Innate lymphoid cells support regulatory T cells in the intestine through interleukin-2

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Interleukin (IL)-2 is a pleiotropic cytokine that is necessary to prevent chronic inflammation in the gastrointestinal tract1–4. The protective effects of IL-2 involve the generation, maintenance and function of regulatory T (Treg) cells5–8, and the use of low doses of IL-2 has emerged as a potential therapeutic strategy for patients with inflammatory bowel disease9. However, the cellular and molecular pathways that control the production of IL-2 in the context of intestinal health are undefined. Here we show, in a mouse model, that IL-2 is acutely required to maintain Treg cells and immunological homeostasis throughout the gastrointestinal tract. Notably, lineage-specific deletion of IL-2 in T cells did not reduce Treg cells in the small intestine. Unbiased analyses revealed that, in the small intestine, group-3 innate lymphoid cells (ILC3s) are the dominant cellular source of IL-2, which is induced selectively by IL-1β. Macrophages in the small intestine produce IL-1β, and activation of this pathway involves MYD88- and NOD2-dependent sensing of the microbiota. Our loss-of-function studies show that ILC3-derived IL-2 is essential for maintaining Treg cells, immunological homeostasis and oral tolerance to dietary antigens in the small intestine. Furthermore, production of IL-2 by ILC3s was significantly reduced in the small intestine of patients with Crohn’s disease, and this correlated with lower frequencies of Treg cells.

To determine whether IL-2 is constitutively required for the maintenance of Treg cells and immunological homeostasis in the intestine, we administered isotype-control or anti-IL-2 neutralizing antibodies every other day to adult mice for two weeks. Within this short time period, the neutralization of IL-2 promoted an enlargement of the spleen and mesenteric lymph nodes, and caused significant reductions of Treg cells and significant increases in the proliferation of CD4+ T cells throughout the gastrointestinal tract and associated lymphoid tissues, including the mesenteric lymph nodes, large intestine and small intestine (Extended Data Fig. 1a–g). Blockade of IL-2 resulted in significantly enhanced IFN-γ production by CD4+ T cells in both the small and large intestine, as well as increased IL-17A production in the large intestine (Extended Data Fig. 1h–k). Previous studies have suggested that CD4+ T cells are the dominant cellular source of IL-21,2. Therefore, we generated mice with a lineage-specific deletion of IL-2 in T cells by crossing IL-2–floxed mice10 with Lckcre mice. LckcreIl2−/− mice exhibited a complete loss of IL-2 protein staining in T cells, and we observed a significant reduction in the number of Treg cells, and an increase in CD4+ T cell proliferation and effecter function in the mesenteric lymph nodes and large intestine (Extended Data Fig. 2a–g). By contrast, deletion of T-cell-derived IL-2 did not alter Treg cells, CD4+ T cell proliferation or effector cytokine production within the small intestine (Extended Data Fig. 2h–k). Collectively, these data demonstrate that T-cell-derived IL-2 is required for maintaining immunological homeostasis in lymphoid tissues and the large intestine but is dispensable in the small intestine, which indicates the existence of other critical cellular sources of IL-2.

We next examined the relevant cellular sources of IL-2 in the healthy mammalian small intestine by unbiased flow cytometry analyses. Notably, we observed that the major population of IL-2+ cells lack lineage markers for T cells, B cells, macrophages and dendritic cells, but are CD127+, CD90.2+ and express retinoic-acid-related orphan receptor-γt (ROR-γt) (Fig. 1a), which represents a phenotype that is consistent with ILC3s11–13. To directly compare ILC2 transcript levels between CD4+ T cells and ILC3s in the healthy small intestine, we performed RNA sequencing on sorted cell populations. In comparison to differentially expressed genes found in ILC3s (Rorc, Il1r2, Il2rg also known as Il1r1 and Il23r) or CD4+ T cells (Gldb), expression of Il2 was more highly enriched in ILC3s (Fig. 1b). Significantly higher expression of Il2 was confirmed in ILC3s relative to CD4+ T cells, dendritic cells or B cells after quantitative PCR analysis of populations that were purified from the healthy mouse small intestine (Fig. 1c). ILC3s were the most-abundant IL-2+ cell type in terms of frequency and total cell number among other subsets of innate lymphoid cells and total CD4+ T cells from the small intestine (Fig. 1d–f, Extended Data Fig. 3), and were present at higher cell numbers than effector and memory CD4+ T cells (Extended Data Fig. 4a). This is in contrast to the large intestine, in which the majority of IL-2 was produced by CD4+ T cells and there was a limited presence of innate lymphoid cells that produce IL-2 (Extended Data Fig. 4b–d). ILC3s are a heterogeneous population that includes both CCR6+ lymphoid-tissue-inducer-like ILC3s and T-bet+ ILC3s11–13. IL-2 in the small intestine was produced by both subsets of ILC3s, with a significantly higher frequency of T-bet+ ILC3s that produce IL-2 (Extended Data Fig. 4e). Production of IL-2 by ILC3s was confirmed by flow cytometry analyses of the small intestine of Rag1−/− mice, which revealed that the major population of IL-2+ cells comprises CD127+ CD90.2+ ROR-γt+ ILC3s (Extended Data Fig. 4f–h), which include T-bet+ and CCR6+ ILC3s (Extended Data Fig. 4i, j). Unbiased analyses of the large intestine of Rag1−/− mice indicated that the major population of IL-2+ cells is comprised of innate lymphoid cells (Extended Data Fig. 4k). Furthermore, the frequency of IL-2+ cells that we observed in the small intestine of Rag2−/− mice was significantly reduced in Rag2−/− Il2rg−/− mice deficient in innate lymphoid cells, or Rag2−/− mice depleted of innate lymphoid cells with anti-CD90.2 antibody (Fig. 1g). Collectively, these findings demonstrate that IL-2 is dominantly produced by ILC3s in the healthy small intestine.
ILC3 development and function can be markedly influenced by the intestinal microbiota\(^1\)–\(^13\). To investigate whether the intestinal microbiota also regulates ILC3–intrinsic IL-2 production, we examined the small intestine of conventionally housed specific-pathogen-free mice exposed to broad-spectrum antibiotics and germ-free mice. Notably, germ-free mice or specific-pathogen-free mice that were exposed to broad-spectrum antibiotics displayed significantly decreased IL-2 production by ILC3s, relative to specific-pathogen-free mice (Fig. 2a). To determine the signals that directly induce ILC3–intrinsic IL-2 production, we stimulated sort-purified ILC3s from the small intestine with IL-1\(\beta\), IL-6, IL-23 or the aryl hydrocarbon receptor agonist FICZ, which are all known to directly stimulate ILC3s\(^11\)–\(^13\). Among these, we found that IL-1\(\beta\) selectively induced IL2 transcription and protein production in ILC3s (Fig. 2b, c). We observed comparable results with stimulation using IL-1\(\alpha\) (Fig. 2d). Furthermore, IL-1\(\beta\) was sufficient to significantly boost IL-2 production in ILC3s from germ-free mice and specific-pathogen-free mice exposed to broad-spectrum antibiotics (Fig. 2e, f).

