

IL-9 and Blimp-1 protect the transcriptional identity of group 2 innate lymphocytes in allergic asthma

Received: 2 April 2025

Accepted: 24 March 2026

Published online: 24 April 2026

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Allergic asthma is driven by type 2 immune responses, including type 2 innate lymphoid cells (ILC2s). Although ILC2s are activated by the tissue alarmins interleukin (IL)-33 and IL-25, these signals do not intrinsically enforce type 2 identity and the mechanisms that maintain type 2 cytokine expression remain unclear. Here we show that allergen-induced IL-33 and IL-25 rapidly induce IL-9, which in turn upregulates the transcriptional repressor Blimp-1 in ILC2s. Blimp-1 sustains type 2 immunity by directly repressing type 1 inflammatory programs, including expression of interferon- γ and tumor necrosis factor. Deletion of Blimp-1 in ILC2s increased type 1 cytokine production and reduced IL-5 and IL-13 expression, eosinophil recruitment and mucus production in the lung. In contrast, IL-9 expression was enhanced in the absence of Blimp-1, leading to increased mast cell recruitment. Together, these findings identify Blimp-1 as a key regulator of ILC2 transcriptional fidelity that stabilizes type 2 inflammation while constraining divergent inflammatory programs during allergic responses.

Allergic asthma is a chronic inflammatory lung disease characterized by airway obstruction, mucus production and bronchial hyperreactivity¹. Type 2 cytokines including interleukin (IL)-5, IL-9 and IL-13 drive inflammation and are produced by both T helper 2 (T_H2) cells and type 2 innate lymphoid cells (ILC2s). ILC2s respond to local tissue damage in the lung and amplify T_H2 cell responses through type 2 cytokine production^{2,3}. ILC subsets (ILC1, ILC2 and ILC3) parallel CD4 T cell lineages (T helper 1 (T_H1), T_H2 and T helper 17 (T_H17) cells), where lineage-defining transcription factors limit alternative cell states⁴. Inflammatory states associated

with type 1 responses such as influenza can shift ILC2s toward an ILC1 state, suggesting that ILCs are capable of plasticity, defined as the potential to express cytokines of an alternative cell state given the appropriate signals^{5–7}. How ILCs maintain their transcriptional state after activation by tissue alarmins is not well understood.

B lymphocyte-induced maturation protein-1 (Blimp-1) is a transcriptional repressor with wide-ranging roles in immune cells. Initially identified as the master regulator of plasma cells⁸, Blimp-1 also plays important roles in shaping CD8 T cell effector, exhaustion and memory

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responses, as well as CD4 T cells and natural killer (NK) cells, where it helps regulate effector differentiation and function^{9,10}. We recently reported an unexpected role for Blimp-1 in the context of inhaled allergens where Blimp-1 was required for the development of T_H2 cells that migrate to the lung and cause allergic asthma^{11,12}. Given the shared features between T_H2 cells and ILC2s in the context of allergic asthma, we hypothesized that Blimp-1 may also play a role within the ILC2 compartment in response to allergens. Although Blimp-1 expression in a subset of IL-10-producing ILC2s has been reported¹³, the specific role of Blimp-1 in ILC2s has not been thoroughly investigated.

Here we show that Blimp-1 maintains the type 2 inflammatory identity of ILC2s during tissue inflammation. Tissue damage signals IL-33 and IL-25 promote expression of IL-9, which is necessary and sufficient to induce Blimp-1. ILC2 cells deficient in Blimp-1 exhibited an increase in tumor necrosis factor (TNF), interferon- γ (IFN γ) and other type 1 inflammatory cytokines and chemokines, leading to a decrease in type 2 inflammation and pathophysiological features of allergic asthma. Our study suggests that, in response to tissue inflammation, ILC2s require transcriptional repression of alternative fates to limit plasticity. Finally, this study adds ILC2s to the cell types in which the transcriptional repressor Blimp-1 plays important roles in effector differentiation.

Results

Blimp-1 is induced in activated ILC2s by alarmins or allergens

Given the importance of ILC2s in allergic lung inflammation, we considered that Blimp-1 may play a role in ILC2s. Using Blimp-1 YFP reporter mice, we measured the expression of Blimp-1 in ILC2s after chronic house dust mite (HDM) exposure. Blimp-1 was significantly induced in ILC2s and increased with additional doses of HDM, especially on rechallenge (Extended Data Fig. 1a–f). As HDM responses are largely CD4 T cell dependent, we examined papain, a cysteine protease that robustly activates ILC2s^{14,15}. Three days of intranasal (i.n.) papain induced lung inflammation, eosinophil recruitment and ILC2 proliferation (Extended Data Fig. 1g–j). Blimp-1 was robustly expressed after papain as measured by YFP in ST2⁺ KLRG1⁺ GATA3⁺ ILC2 cells (Fig. 1a,b and Supplementary Fig. 1). Proliferation of ILC2s was similar in both Blimp-1-expressing and Blimp-1-nonexpressing populations (Extended Data Fig. 1k). ILC2s activated by papain rapidly produce IL-5 and IL-13. Blimp-1 was not specific to cytokine-expressing cells; however, Blimp-1⁺ cells produced slightly more IL-5 and IL-13 than Blimp-1[−] cells (Fig. 1c–e). Similar patterns were observed after HDM, indicating that Blimp-1 is part of the ILC2 effector program (Extended Data Fig. 1l)¹⁶. We wondered whether repeated stimulation via HDM drove more Blimp-1 even though ILC2s are rapidly activated to secrete cytokines. We found that papain induced more Blimp-1 than HDM after an equal number of exposures (Extended Data Fig. 1m), suggesting that chronic exposure to HDM promotes more Blimp-1 and that differences between allergens impact the overall expression of Blimp-1.

We next tested other settings of ILC2 activation in vivo. IL-33 and IL-25 are alarmins produced in response to helminth infection and allergen inhalation that potentially activate ILC2s¹⁷. IL-33 and IL-25 i.n.

robustly induced Blimp-1 in ILC2s similar to papain and HDM (Fig. 1f–g). To test whether Blimp-1 is expressed in ILC2s responding to helminth infection, we used *Nippostrongylus brasiliensis* as a model that includes infection of both the lung and the gastrointestinal tract¹⁸. Similar to the above models, Blimp-1 was expressed in lung ILC2s 5 d after helminth infection (Fig. 1h,i), suggesting that Blimp-1 is universally expressed in effector ILC2s. We next assessed expression of Blimp-1 in inflammatory ILC2s (iILC2s; CD90.2^{hi}KLRG1^{hi}) versus natural ILC2 cells (nILC2s; CD90.2^{hi}KLRG1^{lo}). The iILC2s are recruited to the lung from the gastrointestinal tract in response to inflammatory signals and are distinct from nILC2 cells that reside in the lung^{19,20}. The iILC2s were readily found in the lung after IL-25 administration intraperitoneally (i.p.) (Fig. 1j). Blimp-1 was upregulated in both iILC2s and nILC2s; however, iILC2s expressed slightly more Blimp-1 than nILC2s (Fig. 1k,l). ILC2s are also recruited from the bone marrow (BM) and can be blocked with FTY720, an agonist of S1P1²¹. Blocking ILC2 recruitment with FTY720 after IL-33 treatment reduced the total number of ILC2s in the lung, whereas precursors (common lymphoid progenitors, CLP) were increased in the BM (Extended Data Fig. 2a–c). Blocking recruitment of ILC2s did not affect the percentage of Blimp-1⁺ cells in the lung, despite their reduced number (Extended Data Fig. 2d–f). These data suggested that Blimp-1 is not specific to a subset of ILC2s, but rather expressed as ILC2s differentiate into effector cells, whether they are activated locally in the tissue or recruited from another site. Finally, we assessed Blimp-1 in ILC2s at other tissue sites. Alarmins induced Blimp-1 in intestinal and skin-associated ILC2 populations (Fig. 1m and Extended Data Fig. 2i), suggesting that upregulation is a global feature across barrier and mucosal tissue sites in response to inflammatory tissue alarmins²².

