Tissue signals imprint ILC2 identity with anticipatory function

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Group 2 innate lymphoid cells (ILC2s) are distributed systemically and produce type 2 cytokines in response to a variety of stimuli, including the epithelial cytokines interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP). Transcriptional profiling of ILC2s from different tissues, however, grouped ILC2s according to their tissue of origin, even in the setting of combined IL-25-, IL-33-receptor-, and TSLP-receptor-deficiency. Single-cell profiling confirmed a tissue-organizing transcriptome and identified ILC2 subsets expressing distinct activating receptors, including the major subset of skin ILC2s, which were activated preferentially by IL-18. Tissue ILC2 subsets were unaltered in number and expression in germ-free mice, suggesting that endogenous, tissue-derived signals drive the maturation of ILC2 subsets by controlling expression of distinct patterns of activating receptors, thus anticipating tissue-specific perturbations occurring later in life.

ILC2s are defined by their ability to produce type 2 cytokines, in particular IL-5 and IL-13, by integrating inputs from multiple ligands, including cytokines, neuropeptides, and eicosanoids. The epithelial cytokines IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) are powerful ILC2 activating ligands, and mice deficient in these signaling pathways display substantial compromise in the capacity of mature lung ILC2s to generate type 2 cytokines in response to helminths and allergens. Recent analyses of murine and human ILCs have indicated transcriptomic heterogeneity of ILC2s in tissues; however, direct comparisons of ILC2s among normal resting tissues are limited and complicated by sorting strategies that rely on surface markers that are variably expressed by ILCs. Furthermore, functional characterizations of the signals that establish the homeostatic activation profiles of ILC2s across and within disparate tissues, particularly in skin, are lacking.

In this study, we combine analysis of mouse cytokine reporter alleles with RNA-sequencing approaches to comprehensively assess the constitutively function-marked populations of ILC2s present in resting bone marrow and peripheral tissues. We further analyze germ-free mice and mice triple-deficient in IL-33, IL-25, and TSLP signaling to uncover key determinants of homeostatic effector cytokine function, which remains intact even in the absence of commensal microbiota, as assessed by the ILC2-defining cytokines IL-5 and IL-13. Notably, although IL-5+ ILC2s required IL-33, IL-25, and TSLP signaling for optimal homeostatic type 2 cytokine competency in a tissue-dependent manner, the transcriptional signatures imprinted by each tissue in which ILC2s reside dictated their identity. Receptiveness to independent activating signals was segregated by tissue, as highlighted by skin ILC2s, which, like smaller ILC2 subsets in lung and bone marrow, expressed the IL-18 receptor and produced type 2 cytokines in response to IL-18. In the absence of IL-18, this ILC2 subset was functionally impaired both in the steady-state and after inflammatory skin challenge. The expression of activating receptors by a tissue-specific program, even in the absence of ligand-receptor signals, reveals an anticipatory logic underpinning the roles for type 2 immune responses among different organs and tissues.

Results

Basal activation defines tissue-resident ILC2s. The epithelial cytokines IL-33, IL-25, and TSLP are important activating signals for ILC2s in homeostasis and in response to tissue injury. To assess the impact of combined TSLP, IL-33, and IL-25 deficiency on ILC2s under steady-state conditions, we analyzed ILC2s from multiple tissues in 8- to 12-week-old YRS mice. These mice express reporter alleles for arginase-1 (Yarg), IL-5 (Red5, also called R5), and IL-13 (Smart13, also called S13 or hucD4), which are highly expressed among resting (YR13) and activated (S15) ILC2s; cells from YRS mice triple-deficient in TSLP receptor, IL-33 receptor, and IL-25 (TKO-YRS; Fig. 1a) were collected and analyzed similarly (below). Among lineage-negative (Lin−) cells from YRS mice, we assessed R5+ ILC2s in the lung, gut (small intestine lamina propria), fat (perigonadal adipose tissue), and skin; the latter had diminished Yarg expression and variable expression of other common ILC2 surface markers. ILC2s from bone marrow expressed Yarg but were R5− (Fig. 1b and Supplementary Fig. 1). These findings are consistent with previous characterizations of these reporter alleles. Although bone marrow ILC2s did not spontaneously express the R5 or Smart13 reporter alleles as in other tissues, stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin resulted in robust IL-5 and IL-13 release and expression of cytokine reporter alleles in >90% of these cells, thereby confirming the identity of...
the Yarg\textsuperscript{+} bone marrow ILC2 population by their ability to produce hallmark ILC2 cytokines (Fig. 1c–e).