Gene-expression analyses of intestinal phagocytes revealed that the majority of IL-1\(\beta\) in the small intestine is produced by macrophages (Fig. 2g). Consistent with this, macrophages isolated from the small intestine of germ-free mice exhibited significantly reduced IL-1\(\beta\) expression and could be partially restored by mono-colonization with segmented filamentous bacteria (Fig. 2h), a component of the mouse microbiota that is known to colonize the distal small intestine, induce IL-1\(\beta\), activate ILC3s and promote expansion of T\(\text{reg}\) cells\(^14\)–\(^16\). Mono-colonization with segmented filamentous bacteria was sufficient to significantly induce ILC3–derived IL-2 production (Fig. 2i). To explore the sensing modules that are required to promote the IL-1\(\beta\)–ILC3–IL-2 axis, we examined Myd88\(^{−/−}\) mice and observed a significant reduction in expression of IL-1\(\beta\) by macrophages (Fig. 2j).
CD11b+CD64+*F4/80+*, conventional dendritic cell 1 (cDC1) (CD45+CD11c+MHCI*II*CD64+*XCR1+CD172a+) and conventional dendritic cell 2 (cDC2) (CD45+CD11c+MHCI*II*CD64+*XCR1−CD172a+) in small-intestinal lamina propria cells (n = 6). Il1b expression was normalized to Gapdh. h, i, Quantitative PCR analysis of Il1b transcript in sort-purified small-intestinal macrophages (h) or flow cytometry analysis of IlC3-derived IL-2 expression (i) in specific-pathogen-free, germ-free and segmented filamentous bacteria (SBF) mono-colonized mice (n = 6). j, Quantitative PCR analysis of Il1b transcript in sort-purified small-intestinal macrophages in wild-type (WT), Myd88−/− and Nod2−/− mice (n = 3). k, Flow cytometry analysis of small-intestinal IL-2+ ILC3s in wild-type mice, Nod2−/− mice and Nod2−/− mice after ex vivo culture (n = 8). Data in a, c, f, h and i are representative of two or three independent experiments with similar results (at least three mice per group). Data in b, d, g and k are pooled from two independent experiments. Results are shown as mean ± s.e.m. Statistics are calculated by paired or unpaired two-tailed Student’s t-test.
with an adoptive transfer of unfractinated wild-type CD4+ T cells. In comparison to Il2f/fRag1−/− recipient mice, Rorc−/−Il2f/fRag1−/− recipient mice exhibited rapid and substantial weight loss, shorter colon length, spontaneous colonic inflammation characterized by increased inflammatory cell infiltration and significantly increased levels of faecal lipocalin-2 (Extended Data Fig. 9a–d). Notably, we observed significantly decreased numbers of colonic Treg, cells associated with significantly increased numbers of IL-17A+ IFN-γ+ and IL-17A− IFN-γ− T cells in the large intestine, as well as increased numbers of Treg cells in the small intestine of Rorc−/−Il2f/fRag1−/− recipient mice relative to controls, even though both groups exhibited comparable small-intestinal Treg cells (Extended Data Fig. 9a–h). These findings suggest a model in which ILC3-derived IL-2 can also modulate immunological homeostasis and prevent inflammation throughout the entire gastrointestinal tract in the context of lymphopenia.

Finally, to investigate whether ILC3s support Treg cells in the human intestine, we examined the frequency of these populations in intestinal biopsies from healthy controls and patients with Crohn’s disease. As previously reported26,27, we observed significantly reduced frequencies of Treg cells and ILC3s in the small intestine of patients with Crohn’s disease relative to healthy controls, and—notably—we also identified a significant positive correlation between the frequencies of these two cell types within the same biopsy (Fig. 4a–c). To determine whether the impaired responses of Treg cells are associated with reduced ILC3-derived IL-2, we sort-purified ILC3s from resected small intestinal tissues of patients with Crohn’s disease and identified significantly reduced levels of IL2 transcript isolated from inflamed versus non-inflamed regions (Fig. 4d). In addition, IL-2+ ILC3s were significantly reduced in terminal-ileum biopsies from patients with Crohn’s disease relative to healthy controls, and no significant change was observed in IL-2+ CD4+ T cells (Fig. 4e, f). These data indicate that—similar to their role in mice—ILC3s support Treg cells within the human small intestine.
intestine, and suggest that this pathway becomes dysregulated in the context of Crohn's disease.

Collectively, our studies reveal a pathway of immune regulation that uniquely occurs in the healthy small intestine (Extended Data Fig. 10). This pathway is continuously required, involves MYD88- and NOD2-dependent microbial sensing by macrophages, production of IL-1β and induction of ILC3-derived IL-2. Subsequently, this pathway supports intestinal Treg cells, immunological homeostasis and oral tolerance. Our findings in mouse models translated into human samples, and indicate that impaired ILC3-derived IL-2 is linked to a reduction of Treg cells and impaired immune regulation within the small intestine of patients with Crohn's disease. Our results will inform ongoing strategies of therapeutically administering low doses of IL-2 to patients with inflammatory bowel disease, or to individuals with lymphopenia, immune deficiencies or who have been treated with chemotherapeutic agents. ILC3-derived IL-2 does not exhibit functional redundancy or hierarchies with previously known pathways by which ILC3s regulate adaptive immunity in the large intestine. 4–8,30. Finally, our results substantially advance our understanding of the role and regulation of IL-2 throughout the gastrointestinal tract, and identify a previously unappreciated direct communication between ILC3s and Treg cells.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source online content unappreciated direct communication between ILC3s and Treg cells.