IL-33 and IL-25 indirectly induce Blimp-1 in activated ILC2s

We next asked what factors support the upregulation of Blimp-1 in ILC2s. As IL-33 and IL-25 induced Blimp-1 in ILC2s in vivo, we reasoned that stimulation of ILC2s in vitro with these alarmins may also upregulate Blimp-1. Sorted lung ILC2s from naive Blimp-1 YFP mice were stimulated for 3 d with IL-33 or IL-25, with IL-2 to support cell survival (Supplementary Fig. 2). IL-33 or IL-25 potentially induced Blimp-1 in nearly all ILC2s compared to IL-2 alone (Fig. 2a,b). ILC2s sorted from the dermis and small intestine lamina propria (siLP) stimulated with IL-33 also readily expressed Blimp-1 (Extended Data Fig. 2j–m). Time-course analysis demonstrated that IL-33 could induce Blimp-1 as early as 8 h and >50% of ILC2s were Blimp-1⁺ by 24 h (Fig. 2c,d). However, IL-5 and IL-13 were still detectable before Blimp-1, as early as 4 h after IL-33 stimulation, suggesting that Blimp-1 was not associated with type 2 cytokine expression, in line with expression in vivo in both type 2 cytokine-positive and type 2 cytokine-negative cells (Extended Data Fig. 3a,b). Thymic stromal lymphopoietin (TSLP) and neuromedin U (NMU) are also known activators of ILC2s; however, neither induced Blimp-1 above IL-2 alone (Extended Data Fig. 3c,d).

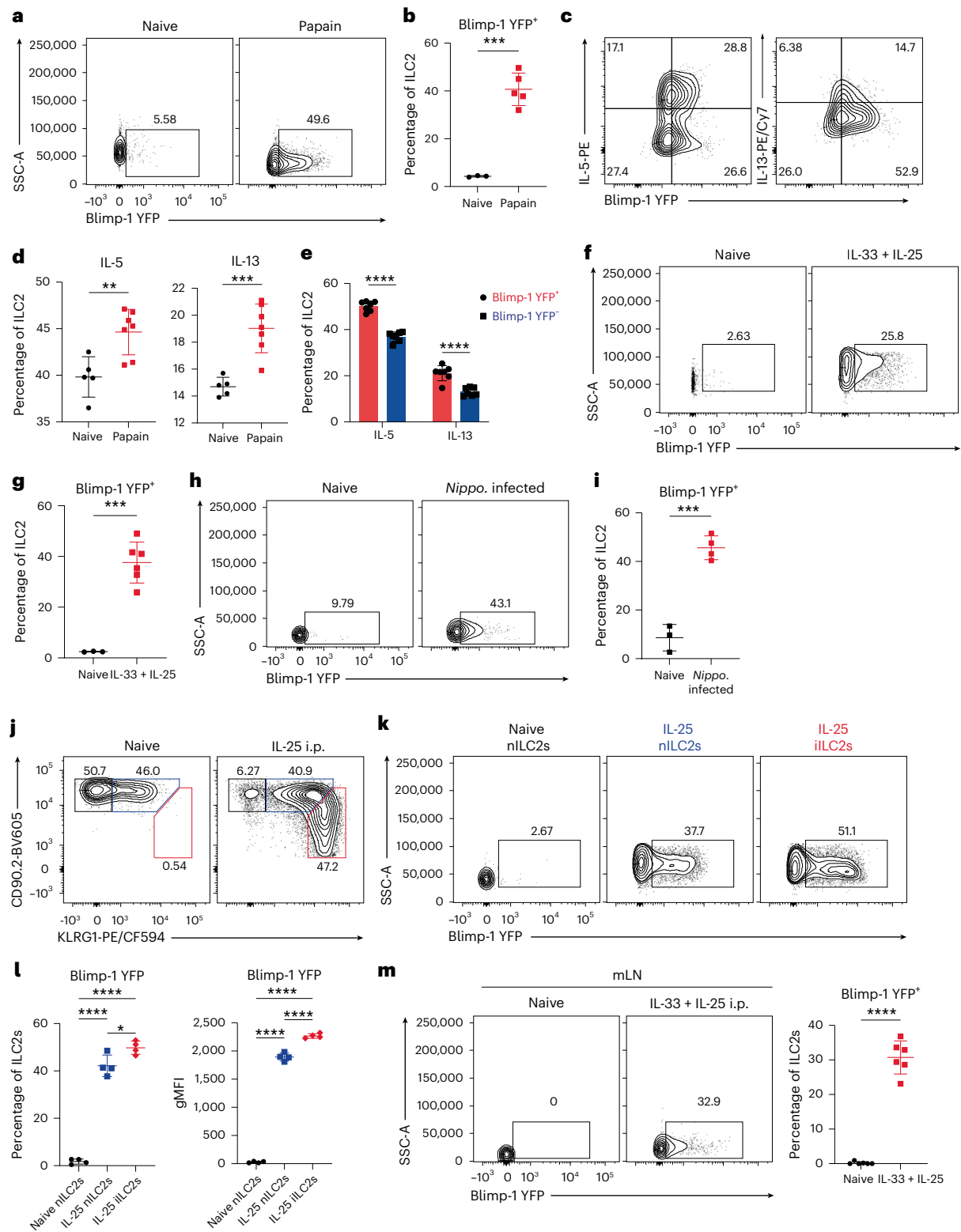
In T cells, Blimp-1 is driven by cytokine–JAK–STAT pathways such as IL-12–STAT4 in T_H1 cells and IL-10–STAT3 in T_H2 cells^{23,24}. To test whether Blimp-1 is also driven by STAT pathways in ILC2 cells, sorted lung ILC2s

Fig. 1 | Blimp-1 is expressed in lung ILC2s in response to tissue inflammation and helminth infection. **a**, Representative flow plot of Blimp-1-YFP in ILC2s. **b**, Quantification of **a** ($n = 3–5$ mice per group). **c**, Representative flow plots of Blimp-1-YFP and IL-5. **d**, Quantification of IL-5⁺ (left) or IL-13⁺ (right) ILC2 from **c** ($n = 5–7$ mice per group). **e**, Percentage of IL-5 and IL-13 expression in Blimp-1 YFP⁺ ILC2 or Blimp-1 YFP[−] ILC2 ($n = 7$). **f**, Representative flow plots of Blimp-1 YFP in ILC2s isolated after IL-33+IL-25. ($n = 3–6$ mice per group). **g**, Quantification of Blimp-1 YFP+ ILC2s from **f**. **h**, Representative flow plots of Blimp-1 YFP in ILC2s isolated from *N. brasiliensis* infection. **i**, Quantification of Blimp-1 YFP+ ILC2s from **h**. **j**, Representative flow plot for iILC2 gating after IL-25 i.p. ($n = 4$). **k**, Percentage of Blimp-1 YFP in ILC2 subsets from **j**. **l**, Quantification of Blimp-1 YFP percentage (left) or geometric mean fluorescence intensity (gMFI) (right) in nILC2s and iILC2s ($n = 4$). **m**, Percentage of Blimp-1 YFP in gut ILC2s after IL-33

and IL-25 i.p. ($n = 6$) (left) and quantification (right). Each point represents one individual sample. The data are shown as mean $\pm$ s.d. and represent two or three independent experiments. A two-tailed, unpaired Student's *t*-test was performed for **b**, **d**, **g**, **i** and **m**. Šidák's multiple-comparison test for **e** and Tukey's multiple-comparison test for **l**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. The specific *P* values are as follows: for **b**: $P = 0.0001$; for **d**: left, $P = 0.0055$; right, $P = 0.0005$; for **e**: comparison between IL-5, $P < 0.0001$, comparison between IL-13, $P < 0.0001$; for **g**: $P = 0.0002$; for **i**: $P = 0.0002$; for **l**: left, IL-25 iILC2s versus IL-25 nILC2s, $P = 0.0184$, IL-25 iILC2s versus naive nILC2s, $P < 0.0001$, IL-25 nILC2s versus naive nILC2s, $P < 0.0001$; right, IL-25 iILC2s versus IL-25 nILC2s, $P < 0.0001$, IL-25 iILC2s versus naive nILC2s, $P < 0.0001$, IL-25 nILC2s versus naive nILC2s, $P < 0.0001$; and, for **m**, $P < 0.0001$. *Nippo.*, *Nippostrongylus brasiliensis*.