The widespread distribution of R5\textsuperscript{+} ILC2s in resting tissues suggested that ILC2s were activated by stimuli in the absence of apparent exogenous challenge. To decipher these signals, we performed RNA sequencing (RNA-seq) to compare the transcriptomes of purified R5\textsuperscript{+} ILC2s isolated from lung, gut, fat, and skin against Yarg (Arg1) triple-deficient TKO-YRS mice.

Representative flow cytometry (c) and percentage (d) of sorted Yarg\textsuperscript{+} bone marrow (BM) ILC2 expressing R5 and SmartI3 (S13) reporter alleles before and after stimulation with ionomycin (Ion) and PMA. e, IL-5 and IL-13 in supernatants of Yarg\textsuperscript{+} BM ILC2s. Data in b and c are representative of 3 independent experiments and in d–f represent biological replicates (n = 3, BM; n = 5, lung; n = 6, fat, gut, skin).
of steady-state tissue ILC2s resembled the shared transcriptional programs of tissue-effector ILC2s and CD4+ helper T cells (T(H)2) cells elicited by type 2 immune stimulation15,17.

Basal ILC2 activation is independent of microbiota. Basal stimulation by commensal microbes has been implicated in epigenetic and transcriptional alterations among intestinal ILC subsets26,27. With this in mind, we examined the tissue ILC2 activation program in germ-free mice as compared with mice housed in specific-pathogen-free (SPF) barrier conditions. Similar numbers of ILC2s (defined in lungs, skin, fat, and bone marrow as Lin−CD45+Thy1.2−CD25+, or in gut as Lin−CD45+KLRG1+IL-17Rb−, in the absence of the fluorescent reporters) were recovered from the lungs, skin, gut, and bone marrow of germ-free and SPF mice (Fig. 2a–c and Supplementary Fig. 3a,b). Additionally, steady-state eosinophil numbers, which depend on ILC2-derived IL-57, were not significantly different in germ-free animals as compared to SPF controls (P = 0.0661; Fig. 2d), indicating that although the presence or absence of particular commensal microbes can alter intestinal ILC subset composition26, these changes do not significantly impact cytokine-influenced functional outcomes of the tissue ILC2 activation program. In confirmation of these findings, we detected similar expression of IL5 transcripts in comparing germ-free and SPF ILC2s isolated from bone marrow, fat, lung, gut, and skin (Fig. 2e). Transcriptional abundance for Gata3 and Arg1, and for receptor components for tissue-derived cytokines, such as Il1rl1 (encoding the IL-33 receptor subunit T1/ST2), Il17rb (encoding the IL-25 receptor subunit IL17RR), and Tnafip3 (encoding the ubiquitin-modifying enzyme A20 induced by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling), were also comparable (Supplementary Fig. 3c–g). Thus, tissue programs driving peripheral ILC2 activation are independent of commensal microbes and are likely driven by tissue-intrinsic signals.

Distinct tissue signals drive resident ILC2 activation. Tissue-derived signals, such as the cytokines IL-25, IL-33, and TSLP, control type 2 cytokine production from ILC2s in peripheral tissues in response to infectious or homeostatic perturbations28,29,30,31, and they synergize with other ILC2 activation signals such as leukotrienes32 and neuropeptides33. We assessed whether these three signals contributed to the resting activation profile of ILC2s by examining multiple tissues from triple-reporter YRS and TKO-YRS mice, which lack responsiveness to TSLP, IL-33, and IL-25 cytokine signals. Notably, TKO-YRS mice harbored normal numbers of total ILC2s in lung, fat, and gut (lung, P = 0.1024; fat, P = 0.6638; gut, P = 0.0636; Fig. 3a–c), although the numbers of activated IL-5-expressing R5+ ILC2s were significantly reduced in these peripheral tissues (lung P = 1.6244 × 10−2; fat, P = 0.0009; gut, P = 6.0366 × 10−14; Fig. 3d–f and Supplementary Fig. 4), extending prior observations of lung ILC2s’. Peripheral eosinophil numbers were also diminished in TKO-YRS mice, consistent with the role for ILC2s in sustaining systemic IL-5 (Fig. 3g). The magnitude of the reduction in R5+ ILC2s, however, was not consistent among peripheral tissues; notably, the skin contained comparable numbers of R5+ ILC2s in wild-type YRS and in TKO-YRS mice (Fig. 3h), indicating that signals other than IL-25, IL-33, and TSLP sustain steady-state IL-5 production from skin ILC2s.