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**Author contributions**

L.Z. and G.F.S. conceived the project. L.Z. performed most experiments and analysed the data. F.T., C.C., N.J.B., J.G., H.K. and E.K.S. helped with experiments. G.G.P. performed bioinformatics analyses. J.R.K., R.N.B., M.A.S. and R.E.S. provided human samples, scientific advice and expertise. E.V., L.Z. and G.F.S. wrote the manuscript, with input from all the authors.

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L.Z. and G.F.S. conceived the project. L.Z. performed most experiments and analysed the data. F.T., C.C., N.J.B., J.G., H.K. and E.K.S. helped with experiments. G.G.P. performed bioinformatics analyses. J.R.K., R.N.B., M.A.S. and R.E.S. provided human samples, scientific advice and expertise. E.V., L.Z. and G.F.S. wrote the manuscript, with input from all the authors.

**Competing interests** The authors declare no competing interests.

**Additional information**

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

Mice. Wild-type, Rag1−/−, THY1.1, OT-II, Lck−/−, IL17ra−/−, H2-Ab1−/−, Csf2−/−, Myd88−/− and Nod2−/− mice on a C57BL/6 background were purchased from Jackson Laboratory. Rag2−/− and Rag2−/−Il2ra−/− on a C57BL/6 background were purchased from Taconic Farms. C57BL/6 Rag1−/− mice and Rorc(gammat)-Gfp+ mice were provided by C.E. C57BL/6 Il2rα−/− mice were provided by K.A.S. C57BL/6 Ncr1−/− were provided by E.V., and only heterozygous Ncr1+/- mice were used in this study. All mice were bred and maintained in specific-pathogen-free facilities at Weill Cornell Medicine and littermates were used as controls in all experiments. C57BL/6 germ-free mice were maintained at the gnotobiotic facility at Weill Cornell Medicine. Sex and age-matched mice between 8 and 14 weeks of age were used for experiments if not otherwise indicated. Sex was not found to influence results of any experiments. No mice were excluded from the analysis unless clearly indicated. All mouse experiments were approved by, and performed in accordance with, the Institutional Animal Care and Use Committee guidelines at Weill Cornell Medicine.

In vivo administration of antibodies and antibiotics. Anti-IL-2 monoclonal antibodies (JES6-1A2 and S4B6-1, BioXcell) were administered intraperitoneally every other day at a dose of 300 μg (150 μg JES6-1A2 plus 150 μg S4B6-1) per mouse starting on day 0 and ending on day 14. Anti-CD90.2 monoclonal antibody (30H12, BioXcell) was administered intraperitoneally every 3 days at a dose of 250 μg per mouse starting on day 0 and ending on day 14, as previously described.1 Anti-NK1.1 antibody (PK136, BioXcell) was administered intraperitoneally every 3 days at a dose of 250 μg per mouse starting on day 0 and ending on day 14. A cocktail of antibodies (0.25 mg/ml of vancomycin, 0.5 mg/ml of ampicillin, neomycin, gentamicin and metronidazole, and 4 mg/ml sucrose) was continuously administered via drinking water for two weeks.

Isolation of cells from the intestinal lamina propria of mice and humans. Intestines of mice were removed, cleaned from remaining fat tissue and washed in ice-cold PBS (Corning). Peyer’s patches on the small intestine were identified in ice-cold PBS and centrifuged at 12,000 r.p.m. to remove aggregates, and the resulting cell suspension caliper. OV A-specific ear swelling was calculated as (right ear thickness (grade VI, Sigma Aldrich) in 200 μg OV A (Grade III, Sigma Aldrich) 20 mg per mouse every other day, and simultaneously fed ad libitum with OVA in drinking water (10 mg/ml for 12 days). Treg cell conversion of naive OT-II cells was analysed in the small intestine on day 13. CD3+ CD4+ T cells were sorted from the spleen and lymph nodes of THY1.1+ OT-II T cells and adoptively transferred by retro-orbital injection into recipient mice (Il2Rα−/− and Ncr1+/-) mice. One day later, recipient mice were gavaged with OVA (grade V, Sigma Aldrich) 20 mg per mouse every other day, and simultaneously fed ad libitum with OVA in drinking water (10 mg/ml for 12 days). Treg cell conversion of naive OT-II cells was analysed in the small intestine on day 13.
supernatant was collected. Afterwards, a sandwich ELISA was performed using mouse lipocalin-2/NGAL DuoSet ELISA (R&D Systems), according to the manufacturer’s instructions.

**Regulatory T cell suppression assay.** Dendritic cells (CD11c+ MHCII+) or naive T cells (CD3+CD4+CD25-CD45RBhigh) were sort-purified from the spleen of wild-type or THY1.1+ mice, respectively. Small-intestinal Treg cells (CD45+CD3+CD4+CD25+) were sort-purified from either Nr1I+Il2rg mice or littermate controls, and were subsequently found to be at least 95% FOXP3+ T cells. Dendritic cells were plated at 4 × 10^3 per well in the presence of 1 μg/ml soluble purified anti-CD3 antibody (clone 145-2C11, BD Biosciences). CFSE-labelled T cells (2 × 10^4 per well) were mixed with Treg cells at defined ratios and co-cultured with dendritic cells in the presence of anti-CD3. After a three-day culture, T cell proliferation was measured by CFSE dilution via flow cytometry. T reg cell suppression was calculated by gating on T effector cells, and quantifying the percentage of CFSE dilution in comparison to cells cultured in the absence of Treg cells.

**Histological staining.** Tissue samples from the intestines of mice were fixed with 4% paraformaldehyde, embedded in paraffin and 5-μm sections were stained with haematoxylin and eosin.