from naive Blimp-1 YFP mice were stimulated *in vitro* with IL-33 for 24 h with or without the JAK inhibitor ruxolitinib, which specifically binds and inhibits JAK1 and JAK2, effectively eliminating all downstream STAT activation²⁵. Inhibition of STAT pathways via the JAK inhibitor completely ablated Blimp-1 expression (Fig. 2e,f) suggesting that the JAK–STAT pathways are critical for Blimp-1 upregulation. IL-33 and IL-25 have been reported to activate STATs; however, we were unable to detect canonical phospho-STAT3 or STAT5 activation downstream of these alarmins in ILC2s (Extended Data Fig. 3e–g).

Given the timing of Blimp-1 upregulation, we hypothesized that Blimp-1 may be indirectly regulated by another factor rapidly upregulated in activated ILC2s in response to alarmins. To test this, we mixed IL-33 receptor (ST2)-deficient ILC2s 1:1 with ST2-intact cells and stimulated them with IL-33. ST2-deficient ILC2s cultured alone were unable to induce Blimp-1 and failed to become activated, whereas ST2-intact cells robustly expressed Blimp-1 as expected (Fig. 2g). ST2-deficient ILC2s cultured together with ST2-intact cells produced a significant amount of Blimp-1, although less than ST2-intact cells (Fig. 2g,h).



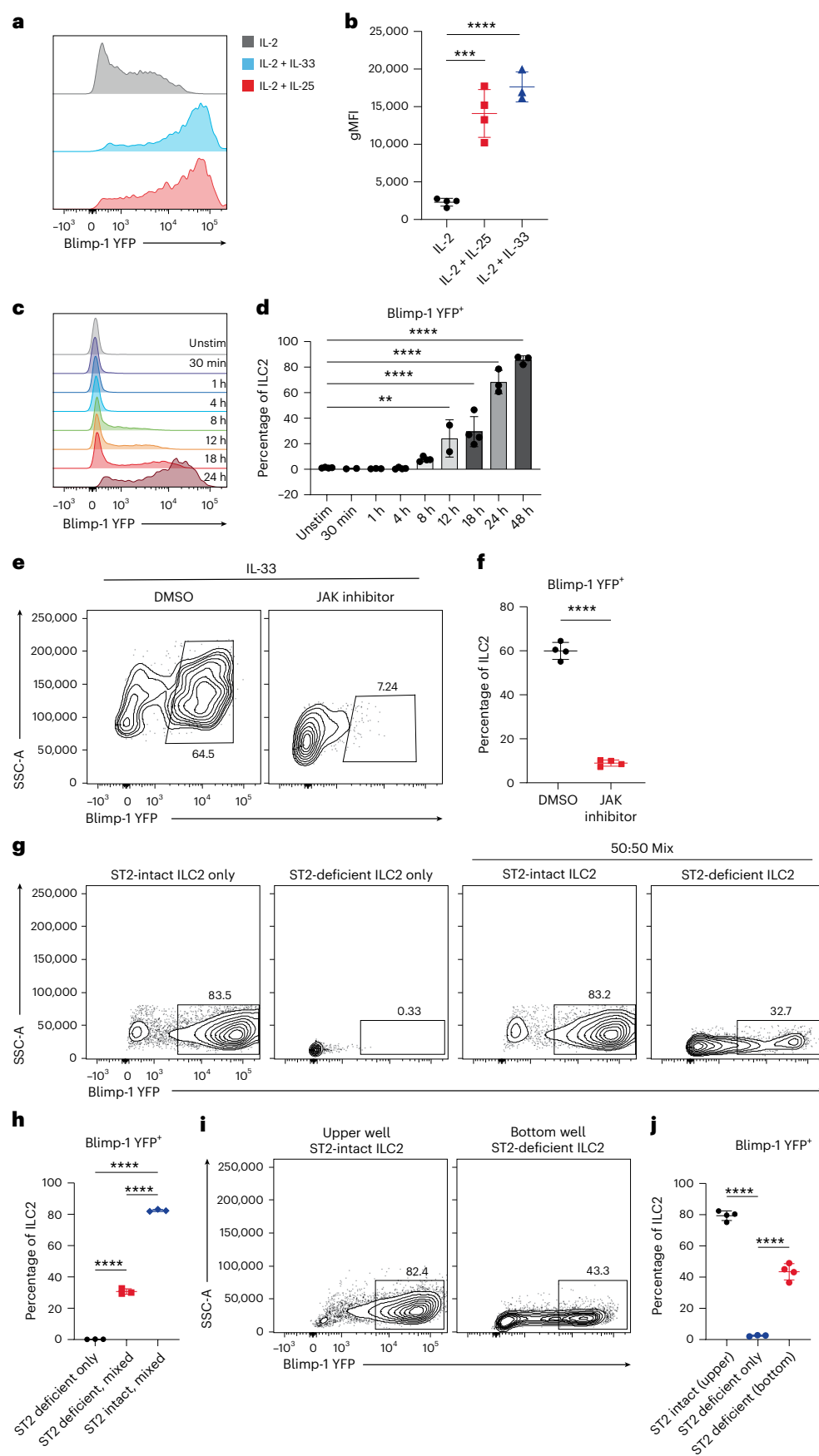


Fig. 2 | IL-33 and IL-25 indirectly upregulate Blimp-1 in ILC2s. **a**, Histogram for Blimp-1 YFP in ILC2s after stimulation with IL-33 or IL-25 for 3 d. **b**, Quantification of Blimp-1 gMFI from **a** ($n = 4$). **c**, Histogram of Blimp-1 expression in ILC2s at indicated timepoints stimulated with IL-33 + IL-25. **d**, Quantification of YFP⁺ cells in **c** ($n = 4$). **e**, Percentage of Blimp-1 YFP in stimulated ILC2s when treated with JAK inhibitor. **f**, Quantification of **e** ($n = 4$). **g**, Percentage of Blimp-1 YFP in ILC2s from ST2-deficient and ST2-intact ILC2 cell-mixing experiment. **h**, Quantification of **g**. **i**, Percentage of Blimp-1 YFP in ILC2s from ST2-deficient and ST2-intact ILC2 transwell assay. **j**, Quantification of **i**. Each point represents one individual sorting sample from two pooled animals. The data are shown as mean $\pm$ s.d. and represent two or three independent experiments. A two-tailed, unpaired

Student's *t*-test was performed for **f**, Dunnett's multiple comparisons test for **b**, **d** and **j** and Tukey's multiple-comparison test for **h**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. The specific *P* values are as follows: for **b**: IL-2 versus IL-2 + IL-25, $P = 0.0001$, IL-2 versus IL-2 + IL-33, $P < 0.0001$; for **d**, unstimulated versus 12 h, $P = 0.0028$, unstimulated versus 18 h, $P < 0.0001$, unstimulated versus 24 h, $P < 0.0001$, unstimulated versus 48 h, $P < 0.0001$; for **f**, $P = 0.0002$; for **h**: ST2-deficient ILC2s, mixed versus ST2-intact ILC2s, mixed, $P < 0.0001$, ST2-deficient ILC2s, mixed versus ST2-deficient ILC2s only, $P < 0.0001$, ST2-intact ILC2s, mixed versus ST2-deficient ILC2s only, $P < 0.0001$; and, for **j**: ST2-deficient only versus transwell ST2-deficient, $P < 0.0001$ and ST2-deficient only versus transwell ST2-intact, $P < 0.0001$. DMSO, dimethyl sulfoxide; Unstim, unstimulated.