To explore this further, we compared the transcriptional profiles of ILC2s from YRS and TKO-YRS mice isolated from multiple tissues. In agreement with the reporter gene expression and in agreement with prior studies28,29,30, type 2 cytokine transcripts were reduced in TKO-YRS ILC2s isolated from lung, fat, and gut (Supplementary Fig. 5d,e). Clustering based on the top 1,000 differentially expressed genes, however, revealed that the major transcriptional signatures
from wild-type and TKO ILC2s were tissue-specific and independent of the presence or absence of these epithelial cytokines (Fig. 4a and Supplementary Fig. 5a–e). Among wild-type R5+ or Yarg+ ILC2s, tens to hundreds of transcripts were differentially expressed by ILC2s collected from single tissues as compared to all other tissues, with gut and skin ILC2s showing the greatest numbers of divergent transcripts from the other groups. Notably, we observed enhanced Il17rb expression in gut ILC2s but substantially increased...
**Il18r1** expression in skin ILC2s (Fig. 4b, Supplementary Fig. 5g, and Supplementary Table 1). Collectively, these findings suggest that distinct tissue-derived factors contribute to ILC2 subset activation in the steady state.

**Single-cell RNA-sequencing distinguishes ILC2 subsets.** To explore further the nature of ILC2 tissue signatures and to discern whether these differences identified by bulk RNA-seq resulted from contamination by nonhematopoietic tissue cells, we performed single-cell RNA-sequencing (scRNA-seq) on 35,396 sorted ILC2s from multiple tissues showed segregation by tissue, which could be further subclustered within each tissue, revealing novel intratissue ILC2 subsets (Fig. 5a and Supplementary Fig. 6a–c). We observed minimal (<2%; Supplementary Fig. 6b) contamination by non-ILC2 cells, and, in agreement with the bulk sequencing results, common transcripts such as *Gata3, Il7r*, and *Crlf2* (encoding the TSLP receptor subunit TSLPR) were expressed by the majority of ILC2s across tissues, whereas other transcripts were enriched in particular tissues (Fig. 5b–f and Supplementary Figs. 6c and 7), consistent with tissue-specific functional roles. *Il1r1*, encoding an IL-33 receptor subunit, was enriched among fat and lung ILC2s, corresponding to a loss of IL-5 reporter R5 expression in ILC2s from these tissues in mice lacking this receptor (Fig. 5d and Supplementary Fig. 4). The gene encoding IL-25R, *Il17rb*, was abundant among gut ILC2s and matched the loss of R5 expression predominantly seen among small-intestinal ILC2s in mice lacking IL-25 (Fig. 5e and Supplementary Fig. 4). These findings are consistent with prior studies linking IL-33 to steady-state ILC2 function in fat and lung and IL-25 to small-intestinal ILC2 function. In contrast, skin ILC2s showed comparatively low expression of both IL-25 and IL-33 receptors, as assessed both by flow cytometry and transcript analysis, and consistent with unaltered IL-5 expression among TSK skin ILC2s (Fig. 5d,e,g, Supplementary Figs. 4 and 6c, and Supplementary Table 1). Of note, skin ILC2s expressed IL-18R1 (CD218) cell surface protein and *Il18r1* transcripts, even in germ-free mice (Supplementary Fig. 3h). In agreement with the bulk RNA-seq results, IL-18 receptor subunit expression was also enriched in subsets of lung and bone marrow ILC2s identified by scRNA-seq and flow cytometry (Fig. 5h and Supplementary Fig. 6b,c). These findings raise the possibility that IL-18 could influence ILC2 function in a tissue- and subset-specific manner.