**RNA sequencing.** ILC3s (CD45+CD3- RORγtGFP+CD127+) and CD4+ T cells (CD45+CD3+CD4+) were sort-purified from small intestine of Rorc(gammat)-GfpTG mice. Sorted cells were used to prepare RNA sequencing libraries by the Epigenomics Core at Weill Cornell Medicine, using the Clontech SMARTer Ultra Low Input RNA Kit V4 (Clontech Laboratories). Sequencing was performed on an Illumina HiSeq 2500, yielding 50-bp single-end reads. Raw sequencing reads were demultiplexed with Illumina CASAVA (v.1.8.2). Adapters were trimmed from reads using FLEXBAR (v.2.4) and reads were aligned to the NCBI GRCm38/mm10 mouse genome using the STAR aligner (v.2.3.0) with default settings. Reads per gene were counted using Rsubread. One sample of the CD4+ T cell group was removed, as its library size was anomalously small compared to those of the other samples. Genes with at least ten counts in each sample were considered for further analysis. Differential expression was assessed using DESeq2 version 1.14.0 with default parameters and with a false discovery rate of 0.1.

**Statistical analysis.** P values of mouse datasets were determined by paired or unpaired two-tailed Student’s t-test with a 95% confidence interval. Variance was analysed using F-test. Welch’s correction was performed in case of unequal variance. Where appropriate, Mann–Whitney U-test, Wilcoxon matched-pairs test or two-way ANOVA followed by Bonferroni post-tests were performed. All statistical tests were performed with Graph Pad Prism v.6 software. P values of less than 0.05 were considered to be significant.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**

RNA sequencing data are available at Gene Expression Omnibus under accession number GSE126580. All datasets generated and/or analysed during the current study are presented in this published article, the accompanying Source Data or Supplementary Information, or are available from the corresponding author upon reasonable request.
Extended Data Fig. 1 | IL-2 blockade results in disrupted T cell homeostasis throughout the intestinal tract and associated lymphoid tissues. a–c. Sex- and age-matched C57BL/6 mice were treated with anti-IL-2 monoclonal antibodies every other day for two weeks, and examined for the size of the spleen and mesenteric lymph nodes (mLN) (a), the frequency of Treg cells (b) and Ki-67+CD4+ T cells (c) of mesenteric lymph nodes by flow cytometry (n = 10). d–g. Mice from a–c were also analysed for the frequencies of Treg cells (d) and Ki-67+CD4+ T cells (e) in large-intestinal lamina propria cells, and for the frequencies and numbers of Treg cells (f) and Ki-67+CD4+ T cells (g) in small-intestinal lamina propria cells by flow cytometry (n = 10). h–k. Mice from a–c were analysed for the frequency of TH1 cells (h) and TH17 cells (i) in large-intestinal lamina propria cells, and the frequencies and numbers of TH1 cells (j) and TH17 cells (k) in small-intestinal lamina propria cells by flow cytometry (n = 10). Data in a are representative of two independent experiments with similar results. Data in b–k are pooled from two independent experiments. Results are shown as mean ± s.e.m. All statistics are calculated by unpaired two-tailed Student's t-test. LI, large intestine; SI, small intestine.
Extended Data Fig. 2  | T-cell-derived IL-2 is essential for maintaining immunological homeostasis in the mesenteric lymph node and large intestine. 

**a.** Sex- and age-matched Il2f/f and Lckcre-I12f mice were examined for the deletion efficiency of IL-2 in CD4+ T cells in the large and small intestines. 

**b–c.** Mice in **a** were examined for the frequency of Treg cells (b) and Ki-67+CD4+ T cells (c) from the mesenteric lymph nodes by flow cytometry (n = 6). 

**d–g.** Mice in **a** were analysed for the frequencies and numbers of Treg cells (d), Ki-67+CD4+ T cells (e), T11 cells (f) and TH17 cells (g) of large-intestinal lamina propria cells by flow cytometry (n = 6). 

**h–k.** Mice in **a** were analysed for the frequencies and numbers of Treg cells (h), Ki-67+CD4+ T cells (i), T11 cells (j) and TH17 cells (k) of small-intestinal lamina propria cells by flow cytometry (n = 6). Data in **a** are representative of two independent experiments with similar results. Data in **b–k** are pooled from two independent experiments. Results are shown as mean ± s.e.m. All statistics are calculated by unpaired two-tailed Student’s t-test.
Extended Data Fig. 3 | Gating strategy to analyse subsets of innate lymphoid cells and CD4⁺ T cells in the small intestine. Gating strategy for flow cytometry analysis of innate lymphoid cells and CD4⁺ T cells in small-intestinal lamina propria cells. Lineage 1, CD11b, CD11c and B220; lineage 2, CD3ε, CD5 and CD8α. CD4⁺ T cells were identified as CD45⁺ Lineage 2⁺ CD4⁺; group-1 innate lymphoid cells were identified as CD45⁺ Lineage⁻ CD127⁺ CD90.2⁺ T-bet⁺ RORγt⁻; group-2 innate lymphoid cells were identified as CD45⁺ Lineage⁻ CD127⁺ CD90.2⁺ GATA3⁺; ILC3s were identified as CD45⁺ Lineage⁻ CD127⁺ CD90.2⁺ RORγt⁺; and subsets of ILC3s further identified as CCR6⁺ T-bet⁻ ILC3s or CCR6⁻ T-bet⁺ ILC3s.
Extended Data Fig 4 | See next page for caption.
Extended Data Fig 4 | IL-2+ cells in the large intestine of wild-type mice and in the small and large intestines of Rag1−/− mice. a, Flow cytometry plots with graph of frequency and numbers of IL-2 in ILC3s and effector and memory (E/M) CD4+ T cells (CD3ε+CD4+FOXP3−CD44hiCD62Llow) in small-intestinal lamina propria cells of wild-type mice (n = 8). b, Flow cytometry plots show IL-2+ cells in large-intestinal lamina propria cells of C57BL/6 mice. Lineage 1, CD11b, CD11c and B220; lineage 2, CD3ε, CD5 and CD8α. c, d, Flow cytometry plots with graph of frequency (c) and absolute numbers (d) of IL-2+ cells in large-intestinal lamina propria cells of C57BL/6 mice (n = 6). e, The frequency and number of IL-2+ subsets of ILC3s in small-intestinal lamina propria cells of C57BL/6 mice (n = 8). f, Flow cytometry plots show IL-2+ cells in small-intestinal lamina propria cells of Rag1−/− mice. g, h, Flow cytometry plots with graph of frequency (g) and absolute numbers (h) of IL-2+ cells in small-intestinal lamina propria cells of Rag1−/− mice (n = 5). i, j, Flow cytometry plots with graph of frequency (i) and absolute numbers (j) of IL-2+ subsets of ILC3s in small-intestinal lamina propria cells of Rag1−/− mice (n = 5). k, Flow cytometry plots show IL-2+ cells in large-intestinal lamina propria cells of Rag1−/− mice. Lineage 1, CD11b, CD11c and B220; lineage 2, CD3ε, CD5 and CD8α. Data in b and f–k are representative of two independent experiments with similar results. Data in a and c–e are pooled from two independent experiments. Results are shown as mean ± s.e.m. Statistics are calculated by paired or unpaired two-tailed Student's t-test.
Extended Data Fig. 5 | Natural killer cells and group-1 innate lymphoid cells are dispensable for maintenance of Treg cells in the small intestine. 