Using a transwell system to prevent cell–cell contact, ST2-deficient ILC2s still produced significant Blimp-1, indicating regulation by secreted factors (Fig. 2i,j). These data suggest that IL-33 and IL-25 indirectly drive Blimp-1 in ILC2s, likely through upregulation of a secreted factor that can activate STAT family members.

IL-9 is necessary and sufficient for Blimp-1 induction in ILC2s

To determine candidate secreted factors from ILC2s capable of promoting Blimp-1, we performed RNA sequencing (RNA-seq) comparing unstimulated ILC2s ex vivo from lungs with ILC2s stimulated with IL-33 for 24 h. Secreted protein genes that were significantly upregulated were compared to expression of their cognate receptors on unstimulated ILC2s (Fig. 3a,b). Based on these results, we selected the cytokines IL-9, IL-24 and IL-3 for further examination. ILC2s from Blimp-1 YFP mice were stimulated for 24 h with each candidate cytokine and compared to IL-2 alone. IL-9 drove significantly more Blimp-1 than IL-3 and IL-24 (Fig. 3c,d). Furthermore, ILC2s rapidly produced IL-9, because nearly 85% of ILC2s were positive after 4 h of stimulation, in line with the timeline to subsequently induce Blimp-1 (Fig. 3e,f).

To investigate whether IL-9 is necessary for Blimp-1 expression, we performed transwell assays as above with ST2-deficient ILC2s in the lower well and ST-intact ILC2s in the upper well, followed by IL-33 stimulation with or without blocking anti-IL-9. IL-9 blockade completely abrogated Blimp-1 expression in both ST2-intact and ST2-deficient ILC2s, demonstrating that IL-9 was essential for Blimp-1 expression in ILC2 cells (Fig. 3g,h). Next, we administered IL-33 + IL-25 i.n. with or without IL-9 blockade. Blimp-1 expression in ILC2s in the lung was completely abolished when IL-9 was blocked (Fig. 3i,j). Finally, to determine whether IL-9 was sufficient to induce Blimp-1 in vivo, we administered recombinant IL-9 i.n. $3 \times$ similar to IL-33 or papain and found that this was sufficient to drive Blimp-1 in ILC2s (Fig. 3k,l). Collectively these data demonstrate that IL-9 is the primary driver of Blimp-1 in ILC2s in response to activation by alarmins.

Blimp-1 suppresses type 1 genes in ILC2s

To determine the function of Blimp-1 in ILC2s, we deleted Blimp-1 in all ILC subsets as well as T cells, B cells and NK cells using Blimp-1^{fllox} mice crossed to IL7R α -Cre (Blimp-1^{IL7R α Cre}) mice. ILC2 numbers in the lung at steady state were similar between control^{IL7R α Cre} and Blimp-1^{IL7R α Cre} mice, indicating that Blimp-1 was not required for the development of ILC2s (Extended Data Fig. 4a). As Blimp-1 was absent in several cell types, we tested the function of Blimp-1 in vitro. Sorted ILC2s from the lungs of

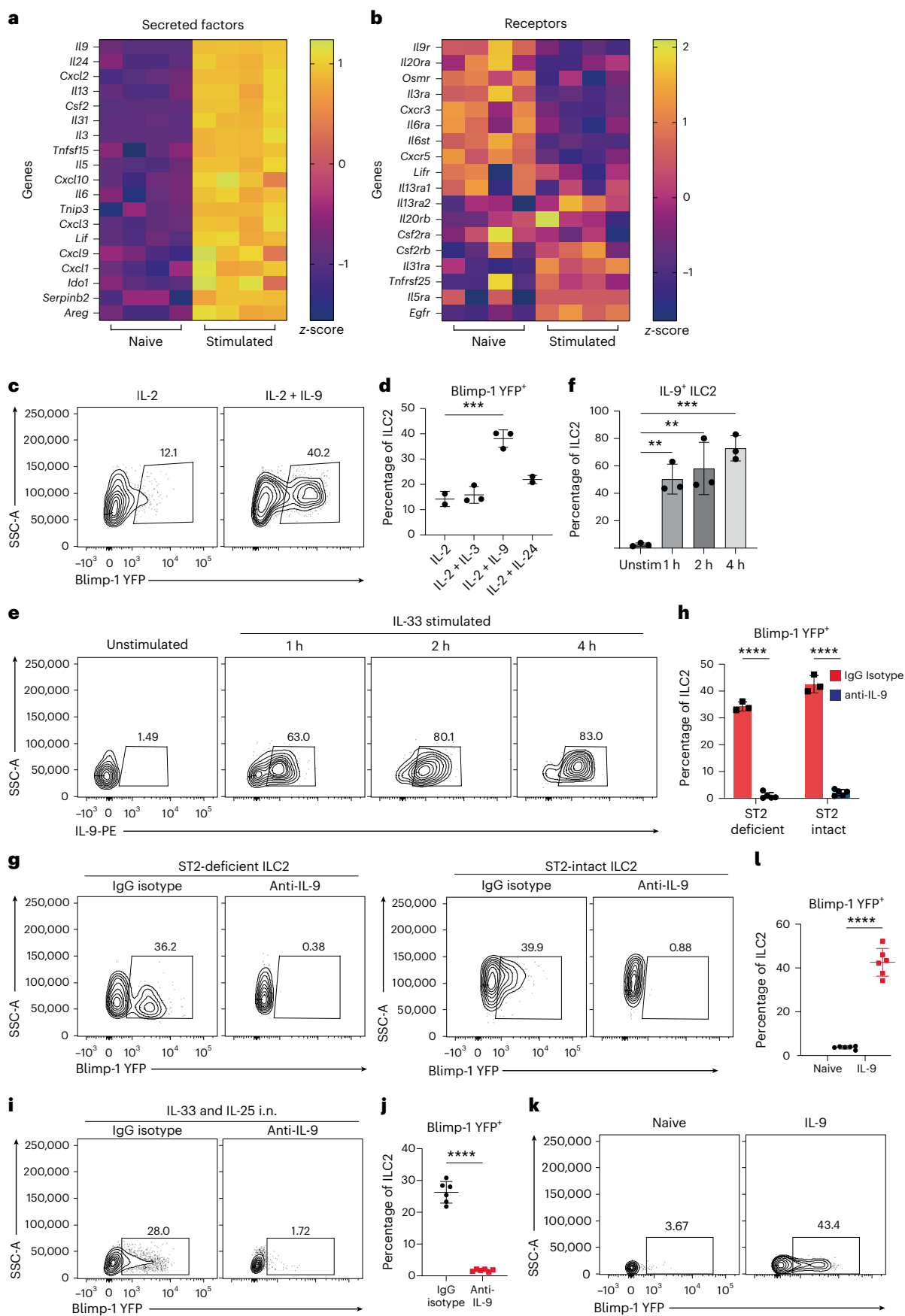
control^{IL7R α Cre} and Blimp-1^{IL7R α Cre} mice were activated with IL-33 and IL-25 followed by RNA-seq and assay for transposase-accessible chromatin using sequencing (ATAC-seq). Differentially expressed genes were defined as $\log_2$ (fold-change) > 2 and adjusted P (P_{adj}) < 0.01 . The number of genes upregulated in Blimp-1-deficient ILC2s was 369, whereas only 89 were downregulated (Fig. 4a and Extended Data Fig. 4b). Gene set enrichment analysis (GSEA) showed that the top pathway enriched in Blimp-1-deficient ILC2s was cytokines and chemokines (Fig. 4b). Closer inspection revealed that several type 1-associated genes were upregulated, including *Tnf*, *Lif*, *Ifng*, *Cxcl9*, *Cxcl10*, *Lta*, *Ltb*, *Csf1* and *Csf2* (Fig. 4c). Type 2 genes *Il4*, *Il5* and *Il13* were not significantly different; however, *Il9* was significantly upregulated, consistent with prior reports that Blimp-1 suppresses IL-9 in CD4 T cells (Fig. 4c,d)²⁶. *Il3*, a cytokine that promotes inflammatory cells including mast cells, was also upregulated in the absence of Blimp-1 (Fig. 4c)²⁷. Several genes of interest were also downregulated, including *Arg1*, *Slc2a3* (Glut3) and *Ccr3* (Fig. 4e). We confirmed an increase in both TNF and IL-9 in Blimp-1-deficient ILC2s at the protein level by intracellular cytokine staining and ELISA for TNF (Fig. 4f and Extended Data Fig. 4c,d). However, we did not detect an increase in IL-9 protein, likely because IL-9 is already robustly induced in ILC2s. In contrast, IFN γ was not upregulated at the protein level (Extended Data Fig. 4e). IFN γ is known to be highly regulated post-transcriptionally, suggesting that additional factors are needed to drive expression not present in this setting²⁸. IL-3, granulocyte–macrophage colony-stimulating factor and CCL2 were upregulated in the supernatant upon ILC2 activation in Blimp-1^{IL7R α Cre} cells (Extended Data Fig. 4f–h). In contrast to RNA-seq data, we found that type 2 cytokines were reduced at the protein level, suggesting the possibility that increased type 1 cytokines could indirectly repress type 2 cytokines (Extended Data Fig. 4i–l).