**IL-18 independently activates ILC18 receptor-bearing ILC2s.** ILC-1 cytokine family members, including IL-18 and IL-33, can induce cytokine expression from lymphoid cells, particularly in the presence of STAT5 activators such as IL-7 and TSLP. To test the function of IL-18 on these IL-18R-bearing peripheral ILC2 subsets, we sorted ILC2s from skin and lung and cultured them with IL-18 and TSLP in vitro. Compared to TSLP alone, ILC2s produced significantly higher amounts of IL-13 in the presence of IL-18 (skin, P = 0.0066; lung P = 0.0021; Fig. 6a), in agreement with a recent characterization of IL-18-responsive ILCs isolated from human tissues. Intradermal injection of IL-18 and TSLP into S13 reporter mice increased IL-13 reporter expression among both skin and
Blunted type 2 skin inflammation in the absence of IL-18. ILC2s have been shown to be increased in lesional skin of patients with atopic dermatitis and in mouse models of atopic dermatitis-like inflammation\(^{18}\). Our findings that IL-18 activated skin ILC2s and that loss of IL-18 led to reduced activation of skin ILC2s in homeostasis (Fig. 6a–d) led us to further examine whether IL-18 contributed to ILC2 function in the context of an in vivo mouse model of skin inflammation. To test this, we used topical application of MC903, a well-characterized mouse model of atopic-like inflammation that has been shown to activate ILC2s and cause eosinophilia, skin swelling, and type 2 inflammation\(^{26}\). Compared to wild-type mice, IL-18-deficient mice exhibited decreased skin tissue accumulation of total ILC2s and of IL-5- and IL-13-producing ILC2s (Fig. 7a–c), which are critical determinants of eosinophil recruitment into tissues\(^{26}\). Indeed, mice deficient in IL-18 also showed a corresponding decrease in the numbers of eosinophils recruited to ear skin tissue in response to MC903 (Fig. 7d). These data suggest that IL-18 not only influences basal ILC2 subset activation in the steady-state but also contributes to its function in models of atopic dermatitis.

**Discussion**

ILC2s are largely tissue-resident cells with a canonical effector function centered on the production of type 2 cytokines, particularly in response to key activating cytokines like IL-33, IL-25, and TSLP. Here we show that expression of these cytokine receptors impacts cytokine discharge from ILC2s, but with varying effects in different tissues, reflecting diversity in receptor expression on ILC2 subsets in different organs. Indeed, some ILC2 subsets, including most skin ILC2s, express low levels of receptors for epithelial cytokines IL-33, IL-25, and TSLP; instead they are activated dominantly by IL-18, which is highly expressed in skin ILC2s and in subpopulations of ILC2s in lung and bone marrow. Strikingly, transcriptomic profiling of ILC2s revealed tissue-specific subsets of cells whose expression patterns remained largely unaffected by the absence of signaling through the three canonical epithelial cytokines. These tissue-specific expression patterns, including the capacity to respond to epithelial cytokines or IL-18, were normal in germ-free and cytokine-deficient mice, uncovering a tissue-derived signature responsible for preemptively establishing responsiveness of ILC2 subsets in different tissues in an anticipatory fashion, since normal receptor expression and tissue distribution occurred even in the absence of ligand-mediated stimulation.

These tissue signatures reveal new potential therapeutic targets among particular cytokine receptor-bearing subsets of ILC2s. Notably, in IL-18-deficient mice, the IL-18R-bearing ILC2s in the skin exhibited a substantial defect in the homeostatic activation of ILC2s in vivo. Moreover, ILC2 proliferation and activation, as well as eosinophil recruitment, were blunted in IL-18-deficient animals after skin challenge with MC903, a well-characterized mouse model of atopic-like dermatitis. These results are particularly noteworthy, as transgenic mice that overexpress IL-18 in keratinocytes develop severe dermatitis\(^{27}\), and elevated IL-18 correlates with disease severity in atopic dermatitis\(^{24,25}\), raising the possibility that targeting IL-18 may represent a means to ameliorate type 2 immune activation in the skin. In addition, given that receptor components for both IL-33 and IL-18 were expressed on some ILC2 subsets, activation by these two signals either alone or in combination may influence allergic diseases like asthma and atopic dermatitis. Indeed, the genes for IL-18R and IL-33R receptors are located together in the genome at an interval linked by genome-wide association studies with risk for allergic diseases in large cohorts\(^{30,31}\).

