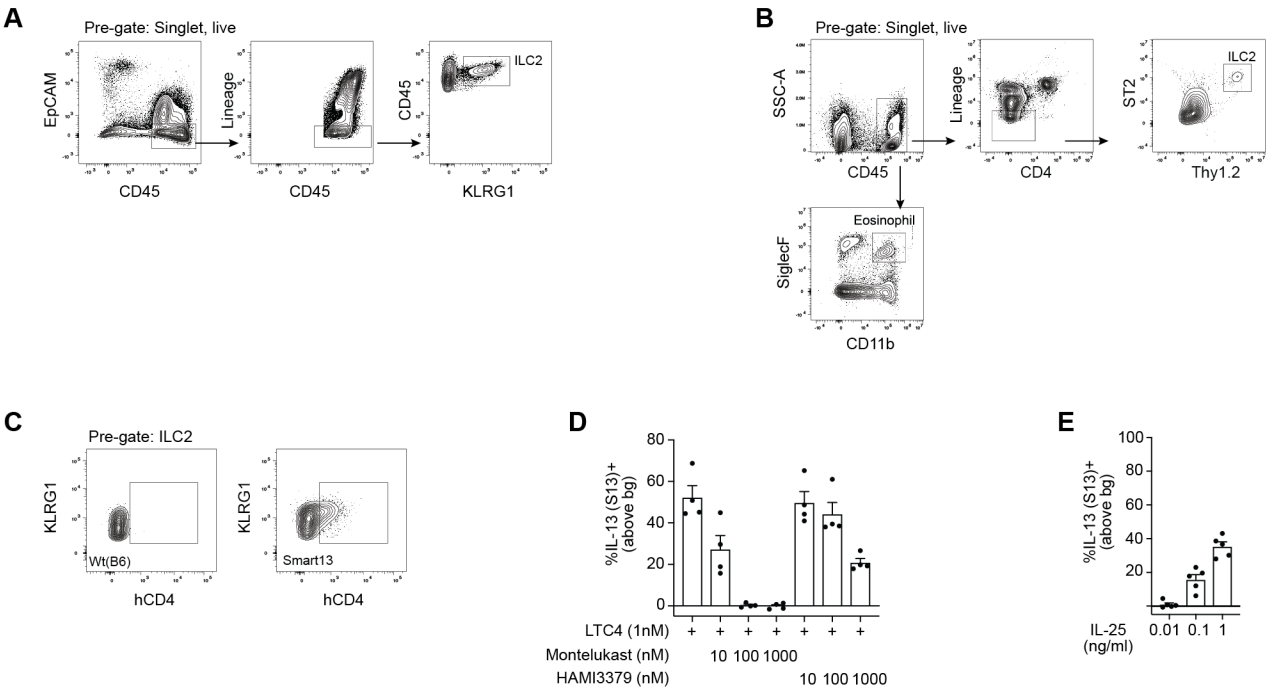

Figure S1. LTC_4-mediated ILC2 activation depends on signaling through the receptor CYSLTR1.

Related to Figure 1.

(A) Gating strategy for identification of ILC2s by KLRG1 expression in the small intestine (SI) lamina propria. **(B)** Gating strategy for identification of ILC2s and eosinophils in the lung. **(C)** Example gating strategy for quantification of IL-13 (S13) reporter expression. **(D)** Sorted SI ILC2s were treated *in vitro* with the indicated inhibitor for 30 minutes prior to stimulation with LTC_4 for 6 hours. IL-13 (S13) expression was subsequently determined by flow cytometry. **(E)** IL-25 dose response for SI ILC2 IL-13 (S13) induction. In (D)-(E) each symbol represents an individual mouse from two or more pooled experiments. bg, background. Graphs depict mean + SEM.