Blimp-1 is a transcriptional repressor known to recruit the histone methyltransferase G9a, which drives methylation of H3K9, a mark of heterochromatin²⁹. To determine the mechanism of Blimp-1-mediated suppression of type 1 genes, we compared open chromatin regions in activated ILC2s from control^{IL7R α Cre} and Blimp-1^{IL7R α Cre} mice identified by ATAC-seq. Blimp-1-deficient ILC2s had increased chromatin accessibility at type 1 cytokine genes, including the *Lta*, *Tnf* and *Ltb* locus and *Ifng* (Fig. 4g). Genome-wide analysis identified increased accessibility at open regions (clusters 1 and 2) but few newly accessible regions, suggesting that Blimp-1 limits gene expression of those genes that may already be accessible (Fig. 4h). ILCs are known to have a chromatin landscape poised for responsiveness³⁰. Motif analysis of regions more

Fig. 3 | IL-9 is necessary and sufficient for Blimp-1 upregulation in ILC2s.

a,b, RNA-seq comparing naive ILC2s with ILC2s stimulated for 24 h with IL-33 for secreted factors (**a**) or receptor expression (**b**). The z-scores were calculated as transcripts per million (TPM). **c**, Percentage of Blimp-1 YFP in ILC2s stimulated with IL-3, IL-9 or IL-24 for 24 h ($n = 3$). **d**, Quantification of **c**. **e**, Percentage of IL-9⁺ ILC2s at indicated timepoints when stimulated with IL-33 ($n = 3$). **f**, Quantification of **e**. **g**, Percentage of Blimp-1 YFP in ILC2s from transwell assay after treatment with anti-IL-9-blocking antibody. **h**, Quantification of **g** ($n = 3$). **i**, Percentage of Blimp-1 YFP in ILC2s after anti-IL-9 blockade in vivo ($n = 6$). **j**, Quantification of **i**. **k**, Percentage of Blimp-1 YFP in ILC2s after IL-9 treatment for 3 d ($n = 6$).

l, Quantification of **k**. Each point represents one individual sorting sample from two pooled animals. The data are shown as mean $\pm$ s.d. and represent three or four independent experiments. A two-tailed, unpaired Student's *t*-test was performed for **j** and **l**, Dunnett's multiple-comparison test for **d** and **f** and Sidák's multiple-comparison test for **h**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. The specific *P* values are as follows: for **d**, IL-2 versus IL-2 + IL-9, $P = 0.0004$; for **f**, unstimulated versus 1 h, $P = 0.003$, unstimulated versus 2 h, $P = 0.0012$, unstimulated versus 4 h, $P = 0.0002$; for **h**, comparison within ST2 knockout (KO), $P < 0.0001$, comparison within ST2 wild-type (WT), $P < 0.0001$; and, for **j**, $P < 0.0001$; for **l**, $P < 0.0001$.