Further study using appropriate fate-mapping tools will be required to determine whether these tissue-specific expression profiles are acquired during fetal or perinatal development when ILC2s become established in tissues\(^{12,15,26}\), and how these programs

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**Fig. 7 | Blunted type 2 skin inflammation in the absence of IL-18.**

**a**–**d**, Total skin ILC2s (Lin−CD45+Thy1.2+CD25−) (a), R5+ ILC2s (Lin−CD45+Thy1.2+Red5+) (b), percentage of R5+ ILC2s expressing S13 reporter allele (c), and total eosinophils (d) in ears of wild type or IL-18 KO mice treated with ethanol (EtOH) or MC903. Data pooled from 2 or 3 individual experiments with n ≥ 6 for each experimental group and are represented as mean ± s.e.m.; *P < 0.05; **P < 0.005, ***P < 0.0005, ****P < 0.0001.
are engaged during differentiation from bone marrow precursors in adults, or after tissue insults like helminths and allergens. Strikingly, ILC2 effector function is expressed postbirth and may create an additional layer of control along with tissue-resident macrophages during this critical period of growth and development, in which cell identity becomes established in temporal proximity to tissue differentiation, and before the maturation of tissue-resident adaptive immunity. Notably, our findings suggest distinct stages in the development of mature tissue ILC2s. ILC2 precursors are first seeded into tissues, where they acquire a tissue-specific transcriptome characterized by expression of distinct subsets of activating receptors and an activated, poised phenotype. The subsequent generation of the canonical type 2 cytokines is then regulated by the generation of distinct sets of ligands that converge on receptors expressed by ILC2s specific to that tissue. How and why these diverse tissue pathways converge on the canonical type 2 cytokines and related effectors in ILC2s distributed across multiple tissues continues to be an important issue that may provide understanding of processes firmly at the nexus of homeostasis and allergic immunity.

Methods

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41590-018-0201-4.

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Author contributions

R.R.-G. and S.J.V.D. designed and performed experiments, analyzed and interpreted the data, and wrote the manuscript. C.S., J.L., J.C.N., H.-E.L., and A.B.M. contributed to experiments. R.R.-G. and S.J.V.D. wrote the supplementary data analysis and expertise. R.M.L. directed the studies and wrote the manuscript with R.R.-G. and S.J.V.D.

Competing interests

The authors declare no competing interests.