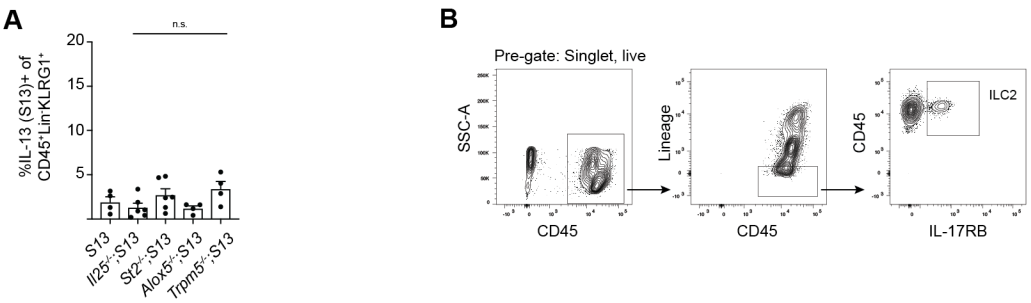

Figure S2. IL-13 expression by small intestine lamina propria ILC2s is restrained during homeostasis. Related to Figure 2.

(A) Quantification of IL-13 (S13) expression by ILC2s in the proximal (first 5cm) small intestine (SI) of naïve mice. ILC2s were identified as CD45+;Lineage-;KLRG1+. **(B)** Gating strategy for identification of ILC2s by IL-17RB expression in the SI lamina propria. In (A) each symbol represents an individual mouse pooled from three experiments. *p < 0.05, **p < 0.01, ***p < 0.001 by one way ANOVA (A) with comparison to S13. n.s., not significant. Graphs depict mean + SEM.

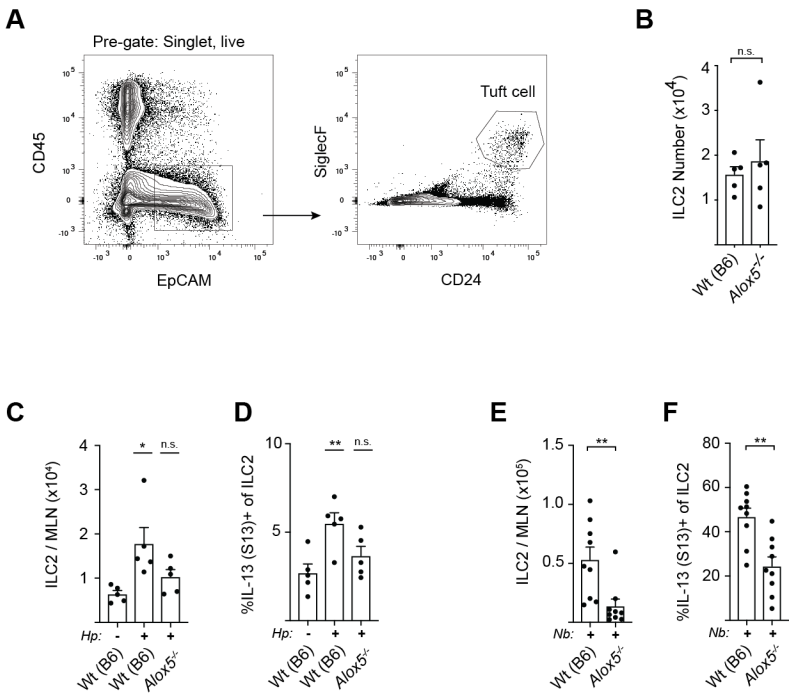

Figure S3. ILC2 number and activation are reduced in the mesenteric lymph nodes of leukotriene-deficient mice following helminth infection. Related to Figure 3.

(A) Gating strategy for identification of tuft cells in Wt(B6) mice. **(B)** ILC2 number in the proximal (first 10cm) small intestine (SI) lamina propria of naïve mice. **(C)** ILC2 number and **(D)** IL-13 (S13) expression in mesenteric lymph nodes four days after *H. polygyrus* infection. **(E)** ILC2 number and **(F)** IL-13 (S13) expression in mesenteric lymph nodes five days after *N. brasiliensis* infection. In (B)-(F) each symbol represents an individual mouse from two or more pooled experiments. *Hp, H. polygyrus*. *Nb, N. brasiliensis*. MLN, mesenteric lymph node. *p < 0.05, **p < 0.01, ***p < 0.001 by Mann-Whitney (B, E, F) or by one way ANOVA (C, D) with comparison to Wt(B6). n.s., not significant. Graphs depict mean + SEM.