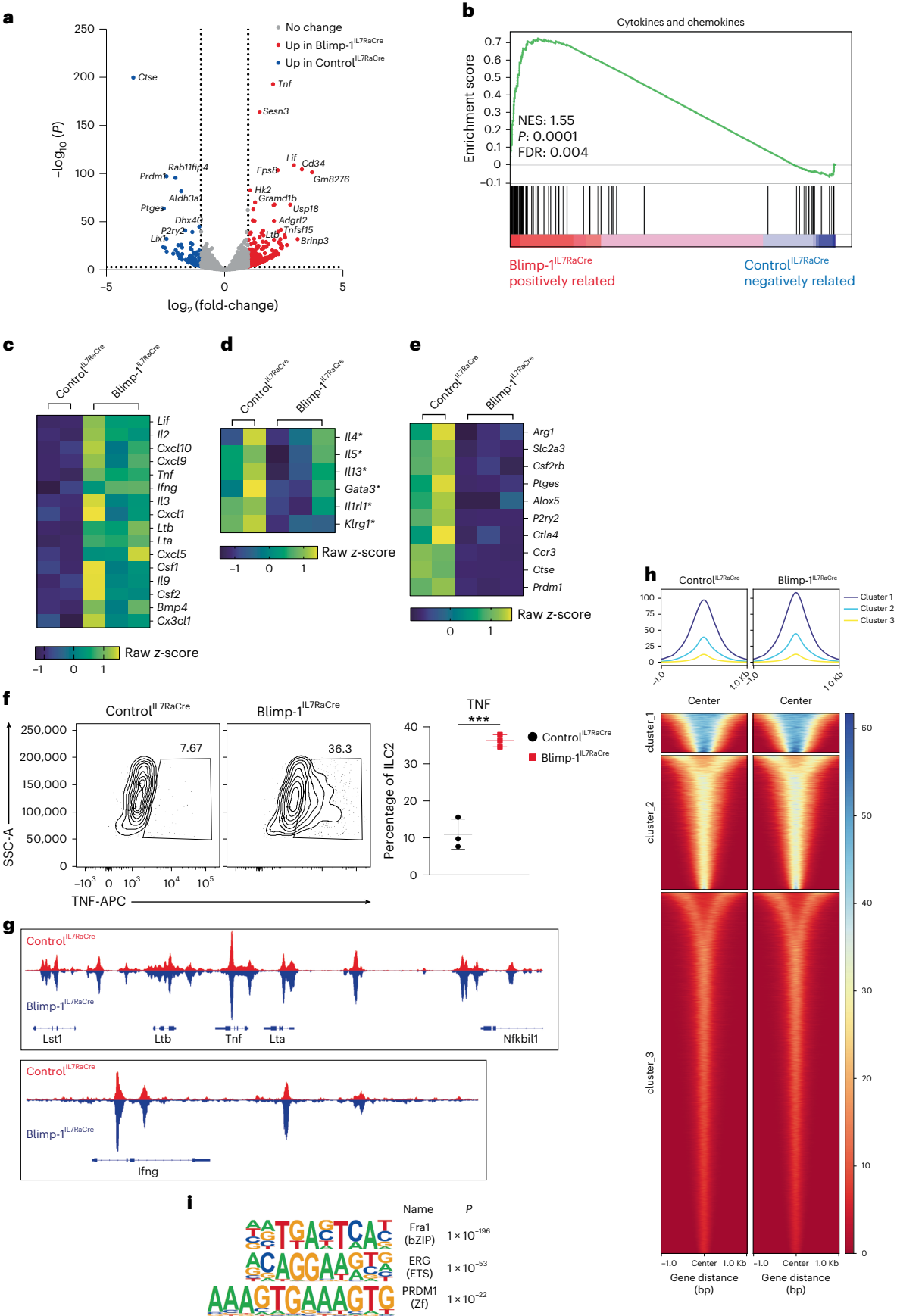


Fig. 4 | Blimp-1 suppresses type 1-related genes in ILC2s. **a**, Volcano plot from RNA-seq comparing control^{IL7RaCre} and Blimp-1^{IL7RaCre} ILC2s. The threshold was set at $\log_2(\text{fold-change}) > 1$ and $\log_{10}(P) < 0.01$. **b**, GSEA plot with enrichment in cytokines and chemokines in Blimp-1^{IL7RaCre} ILC2s. Normalized enrichment score (NES), 1.55; P , 0.0001; false discovery rate (FDR), 0.004. **c–e**, Heatmap for genes that were upregulated (**c**), no change (**d**) or downregulated (**e**) in Blimp-1^{IL7RaCre} ILC2s. The z-scores were calculated as TPM. Genes marked with an asterisk (*) in **d** are not significant. **f**, Percentage and quantification of TNF + ILC2s from control^{IL7RaCre} or Blimp-1^{IL7RaCre} mice with IL-33 and IL-25 ($n = 3$). **g**, Representative ATAC-seq peaks near the TNF locus and the IFN γ locus. **h**, Tornado plot for

ATAC-seq differential peaks comparing Blimp-1-deficient with Blimp-1-intact ILC2s. **i**, Top results from HOMER motif analysis enriched in open chromatin regions of Blimp-1-deficient ILC2s. Each point represents one individual sorting sample from two pooled animals. The data are shown as mean $\pm$ s.d. and represent three or four independent experiments. A two-tailed, unpaired Student's t -test was performed for **f**, the multiple-comparison test with Benjamini–Hochberg procedure for P_{adj} in RNA-seq and the Kolmogorov–Smirnov test for P value in GSEA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The specific P values are as follows: for **f**, $P = 0.0006$.

accessible in Blimp-1-deficient ILC2s identified the Prdm1 consensus sequence as a top hit, suggesting that Blimp-1 mediates repression of type 1 genes directly (Fig. 4i). Collectively, these data suggest that Blimp-1 limits the accessibility and expression of type 1 genes after ILC2 activation, protecting the type 2 transcriptional state of ILC2s under inflammatory conditions.

Blimp-1 promotes type 2 responses in ILC2s by repressing type 1 cytokines

To assess Blimp-1 function in vivo during allergen or alarmin responses, we generated ILC2-specific, Blimp-1-deficient mice using the NMur1-Cre strain which includes eGFP as a marker of NMur1. NMur1 has been observed in non-ILC2 populations in other tissues but is restricted to the ILC2 compartment in the lung (Extended Data Fig. 5a,b). We administered IL-33 and IL-25 i.n. for 3 d and found ILC2 activation, including ST2 and KLRG1, and ILC2 numbers were not affected, indicating that Blimp-1 does not broadly regulate ILC2 activation or expansion (Extended Data Fig. 5c,d). However, Blimp-1 deficient ILC2s produced significantly more IFN γ and TNF compared to control ILC2s (Fig. 5a–d and Supplementary Fig. 3). Despite undetectable IFN γ protein in vitro, in vivo IFN γ was robustly increased which extended to bronchoalveolar lavage (BAL) (Fig. 5e). IL-5 and IL-13 were significantly reduced (Fig. 5f,g), suggesting that they may be indirectly regulated by increased type 1 cytokines. As early responders to tissue damage, ILC2s can influence the inflammatory state of T cell responses. We found increased expression of IFN γ and TNF in both CD4 and CD8 T cells in the lung (Extended Data Fig. 5e–j), although total numbers of both remained the same (Extended Data Fig. 5k,l). Decreased type 2 cytokines led to a reduction in eosinophil recruitment to BAL (Fig. 5h,i); however, mast cells were significantly increased, likely due to increased IL-3 and IL-9, because they can recruit mast cells (Fig. 5j,k)^{27,31}. Neutrophils in the BAL were unaffected (Extended Data Fig. 5m). To assess the impacts on lung inflammation, we performed hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) staining to identify mucus in the airways. Blimp-1^{NMuriCre} mice had reduced lymphocytic infiltration and mucus in the airways compared to control^{NMuriCre} mice (Fig. 5l–n). Collectively, these data indicate that Blimp-1 maintains the type 2 state of ILC2s, whereas its loss promotes a mixed phenotype expressing both type 1 and type 2 cytokines.

We next examined Blimp-1 in ILC2s using clinically relevant allergens, papain and *Alternaria alternata*. Acute models of either allergen produced results similar to IL-33 and IL-25 across most measures. ILC2 numbers were unchanged, but IFN γ and TNF increased at the expense of IL-5 and IL-13 (Fig. 6a–g and Extended Data Fig. 6a–g). Papain

robustly increased IFN γ and TNF, whereas *Alternaria* sp. significantly increased IFN γ , with only a trend in TNF, indicating allergen-specific differences. IL-9 was potentially upregulated in Blimp-1-deficient ILC2s (Extended Data Fig. 6h,i). Despite undetectable levels of IL-9 in the BAL, the shift in cytokines led to decreases in eosinophils, whereas mast cells were increased (Fig. 6h,i and Extended Data Fig. 6j–o). Neutrophils remained unchanged (Extended Data Fig. 6p,q). We also did not observe any changes in NK or ILC1 cells (Extended Data Fig. 6r,s). CD4 and CD8 T cells had increased IFN γ expression but were not changed in total number (Fig. 6j–n) and histological analysis consistently showed a reduction in lymphocytic infiltration and mucus production (Fig. 6o–q and Extended Data Fig. 7a–c). To determine the physiological impact of these changes, we assessed airway hyperresponsiveness (AHR) to methacholine challenge after *Alternaria* sensitization. Despite robust changes by histology, we did not find statistically significant differences in AHR between control^{NMuriCre} and Blimp-1^{NMuriCre} mice (Extended Data Fig. 7d). A recent study demonstrated that IL-9 was a key factor promoting AHR and mast cells are known to be associated with AHR^{32,33}. Thus, despite reduced IL-5, IL-13, eosinophils and lung inflammation, increased IL-9 and mast cell recruitment likely sustain AHR.

To model chronic allergen exposure in asthma, we administered *Alternaria* sp. twice weekly for 3 weeks and compared it to acute daily exposure for 4 d. As expected, the amount of IL-5 and IL-13 from CD4 T cells in chronic exposure far exceeded the amount during acute settings, where almost no cytokines from T cells were detected (Extended Data Fig. 7e–g). No differences in type 2 cytokines from CD4 T cells were observed between control^{NMuriCre} and Blimp-1^{NMuriCre} mice. Eosinophil recruitment was also unaffected, likely due to IL-5 from CD4 T cells (Extended Data Fig. 7h,i). ILC2s from both groups produced IL-5 and IL-13; although IL-5 levels in the chronic setting were comparable to acute administration, IL-13 was greatly increased in ILC2s after chronic *Alternaria* sp. exposure (Extended Data Fig. 7j–l). IFN γ was not detected in ILC2s, whereas TNF was elevated with chronic *Alternaria* sp. exposure, with a nonsignificant increase in Blimp-1^{NMuriCre} mice compared to controls (Extended Data Fig. 7m). High type 2 cytokine production by T cells obscured ILC2-intrinsic effects of Blimp-1 loss, indicating a role for the broader cytokine environment; collectively, these data support a key role for Blimp-1 in acute alarmin responses by maintaining type 2 cytokines and repressing type 1 genes.