Additional information

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Flow cytometry and cell sorting. Single-cell suspensions from bone marrow (BM), lung, adipose tissue, and spleen were prepared as described previously. For skin single-cell suspensions, pooled ear/face skin tissue was minced in RPMI-1640 with 10% FBS, then transferred to a 50-ml conical tube. Collagenase XI (2 mg/mL; Sigma C9407), hyaluronidase (0.5 mg/mL; Sigma H3506), and DNAse I (0.1 mg/mL; Sigma 10104159001) were added. Samples were shaken at 200–250 rpm for 90 min at 37 °C, then dispersed using an automated tissue dispersion device (CellWize, Cellifera, and Biomek 3000). Single-cell suspensions in RPMI-1640 were filtered through a 70-μm cell strainer and washed twice with RPMI containing 10% FBS. The following antibodies, all from BioLegend (unless otherwise specified) were used at 1:100 dilution unless noted: anti-CD3 (17A2), anti-CD4 (RM4–5), anti-CD5 (3D5–7), anti-CD8α (53-6.7), anti-CD11b (M1/70), anti-CD11c (N418), anti-CD19 (ID5), anti-CD25 (PC61), anti-CD44 (IM7), anti-CD45 (30F-11, BD Biosciences), anti-CD49b (DX5; eBiosciences), anti-CD103 (E40.14; BD Biosciences), anti-KLRG1 (2F1; eBiosciences), anti-TSLPR (polyclonal; R&D Systems, diluted 1:20), anti-SiglecF (E50–244; BD Biosciences), α-Arg1 (53-6.7), anti-CD11b (M1/70), α-MHC (mAb C28.5.1; BD Biosciences), anti-CD3 (17A2), anti-CD4 (RM4–5), anti-CD5 (3D5–7), anti-CD8α (53-6.7), anti-CD11b (M1/70), anti-CD11c (N418), anti-CD19 (ID5), anti-CD25 (PC61), anti-CD44 (IM7), anti-CD45 (30F-11, BD Biosciences), anti-CD49b (DX5; eBiosciences), anti-CD103 (E40.14; BD Biosciences), anti-KLRG1 (2F1; eBiosciences), anti-TSLPR (polyclonal; R&D Systems, diluted 1:20), anti-SiglecF (E50–244; BD Biosciences), and α-Arg1 (53-6.7). Cell death exclusion was performed with DAPI (4',6-diamidine-2'-phenylindole dihydrochloride; Roche). Cell counts were performed using flow cytometry counting beads (CountBright Absolute; Life Technologies) per the manufacturer’s instructions.

RNA preparation and qRT-PCR. ILC2s from various tissues (1,000–10,000 cells per sample) were sorted into RLT Plus lysis buffer (Qiagen) and stored at −80 °C, then processed using RNeasy Micro Plus kit (Qiagen) per the manufacturer’s protocol. For qPCR analyses, RNA was reverse transcribed using a SuperScript VILO cDNA synthesis kit (ThermoFisher) and amplified using Power SYBR Green PCR master mix (ThermoFisher) with primers from PrimerBank as listed in Supplementary Table 1.

RNA sequencing. Total RNA was prepared as described above, and quality was assessed by Agilent 2100 Bioanalyser (Agilent Technologies). RNA sequencing libraries were generated using the Illumina TruSeq stranded RNA kit, according to the manufacturer’s protocol (Illumina). Library concentrations were measured using KAPA Library Quantification Kits (Kapa Biosystems), and equal amounts of indexed libraries were pooled and sequenced on the HiSeq 4000 (Illumina). Sequencing yielded ~6.5 billion reads with a mean read depth of 97.7 million reads/sample. Reads were then aligned to the Mus musculus genome, and those that matched uniquely to known mRNAs were used to assess differential expression. Differential gene expression testing was carried out using DESeq2. DESeq2 was used to carry out pairwise comparisons between tissues with the same genotype and between genotypes in the same tissue. Additionally, DESeq2 was run using a multifactorial model to identify the general effect of each tissue on gene expression (bone marrow was modeled as the intercept), the general effect of the knockout (wild-type as the intercept), and an interaction term between the genotype and the tissue.

For single-cell RNA sequencing (scRNA-seq), ILC2s were sorted as described above and loaded with RNA-seq data with DESeq2.

Data and code availability statement. The data and software code that support the findings of this study are available from the corresponding author upon reasonable request.

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- A list of figures that have associated raw data
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All RNA-seq and scRNA-seq data generated in this study are deposited in Gene Expression Omnibus (GEO) under accession code GSE117568. The data that support the findings of this study are available from the corresponding author upon reasonable request.
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**

No statistical methods were used to predetermine sample size. At minimum of 3 individual mice were used for all the experiments and assumed this would be required to recognize differences between genotypes or conditions. Additional details for each figure panel is included in the figure legends.

**Data exclusions**

No data was excluded.

**Replication**

Experiments were replicated at least twice as described throughout the paper and in the Methods.

**Randomization**

Mice of similar ages and sex were used for all the experiments reported. Samples were randomly assigned.

**Blinding**

The investigators were not blinded to the identities of the samples because treatments and data collection were performed by the same people. All samples were collected and analyzed at the same time under the same conditions.