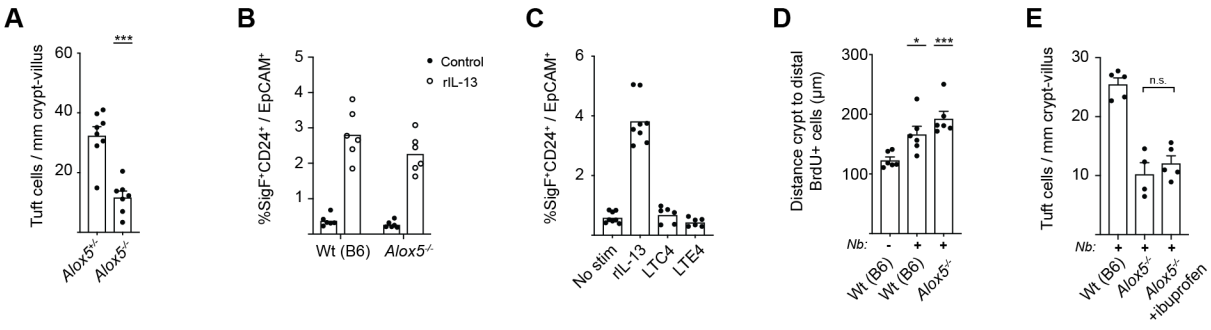

Figure S4. Leukotriene signaling does not directly affect intestinal epithelial cell turnover or differentiation. Related to Figure 4.

(A) Frequency of tuft cells in the distal (last 10cm) small intestine (SI) of littermates after 7 days of *N. brasiliensis* infection. **(B)** Frequency of tuft cells in organoid cultures derived from mice of the indicated genotype left untreated or stimulated with 20ng/ml recombinant IL-13 for one week. **(C)** Frequency of tuft cells in organoid cultures stimulated with 20ng/ml recombinant IL-13, 10nM LTC_4, or 10nM LTE_4 for one week. **(D)** Mice of the indicated genotype were infected with *N. brasiliensis* and injected with 1mg BrdU on day 5 post-infection. After 24 hours the proximal (first 10cm) SI was harvested and BrdU incorporation determined as the distance from the crypt to most distal BrdU+ cell per villus. **(E)** Mice were provided ibuprofen-medicated (1mg/ml) or control drinking water ad libitum starting one day prior to infection and then infected with *N. brasiliensis* for 5 days. Tuft cell frequency in the proximal SI was determined. In (A) and (D)-(E) each symbol represents an individual mouse from two or more pooled experiments. In (B)-(C) symbols represent technical replicates combined from two experiments. *Nb, N. brasiliensis*. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by Mann-Whitney (A) or by one way ANOVA (D-E) with comparison to Wt(B6). n.s., not significant. Graphs depict mean + SEM.