Blimp-1 maintains the type 2 transcriptional state of ILC2s

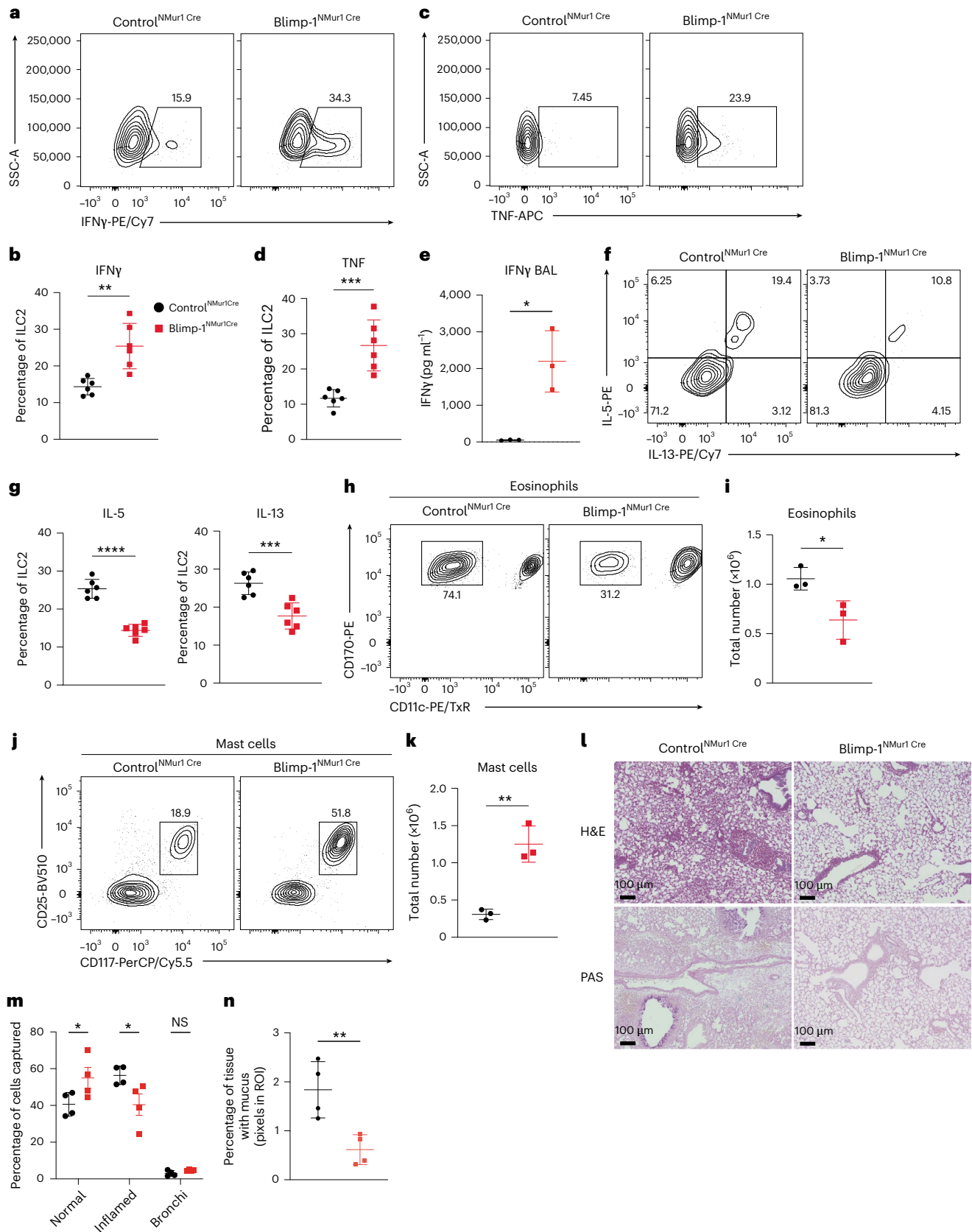
To define how Blimp-1 regulates ILC2 effector differentiation, we performed single-cell RNA-seq (scRNA-seq) of sorted ILC2s from untreated, papain or *Alternaria*-treated control^{NMuriCre} and Blimp-1^{NMuriCre} mice

Fig. 5 | Loss of Blimp-1 shifts the ILC2 cytokine balance in response to tissue alarmins. **a,c**, Representative flow plots of IFN γ^+ (**a**) or TNF $^+$ (**c**) ILC2s in indicated mice after IL-33 + IL-25. **b,d**, Quantification of IFN γ (**b**) or TNF (**d**) from **a** and **c** ($n = 6$). **e**, Quantification of IFN γ in BAL by ELISA ($n = 3$). **f**, Representative flow plots of IL-5 $^+$ IL-13 $^+$ ILC2s in indicated mice after IL-33 + IL-25. **g**, Quantification of IL-5 (left) or IL-13 (right) expression from **f** ($n = 6$). **h,j**, Representative flow plot for eosinophils (**h**) or mast cells (**j**) in indicated mice after IL-33 + IL-25. **i,k**, Quantification of eosinophil number (**i**) or mast cell numbers (**k**) from **h** and **j** ($n = 3$). **l**, Representative H&E and PAS staining of lungs from the indicated mice

after IL-33 and IL-25 i.n. **m,n**, Quantification of inflammation (**m**) and mucus (**n**) from **l** ($n = 4$). Each point represents one individual sample. The data are shown as mean $\pm$ s.d. and represent two independent experiments. A two-tailed, unpaired Student's t -test was performed for **b**, **d**, **e**, **g**, **i**, **k** and **n** and Šidák's multiple-comparison test for **m**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. The specific P values are as follows for **b**: $P = 0.0021$; for **d**: $P = 0.0007$; for **e**: $P = 0.0112$; for **g**: left, $P < 0.0001$, right, $P = 0.0009$; for **i**: $P = 0.0326$; for **k**: $P = 0.0029$; for **m**, normal, $P = 0.0428$, inflamed, $P = 0.0223$, bronchi, $P = 0.9867$; and, for **n**, $P = 0.0095$. NS, not significant; ROI, region of interest.

($n = 2$ per condition). Uniform Manifold Approximation and Projection (UMAP) clustering identified eight clusters, all expressing core ILC2 transcription factors (*Gata3*, *Rora* and *Id2*) and type 2 cytokine genes (*Il13*, *Il5* and *Il4*) (Fig. 7a and Extended Data Fig. 8a,b). Analysis of

clusters by treatment demonstrated distinctions between untreated and allergen-treated mice, likely representing ILC2s at steady state compared to activated ILC2s (Fig. 7b and Extended Data Fig. 8c). The differences between treatment groups aligned with our prior analysis