Reporting for specific materials, systems and methods

**Materials & experimental systems**

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

**Methods**

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

**Unique biological materials**

Policy information about availability of materials

**Obtaining unique materials**

Red5 (IL-5-tdTomato) reporter mice are available upon reasonable request. Smart 13 (IL-13-huCD4) reporter mice (Stock No. 018869) and Yarg (Arg1-YFP) reporter mice (Stock No. 015857) are available at Jackson Laboratories.

**Antibodies**

The following antibodies, all from BioLegend and various Lot #s (unless otherwise specified) were used at 1:100 dilution unless noted: anti-CD3 (Clone 17A2, PE/Cy7 Cat No. 100220), anti-CD4 (Clone RM4-5, BV605 Cat No. 100548, BV711 Cat No. 100548), anti-CD5 (Clone 53-7.3, BV421 Cat No. 100629), anti-CD8a (Clone 53-6.7, BV421 Cat No. 100753, BV785 Cat No.100750), anti-CD11b (Clone M1/70, BV650 Cat No. 101259, AF647 Cat No. 101218), anti-CD11c (Clone N418, BV785 Cat No. 117336, PE/Cy7 Cat No. 117318), anti-CD19 (Clone 6D5, BV605 Cat No. 115538), anti-CD25 (Clone PC61, APC/Fire750 Cat No. 102054), anti-CD44 (Clone IM7, BV650 Cat No. 103057, BV785 Cat No. 103059), anti-CD45 (Clone 30F-11, BD Biosciences, BV711 Cat No. 564357, UV395 Cat No 564279), anti-CD49b (Clone DX5, eBiosciences, eF450 Cat No. 48-5971), anti-CD103 (Clone 2E7, PerCP/Cy5.5 Cat No. 121416), anti-CD127 (Clone A7R34, BV605 Cat No. 135025), anti-CD218 (Clone P3TUNYA, diluted 1:200, eBiosciences, PerCP-eF710 Cat No. 46-5183-82), anti-F4/80 (Clone BM8, PB Cat No. 123124), anti-Grl-1 (Clone R86-86S, PB Cat No. 108430), anti-I-17R8 (Clone 9B10, diluted 1:20, APC Cat No. 146308), anti-NK1.1 (Clone PK136, PB Cat No. 108722), anti-NKp46 (Clone 29A1.4, PB Cat No. 331912), anti-Thy1.2 (Clone 53-2.1, diluted 1:500, BV605 Cat No. 140318), anti-human CD4 (Clone RPA-T4, diluted 1:20, eBiosciences, APC, Cat No. 17-0049), anti-KLRG1 (Clone 2F1, eBiosciences, PerCP/eF710 Cat No. 46-5893-82), anti-TSLPR (polyclonal, R&D Systems, diluted 1:20, APC Cat No. FABS461A), anti-SiglecF (Clone E50-244, BD Biosciences, AF647 Cat No 562680), anti-T1/ST2 (Clone DJ8, MD Biosciences, FITC Cat No. 101001).
Validation

All the antibodies used have undergone extensive validation by the manufacturer.

Animals and other organisms

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

Laboratory animals

Il5Red5, Arg1Yarg, and Il13Smart reporter alleles on wild-type and triple-deficient Crlf2−/−Il25−/−Il1rl1−/− CS7BL/6j backgrounds were bred and maintained as described previously. Il18−/− mice were obtained from The Jackson Laboratory (Stock 004130). Germ-free animals were provided by the UCSF Gnotobiotic Mouse Facility. For all experiments, age-(7-14 weeks) and sex-matched animals were used. All animal procedures were approved by the University of California San Francisco Institutional Animal Care and Use Committee.

Wild animals

This study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

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

See Methods, "Flow Cytometry and Cell Sorting"

Instrument

Becton Dickinson 5-laser LSRFortessa X-20 flow cytometer for analysis and MoFlo XDP (Beckman Coulter) for cell sorting.

Software

BD FACSDiva software (BD Biosciences) for data acquisition and analyzed using FlowJo software version X (Tree Star). Data was graphed using Prism 7 (Graphpad).

Cell population abundance

The purities of sorted cell populations were consistently > 98%.

Gating strategy

The gating strategy is shown in Extended Data Figure 1.

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