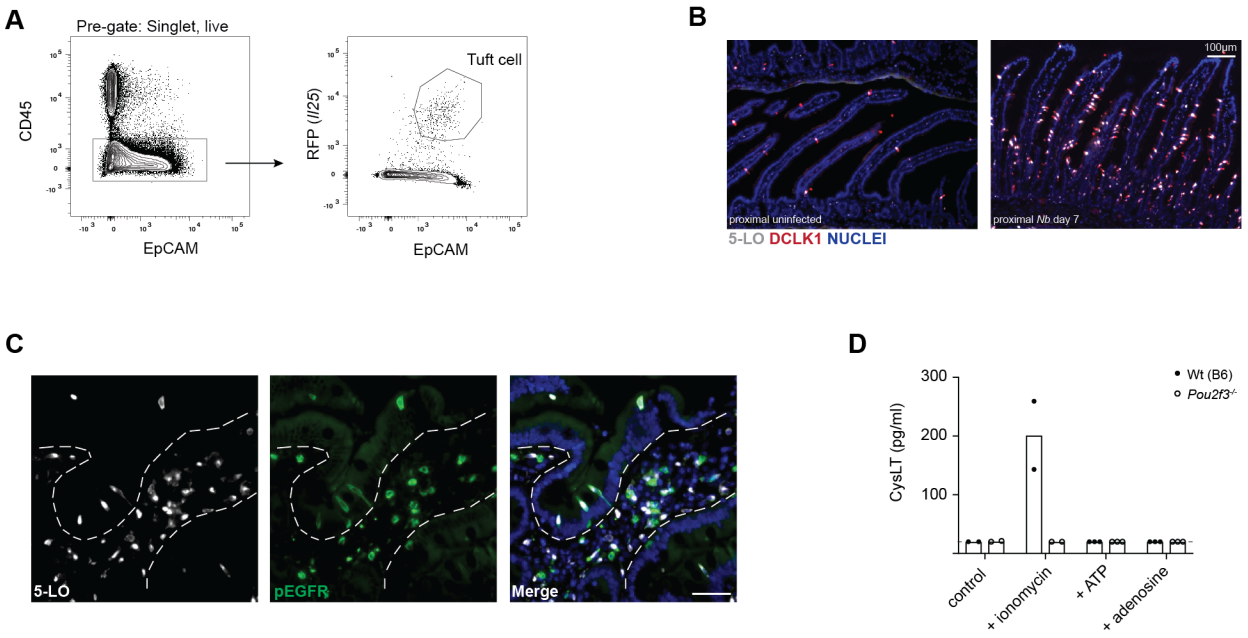

Figure S5. Tuft cells express 5-lipoxygenase, but ATP and adenosine are not sufficient to trigger cysteinyl leukotriene synthesis *in vitro*. Related to Figure 5.

(A) Gating strategy for identification of tuft cells in Flare25 mice. **(B)** Colocalization of 5-lipoxygenase (5-LO) (white) and DCLK1 (red) in the proximal (first 10cm) small intestine (SI) of a naive mouse and after 7 days of *N. brasiliensis* infection. Scale bar is as shown. **(C)** Colocalization of 5-LO (white) and pEGFR (green) in a human duodenal tissue section. Dashed line outlines the basolateral membrane of the epithelial layer. Scale bar: 50µm. **(D)** Cysteinyl leukotriene (CysLT) production in supernatants of intestinal monolayer cultures derived from wildtype mice following 30 minute stimulation with 1µg/ml ionomycin, 100µM ATP, or 100µM adenosine. Dashed line represents limit of detection. In (D) symbols are technical replicates representative of two independent experiments. In (B)-(C) images are representative of two or more independent experiments.

