophils **(G)** among all live cells was assessed by flow cytometry. Data are representative of three (B) two (A) or one (D) experiment or pooled (E-G) from two experiments. Bar graphs depict mean + SEM. Each symbol represents an individual mouse. *, $p < 0.05$ by one-way ANOVA with comparison to colonized Wt(B6).