a, IL-2 was assessed in T-bet+ ILC3s, total ILC3s, CD4+ T cells, natural killer cells, group-1 innate lymphoid cells and dendritic cells in small-intestinal lamina propria cells of Il2f/f and Ncr1creIl2f/f mice. 

b, The number of IL-2+ cells was quantified in small-intestinal lamina propria cells of Il2f/f and Ncr1creIl2f/f mice (n = 4). 

c, d, Sex- and age- matched C57BL/6 mice were treated with anti-NK1.1 monoclonal antibody every three days for two weeks and examined for efficiency of depletion of natural killer cells (c), and for the frequency and number of Treg cells in small-intestinal lamina propria cells (d) (n = 7). Data in a–c are representative of two independent experiments with similar results. Data in d are pooled from two independent experiments. Results are shown as mean ± s.e.m. Statistics are calculated by unpaired two-tailed Student's t-test.
Extended Data Fig. 6  |  ILC3-derived IL-2 is dispensable for the maintenance of small-intestinal Th17 cells, ILC3 homeostasis and large-intestinal Treg cells.  a–d, IL2−/− and Ncr1cam-IL2−/− mice were analysed for the percentage of Treg cells (a), Th1 cells (b), Ki-67+CD4+ T cells (c) and the frequency and cell number of Th17 cells (d), in small-intestinal lamina propria cells at steady state by flow cytometry (n = 8).  e, IL2−/− and Ncr1cam-IL2−/− mice were examined for the frequency and number of Treg cells in large-intestinal lamina propria cells by flow cytometry (n = 8).  f, IL2−/− and Ncr1cam-IL2−/− mice were examined for the frequency and number of ILC3s in small-intestinal lamina propria cells by flow cytometry (n = 8).  g, IL-22 was assessed in ILC3s from small-intestinal lamina propria cells of IL2−/− or Ncr1cam-IL2−/− mice.  h, Representative histograms and bar graph examination of CD25 staining on Treg cells and IL-2+ ILC3s.  i, Representative histograms that demonstrate IL-2 binding capacity and quantification of bound IL-2 mean fluorescence intensity in Treg cells and ILC3s.  j, Experimental design of the delayed-type hypersensitivity model.  Data in f–h are representative of two independent experiments with similar results (at least three mice per group). Data in a–e and i are pooled from two independent experiments. Results are shown as mean ± s.e.m.  Statistics are calculated by paired or unpaired two-tailed Student’s t-test.
Extended Data Fig. 7 | Deletion of ILC3-intrinsic IL-2 affects the population size of peripherally induced Treg cells but not their suppressive capacity. a, b, The frequency of peripheral Treg cells (labelled ‘Nrp-1lo pTregs’) and thymic Treg cells (labelled ‘Nrp-1hi tTregs’) were characterized in small-intestinal lamina propria cells of II1f/f and Ncr1cre-IL1rf/f mice (a) or II2f/f and Ncr1cre-IL2f/f mice (b) (n = 5). c, d, The frequency of subsets of Treg cells were analysed in small-intestinal lamina propria cells of II2f/f and Ncr1cre-II2f/f mice (n = 5). e, Small-intestinal Treg cells were examined for expression of Lag3, Tgfb1, Ctila4, Ebi3 and Il10 in II2f/f and Ncr1cre-II2f/f mice (n = 7). f, g, Sort-purified small-intestinal CD45+CD3+CD4+CD25+ Treg cells were co-cultured with sort-purified CFSE-labelled splenic effector T cells (CD3+CD4+CD25−CD45RBhigh) in the presence of purified splenic dendritic cells and soluble anti-CD3 for three days. CFSE dilution was analysed and quantified (n = 6). Data in a–d and f are representative of two independent experiments with similar results. Data in e and g are pooled from two independent experiments. Results are shown as mean ± s.e.m. Statistics are calculated by unpaired two-tailed Student’s t-test.
ILC3-derived IL-2 does not exhibit functional redundancy or hierarchies with ILC3-specific GM-CSF or MHCII.

**a.** Flow cytometry plots with graph of frequency and quantification of cell numbers of IL-2+ ILC3s in small-intestinal lamina propria cells of wild-type and Csf2−/− mice (n = 8).

**b.** Flow cytometry plots with graph of frequency and quantification of cell numbers of Treg cells and IL-2+ ILC3s in small-intestinal lamina propria cells of H2-Ab1f/f littermate controls and mice lacking ILC3-specific MHCII (MHCIIΔILC3 mice) (n = 7).