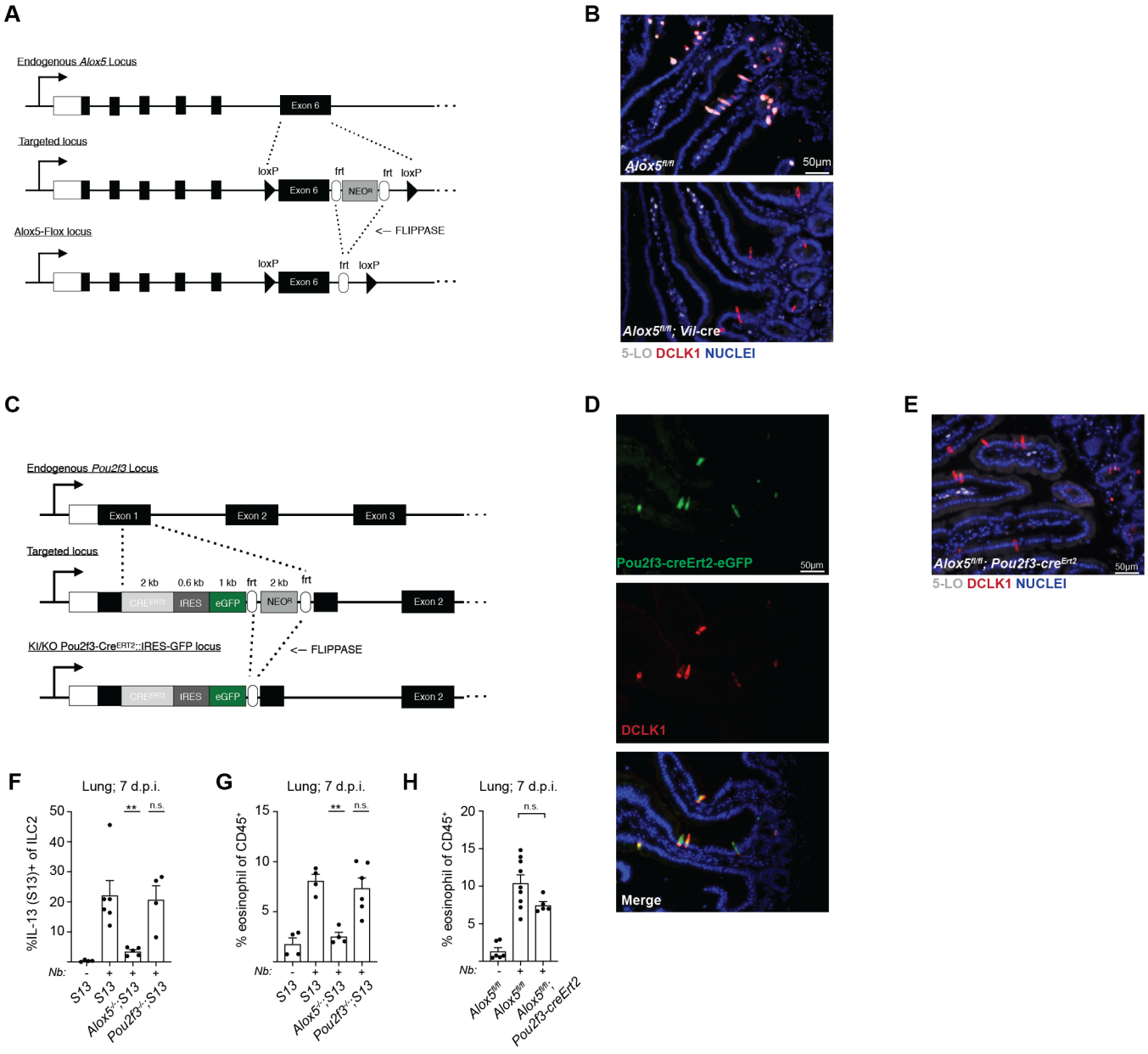

Figure S6. Targeting and validation of the *Alox5*-flox and *Pou2f3*-cre^{Ert2}-eGFP mouse lines. Related to Figure 6.
(A) Gene targeting strategy for the *Alox5*-flox mouse. **(B)** 5-lipoxygenase (5-LO) abrogation in cre-expressing mice was confirmed by staining for 5-LO (white) and DCLK1 (red) in the proximal (first 10cm) small intestine (SI) of *Alox5*$^{fl/fl}$ or *Alox5*$^{fl/fl}$;*Vil*-cre+ mice infected with *N. brasiliensis* for 5 days. 5-LO expression was maintained in the lamina propria of both strains. **(C)** Gene targeting strategy for the *Pou2f3*-cre^{Ert2}-eGFP mouse. **(D)** Colocalization of *Pou2f3*-cre^{Ert2}-eGFP (green) and DCLK1 (red) in the proximal SI of naïve mice. **(E)** 5-LO deletion within tuft cells was confirmed by staining for 5-LO (white) and DCLK1 (red) in the proximal SI of tamoxifen treated *Alox5*$^{fl/fl}$;*Pou2f3*-cre^{Ert2} mice infected with *N. brasiliensis* for 5 days. 5-LO expression was absent in tuft cells but maintained in the lamina propria. **(F)** Frequency of IL-13 (S13)+ ILC2s in the lung after 7 days of *N. brasiliensis* infection. **(G-H)** Eosinophil frequency in the lung after 7 days of *N. brasiliensis* infection. In (F)-(H) each symbol represents an individual mouse from two or more pooled experiments. *Nb, N. brasiliensis*. *p < 0.05, **p < 0.01, ***p < 0.001 by one way ANOVA (F-H) with comparison to infected S13 or *Alox5*$^{fl/fl}$. n.s., not significant. Graphs depict mean + SEM.