**c.** Flow cytometry plots with graph of frequency and quantification of cell numbers of ILC3s (c) in small-intestinal lamina propria cells of H2-Ab1f/f and Ncr1cre-Il2f/f mice (n = 7). Data are pooled from two independent experiments. Results are shown as mean ± s.e.m. Statistics are calculated by unpaired two-tailed Student’s t-test.
Extended Data Fig. 9 | ILC3-derived IL-2 promotes essential immune regulation in the intestine. a–h, CD4+ T cells were adoptively transferred into \( \text{I}l2\text{f}/\text{fRag1}^{-/-} \) or \( \text{R}or\text{c}^{\text{cm}}\text{I}l2\text{f}/\text{fRag1}^{-/-} \) recipient mice. a–d, Recipient mice were examined for changes in weight (a), colon length (b), histological haematoxylin and eosin staining in the terminal colon (c) and lipocalin-2 presence in faecal samples (d) \((n=8)\). e, Flow cytometry plots with graph of percentage and absolute cell number of Treg cells in large-intestinal lamina propria cells in defined recipient mice. f, Absolute cell number of Treg cells in small-intestinal lamina propria cells in defined recipient mice. g, Flow cytometry plots and graph of frequency and absolute number of IFN\(\gamma\)-IL-17A+ and IFN\(\gamma\)+IL-17A+ cells in large-intestinal lamina propria cells in defined recipient mice. h, Cell number of T\(\text{H}1\) and T\(\text{H}17\) cells in small-intestinal lamina propria cells in defined recipient mice. \(n=7\) mice, \(\text{I}l2\text{f}/\text{fRag1}^{-/-}\) group; \(n=8\) mice, \(\text{R}or\text{c}^{\text{cm}}\text{I}l2\text{f}/\text{fRag1}^{-/-}\) (e–h). Data in a–h are pooled from two independent experiments. Results are shown as mean ± s.e.m. Statistics are calculated by unpaired two-tailed Student's \(t\)-test.
Extended Data Fig. 10 | A IL-1β–ILC3–IL-2 circuit is essential for the maintenance of T_{reg} cells and immunological homeostasis uniquely within the small intestine. Here we define a pathway of immune regulation in the small intestine. This pathway is continuously required, and involves MYD88- and NOD2-dependent microbial sensing by macrophages, production of IL-1β and induction of ILC3-derived IL-2 to support the maintenance of peripherally induced intestinal T_{reg} cells. Consequently, this is essential to maintain immunological homeostasis and oral tolerance, and becomes dysregulated in inflammatory bowel disease in humans.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
|-----|-----------|
| ☑ | X The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☑ | X An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☑ | X The statistical test(s) used AND whether they are one- or two-sided |
| ☑ | X Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| ☑ | X A description of all covariates tested |
| ☑ | X A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☑ | X A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☑ | X For null hypothesis testing, the test statistic (e.g. \(F\), \(t\), \(r\)) with confidence intervals, effect sizes, degrees of freedom and \(P\) value noted |
| ☑ | X Give \(P\) values as exact values whenever suitable. |
| ☑ | X For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☑ | X For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☑ | X Estimates of effect sizes (e.g. Cohen's \(d\), Pearson's \(r\)), indicating how they were calculated |
| ☑ | X Clearly defined error bars |
| ☑ | X State explicitly what error bars represent (e.g. SD, SE, CI) |

*Our web collection on [statistics for biologists](#) may be useful.*

Software and code

Policy information about availability of computer code

**Data collection**

FACS Diva software.

**Data analysis**

Flow Cytometry data analyzed by FlowJo V10, Statistical analysis all conducted using GraphPad Prism V6.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNA-seq data will be deposited and made publically available in the Gene Expression Omnibus under accession number GSE126580.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Animal sample size estimates were determined using power analysis (power=0.90% and alpha=0.05) based on the mean and standard deviation from our previous studies and/or pilot studies using at least 3 animals per group. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No samples were excluded from analysis except for one sample of CD4+ T cells group was removed for RNA-seq, as its library size was anomalously small compared to those of the other samples. This is pre-established exclusion criteria for our RNA-seq data sets. |
| Replication | Experiments were repeated with at least two to three biologically independent for all results presented in the manuscript. If the group size was small (due to limited availability of reagents or mouse strains), data from replicate experiments were pooled for graphical representation. All replicates are biological replicates obtained from biologically independent experiments. |
| Randomization | We did not use randomization to assign animals to experimental groups. As whenever possible littermate controls were used, age did not constitute a variable (and was matched for non-littermates) and sex ratios were distributed evenly among experimental groups. Furthermore, we did not observe sex-specific differences in our findings. |
| Blinding | Pathology analysis was single-blind. All other animal studies were not blinded since treatment and experimental analysis could not be separated, blinding of the investigators was not possible. |

Reporting for specific materials, systems and methods

| Materials & experimental systems | n/a | Involved in the study |
|---------------------------------|-----|-----------------------|
| - | | Unique biological materials |
| - | X | Antibodies |
| - | X | Eukaryotic cell lines |
| - | X | Palaeontology |
| - | X | Animals and other organisms |
| - | | Human research participants |

| Methods | n/a | Involved in the study |
|---------|-----|-----------------------|
| - | | ChIP-seq |
| - | | Flow cytometry |
| - | | MRI-based neuroimaging |

Antibodies

Antibodies used

The antibodies are described below. All antibodies were purchased from BD, Thermo Fisher, Biolegend or BioXcell. All antibodies were validated by manufacturers and in previous publications.

Antibodies for mouse flow cytometry:

- B220/RA3-6B2/APC-eF780/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD45R-B220-Antibody-clone-RA3-6B2-Monoclonal/47-0452-82
- CCR6/29-2L17/BV421 or PE/Biolegend/https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd196-ccr6-antibody-7137 or https://www.biolegend.com/en-us/products/pe-anti-mouse-cd196-ccr6-antibody-5220
- CD3e/145-2C11/Percp-Cy5.5/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD3e-Antibody-clone-145-2C11-Monoclonal/45-0041-82
- CD4/GK1.5 or RM4-5/PE-Cy7 or FITC/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD4-Antibody-clone-GK1-5-Monoclonal/11-0041-82
- CD4/GK1.5/BV605/Biolegend/https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd4-antibody-10708
- CD8a/53-6.7/Percp-Cy5.5/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-53-6-7-Monoclonal/45-0051-82
- CD11b/M1/70/APC-eF780/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/47-0112-82

- B220/RA3-6B2/APC-eF780/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD45R-B220-Antibody-clone-RA3-6B2-Monoclonal/47-0452-82
- CCR6/29-2L17/BV421 or PE/Biolegend/https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd196-ccr6-antibody-7137 or https://www.biolegend.com/en-us/products/pe-anti-mouse-cd196-ccr6-antibody-5220
- CD3e/145-2C11/Percp-Cy5.5/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD3e-Antibody-clone-145-2C11-Monoclonal/45-0041-82
- CD4/GK1.5 or RM4-5/PE-Cy7 or FITC/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD4-Antibody-clone-RM4-5-Monoclonal/11-0042-82 or https://www.thermofisher.com/antibody/product/CD4-Antibody-clone-GK1-5-Monoclonal/11-0041-82
- CD4/GK1.5/BV605/Biolegend/https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd4-antibody-10708
- CD8a/53-6.7/Percp-Cy5.5/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-53-6-7-Monoclonal/45-0051-82
- CD11b/M1/70/APC-eF780/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/47-0112-82
CD11c/N418/APC/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD11c-Antibody-clone-N418-Monoclonal/47-0114-82 or https://www.thermofisher.com/antibody/product/CD11c-Antibody-clone-N418-Monoclonal/17-0114-82
CD19/eBio1D3/PE-Cy7/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD19-Antibody-clone-eBio1D3-1D3-Monoclonal/25-0193-82
CD25/PC61.5/AF488/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD25-Antibody-clone-PC61.5-Monoclonal/53-0251-82
CD44/M7/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD44-Antibody-clone-M7-Monoclonal/17-0441-82
CD45/30-F11/BV785/Biolegend/https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-cd45-antibody-10636
CD45RB/C363-16A/APC/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD45RB-Antibody-clone-C363-16A-Monoclonal/17-0455-82
CD62L/MEL-14/PE-Cy7/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD62L-L-Selectin-Antibody-clone-MEL-14-Monoclonal/25-0621-82
CD64/X54-5/7.1/BV421/Biolegend/https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd64-fcgamma-rii-antibody-8992
CD90.2/30-H12/AF700/Biolegend/https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd90-2-antibody-3412
CD127/A7R34/PE-Cy7/Biolegend/https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-cd127-il-7alpha-antibody-6192
CD172a/P84/PE-Dazzle594/Biolegend/https://www.biolegend.com/en-us/products/pe-dazzle-594-anti-mouse-cd172a-sirpalpha-antibody-10804
F4/80/BM8/APC/Thermo Fisher/https://www.thermofisher.com/antibody/product/F4-80-Antibody-clone-BM8-Monoclonal/17-4801-82
F4/80/BM8/APC/Thermo Fisher/https://www.thermofisher.com/antibody/product/F4-80-Antibody-clone-BM8-Monoclonal/17-4801-82
HMC II/MS114.15.2/BV650/Biolegend/https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-mouse-ii-1a-1e-antibody-12085
NK1.1/ PK136/BUV395/BD/http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-mouse-antibodies/cell-surface-antigens/anti-mouse-antibodies/cell-surface-antigens/cell-surface-antigens/buv395-mouse-anti-mouse-nk-1.1-antibody-35/p/56414
Nrp-1/3E12/PE/Biolegend/https://www.biolegend.com/en-us/products/pe-anti-mouse-cd304-neuropilin-1-antibody-8435
TCR\[beta]/H57-597/APC-eF780/Thermo Fisher/https://www.thermofisher.com/antibody/product/TCR-beta-Antibody-clone-H57-597-Monoclonal/47-5961-82
Thy1.1/ OX-7/Percp-Cy5.5/Biolegend/https://www.biolegend.com/en-us/products/percpcyanine55-anti-rat-cd90-mouse-cd901-thy-1-antibody-4514
XCR1/ZET/FITC/Biolegend/https://www.biolegend.com/en-us/products/fcit-anti-mouse-rat-xcr1-antibody-10398
Eomes/Dan11mag/FITC/Thermo Fisher/https://www.thermofisher.com/antibody/product/EOMES-Antibody-clone-Dan11mag-Monoclonal/53-4875-82
Foxp3/FK-165/FITC or eF450/Thermo Fisher/https://www.thermofisher.com/antibody/product/FOX3P-Antibody-clone-FK-165-Monoclonal/48-5773-82 or https://www.thermofisher.com/antibody/product/FOX3P-Antibody-clone-FK-165-Monoclonal/11-5773-82
GATA3/L50-823/BUV395/BD/http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th17-cells/intracellular-markers/cell-signalling-and-transcription-factors/mouse/buv395-mouse-anti-gata3-l50-823-antibody-85438/p/565448
GM-CSF/MP1-22E9/PE/Thermo Fisher/https://www.thermofisher.com/antibody/product/GM-CSF-Antibody-clone-MP1-22E9-Monoclonal/12-7331-82
IFNy/XMG1.2/PE-Cy7/Biolegend/https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-ifn-gamma-antibody-5865
IL-2/JES6-5H4/PE/Biolegend/https://www.biolegend.com/en-us/products/pe-anti-mouse-il-2-antibody-954
IL-6/MP5-20F3/APC/Biolegend/https://www.biolegend.com/en-us/products/apc-anti-mouse-il-6-antibody-7035
IL-17A/Ebiol877/AF488/Thermo Fisher/https://www.thermofisher.com/antibody/product/IL-17A-Antibody-clone-eBio17B7-Monoclonal/11-7177-81
IL-22/IL22JOP/APC/Thermo Fisher/https://www.thermofisher.com/antibody/product/IL-22-Antibody-clone-IL22JOP-Monoclonal/17-7222-82
Ki-67/SolA15/FITC/Thermo Fisher/https://www.thermofisher.com/antibody/product/Ki-67-Antibody-clone-SolA15-Monoclonal/11-5698-82
ROtY/tBD/PE-eF610/Thermo Fisher/https://www.thermofisher.com/antibody/product/ROtY-gamma-7-Antibody-clone-2D2-Monoclonal/61-6981-82
ROtY/tG31-378/BV650/BD/http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th17-cells/intracellular-markers/cell-signalling-and-transcription-factors/mouse/bv650-mouse-anti-mouse-rorgt-q31-378-antibody-85438/p/56414
TNFalpha/eBio176/OX12/Thermo Fisher/https://www.thermofisher.com/antibody/product/TNF-alpha-Antibody-clone-eBio176-Monoclonal/50-5825-82
TNFalpha/eBio176/OX12/Thermo Fisher/https://www.thermofisher.com/antibody/product/TNF-alpha-Antibody-clone-eBio176-Monoclonal/48-7321-82
All mouse antibodies were used at 1:200, except for CCR6, CD64, CD172a, XCR1, GM-CSF, IL-2, IL-6, TNFalpha were used at 1:100.

Antibodies for mouse treatment:
IL-2: S4B6-1 (Cat: BE0043-1) and JES6-1A12 (Cat: BE0043), BioXCell
CD90.2: 30H12 (Cat: BE0066), BioXCell
NK1.1: PK136 (Cat: BE0036), BioXCell

Antibodies for human flow cytometry:
CD3e/UCHT1/APC-eF780/Thermo Fisher/https://www.thermofisher.com/antibody/product/CD3-Antibody-clone-UCHT1-Monoclonal/47-0038-42
CD4/SK3/BV395/BD [http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/surface-markers/human/bv395-mouse-anti-human-cd4-sk3-also-known-as-leu3a/p/563550]
CD11b/CR3M115/FITC/Thermo Fisher [https://www.thermofisher.com/antibody/product/CD11b-activation-epitope-Antibody-clone-CBRM1-5-Monoclonal/11-0113-42]
CD11c/3.9/FITC/Thermo Fisher [https://www.thermofisher.com/antibody/product/CD11c-Antibody-clone-3-9-Monoclonal/11-0116-42]
CD14/TuK4/FITC/Thermo Fisher [https://www.thermofisher.com/antibody/product/CD14-Antibody-clone-TuK4-Monoclonal/12-6140-42]
CD19/HIB19/FITC/Thermo Fisher [https://www.thermofisher.com/antibody/product/CD19-Antibody-clone-HIB19-Monoclonal/11-0199-42]
CD34/581/FITC/Thermo Fisher [https://www.thermofisher.com/antibody/product/CD34-Antibody-clone-581-Monoclonal/12-6140-42]
CD34-581-01
CD45/HI30/BV605/Biolegend [https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-human-cd45-antibody-8521]
CD94/DX22/FITC/Thermo Fisher [https://www.thermofisher.com/antibody/product/CD94-Antibody-clone-DX22-Monoclonal/11-0949-42]
CD117/104D2/Percp-eF710/Thermo Fisher [https://www.thermofisher.com/antibody/product/CD117-c-Kit-Antibody-clone-104D2-Monoclonal/46-1178-42]
CD123/6H6/FITC/Thermo Fisher [https://www.thermofisher.com/antibody/product/CD123-Antibody-clone-6H6-Monoclonal/12-6140-42]
CD127/A019D5/PE-Cy7/Biolegend [https://www.biolegend.com/en-us/products/pe-cy7-anti-human-cd127-il-7alpha-antibody-7216]
FcεR1/AER-37/FITC/Thermo Fisher [https://www.thermofisher.com/antibody/product/FceR1-alpha-Antibody-clone-AER-37-CRA1-Monoclonal/11-5899-42]
Foxp3/PCH101/PE-eF610/Thermo Fisher [https://www.thermofisher.com/antibody/product/FOX3-Antibody-clone-PCH101-Monoclonal/61-4776-42]
IL-2/MQ1-17H12/PE/Thermo Fisher [https://www.thermofisher.com/antibody/product/IL-2-Antibody-clone-MQ1-17H12-Monoclonal/12-7029-42]
NKp44/44.189/eF450/Thermo Fisher [https://www.thermofisher.com/antibody/product/CD336-NKp44-Antibody-clone-44-189-Monoclonal/48-3369-42]

All human antibodies were used at 1:200, except for CD34, CD94, CD117, CD127 and IL-2 were used at 1:100.

All antibodies information (including catalog number) could be easily found via the vendor websites.

Validation

All antibodies commercially available flow cytometry antibodies for staining mouse and human samples and validated by the manufacturer. Vendor websites for antibodies were listed above and the validations can be found there.

Animals and other organisms

Policy information about studies involving animals. ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Wild-type, Rag1-/-, Thy1.1, OT-I, Lckcre, Il1r1f/f, H2-Ab1f/f, Csf2-/-, Myd88-/- and Nod2-/- mice on a C57BL/6 background were purchased from the Jackson Laboratory. Rag2-/- and Rag2-/-/Il2rg-/- on a C57BL/6 background were from Taconic Farms. C57BL/6 Rorc-cre mice and Rorc(γt)-GfpTG mice were provided by G. Eberl. C57BL/6 Il2f/f mice were provided by K. A. Smith. C57BL/6 Ncr1cre were provided by E. Vivier and only heterozygous Ncr1cre mice were utilized in this study. All mice were bred and maintained in specific pathogen-free facilities at Weill Cornell Medicine and littermates were used as controls in all experiments. C57BL/6 germ free mice were maintained at the gnotobiotic facility at Weill Cornell Medicine. Sex- and age-matched animals between 8 and 14 weeks of age were used for experiments if not otherwise indicated. Gender was not found to influence results of any experiments.

Wild animals

No wild animals included.

Field-collected samples

No field-collected samples included.

Flow Cytometry

Plots

 Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Sample preparation is described in methods in the "Isolation of cells from the intestinal lamina propria of mice and humans" section.

Instrument

Fortessa II and Aria II (BD Biosciences)
| Software | Flow cytometry data were collected via Diva (BD Biosciences) and analyzed by FlowJo V10 (TreeStar). |
| --- | --- |
| Cell population abundance | The purities of sorted T cells were more than 98%, cell sorter (FACS Aria II) performance assessed before each sorting run using CS&T beads as per manufacturers instructions. |
| Gating strategy | Based on the pattern of FSC-A/SSC-A, cells in the lymphocyte gate were used for analysis of innate lymphoid cells and T cell subsets. Singlets were gated according to the pattern of FSC-H vs. FSC-A, followed by SSC-W vs. SSC-A. Dead cells were excluded by aqua staining. Positive populations were determined by the specific antibodies, which were distinct from negative populations. |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.