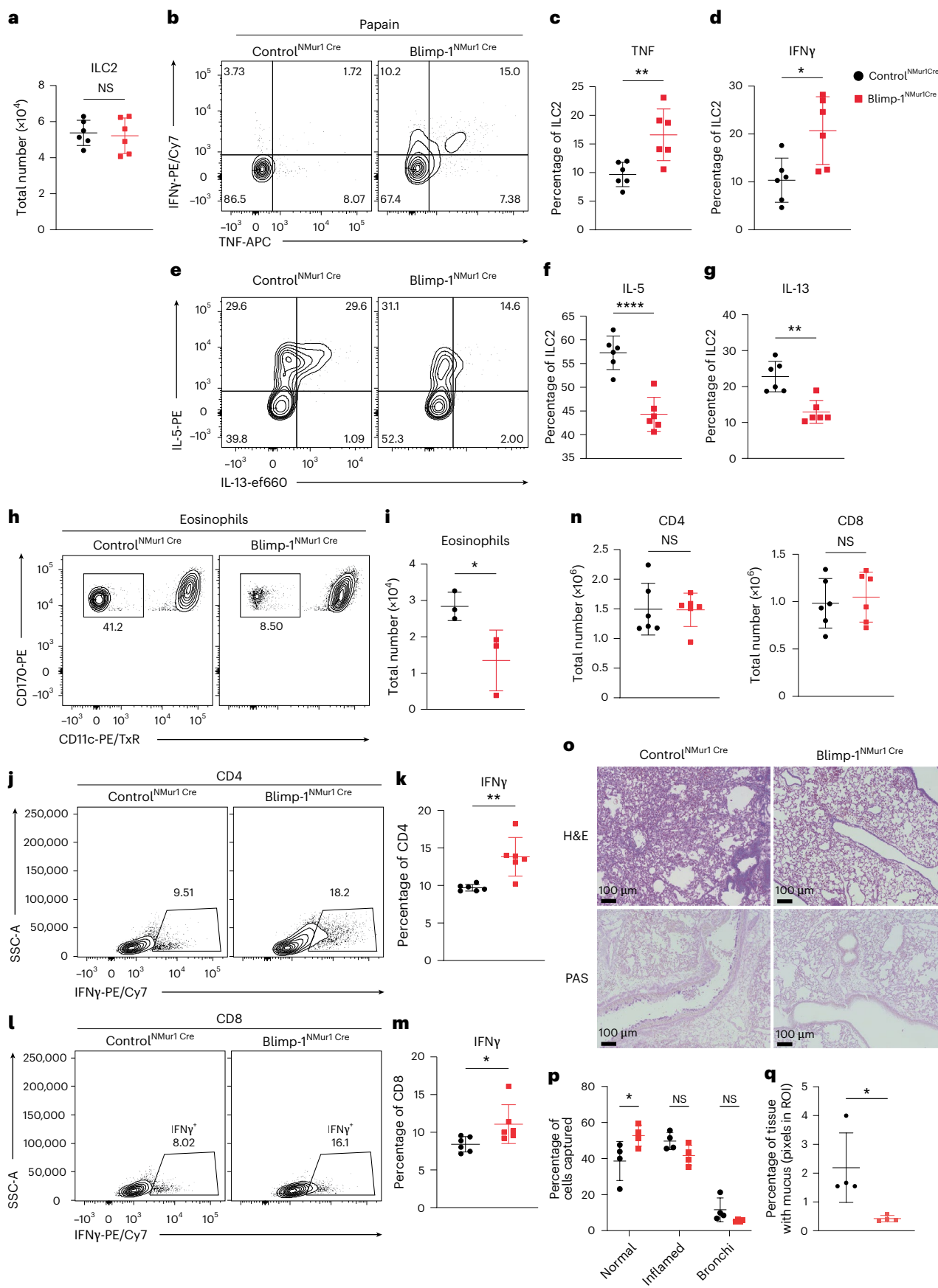


Fig. 6 | Loss of Blimp-1 shifts ILC2 cytokines and limits tissue inflammation to allergens. **a**, Quantification of total ILC2 number. **b**, Representative flow plots of IFN γ TNF $^{+}$ ILC2s in indicated mice after papain ($n = 6$). **c,d**, Quantification of IFN γ (c) or TNF (d) expression from **b**. **e**, Representative flow plots of IL-5 $^{+}$ IL-13 $^{+}$ ILC2s in indicated mice after papain. **f,g**, Quantification of IL-5 (f) or IL-13 (g) expression from **e**. **h**, Representative flow plots of eosinophils from indicated mice after papain ($n = 3$). **i**, Quantification of **h**. **j,l**, Representative flow plots of IFN γ CD4 (j) or CD8 (l) in indicated mice after *Alternaria* sp. ($n = 6$). **k,m**, Quantification of j (k) and l (m). **n**, Quantification of total CD4 (left) or CD8 (right) T cell numbers in indicated mice after papain ($n = 6$). **o**, Representative H&E and PAS staining of

indicated mice after papain. **p,q**, Quantification of inflammation (p) and mucus (q) from **o** ($n = 4$). Each point represents one individual sample. The data are shown as mean $\pm$ s.d. and represent two independent experiments. A two-tailed, unpaired Student's *t*-test was performed for **a**, **c**, **d**, **f**, **g**, **i**, **k**, **m**, **n** and **q** and Šidák's multiple-comparison test for **p**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. The specific *P* values are as follows for **a**: $P = 0.7521$; for **b**, $P = 0.0068$; for **d**, $P = 0.0133$; for **f**, $P < 0.0001$; for **g**, $P = 0.0011$; for **i**, $P = 0.0494$; for **k**, $P = 0.0031$; for **m**, $P = 0.0394$; for **n**, CD4, $P = 0.9591$, CD8, $P = 0.6749$; for **p**, normal, $P = 0.0187$, inflamed, $P = 0.2616$, bronchi, $P = 0.4843$; and, for **q**, $P = 0.0267$.

demonstrating greater effects on TNF in papain compared to *Alternaria* sp. Clusters 0–2 were enriched for untreated ILC2s, whereas clusters 3–7 contained allergen-treated cells, consistent with an activated effector state (Fig. 7b and Extended Data Fig. 8c).

To assess *Prdm1* (Blimp-1) expression, we focused on cells from control^{NMuriCre} mice. *Prdm1* transcripts were sparsely but evenly distributed across all ILC2 clusters (Extended Data Fig. 8d). Consistent with Blimp-1 not defining a distinct ILC2 subset, its expression was observed across both untreated and allergen-treated populations. Further analysis demonstrated that mean expression of *Prdm1* was increased in allergen-treated clusters, especially papain, but that the fraction of cells within each remained similar (Extended Data Fig. 8e). As Blimp-1 expression in ILC2s is somewhat low, drop-out likely contributes to fully capturing the expression dynamics using this method. Thus, Blimp-1 is expressed throughout the ILC2 compartment and activation of ILC2s promotes its upregulation.

We next assessed cluster distribution of control^{NMuriCre} and Blimp-1^{NMuriCre} ILC2s, finding largely overlapping populations with a few distinct clusters (Fig. 7c and Extended Data Fig. 8c). Cluster 5 included control papain-treated mice and Blimp-1-deficient ILC2s from both allergens, whereas cluster 7 was largely composed of Blimp-1-deficient, allergen-treated ILC2s and was distinct from control clusters. Given the differences between untreated and allergen-treated ILC2s, we used trajectory inference (Palantir) to determine how ILC2s may change upon allergen activation, setting cluster 1 as the starting point because it was enriched in untreated mice. Trajectory inference identified two paths that split as cells transitioned away from cluster 0 and terminated in either cluster 6 or cluster 7 (Fig. 7d). A separate trajectory inference algorithm, Diffusion Pseudotime (DPT)³⁴, also identified clusters 6 and 7 as terminal states, where cluster 6 is a terminal state for control ILC2s whereas cluster 7 is a terminal state for Blimp-1-deficient ILC2s (Fig. 7e). Collectively these results suggest that Blimp-1 loss alters the cellular state of differentiated ILC2s, shifting the cells from cluster 6 to cluster 7.

Finally, we assessed Blimp-1 loss on changes in type 1 cytokine production. *Tnf* was increased in Blimp-1-deficient ILC2s from allergen-treated groups compared to controls (Extended Data Fig. 8f). *Ifng* was poorly captured likely due to drop-out (Extended Data Fig. 8f). To determine the effect of IFN γ on ILC2 cells, we utilized a lung IFN γ -response signature module generated in response to infection³⁵ and found that Blimp-1-deficient ILC2s from treated mice had greater enrichment of genes upregulated in response to IFN γ (Fig. 7f,g and Extended Data Fig. 8g). Thus, signatures associated with type 1 cytokines are

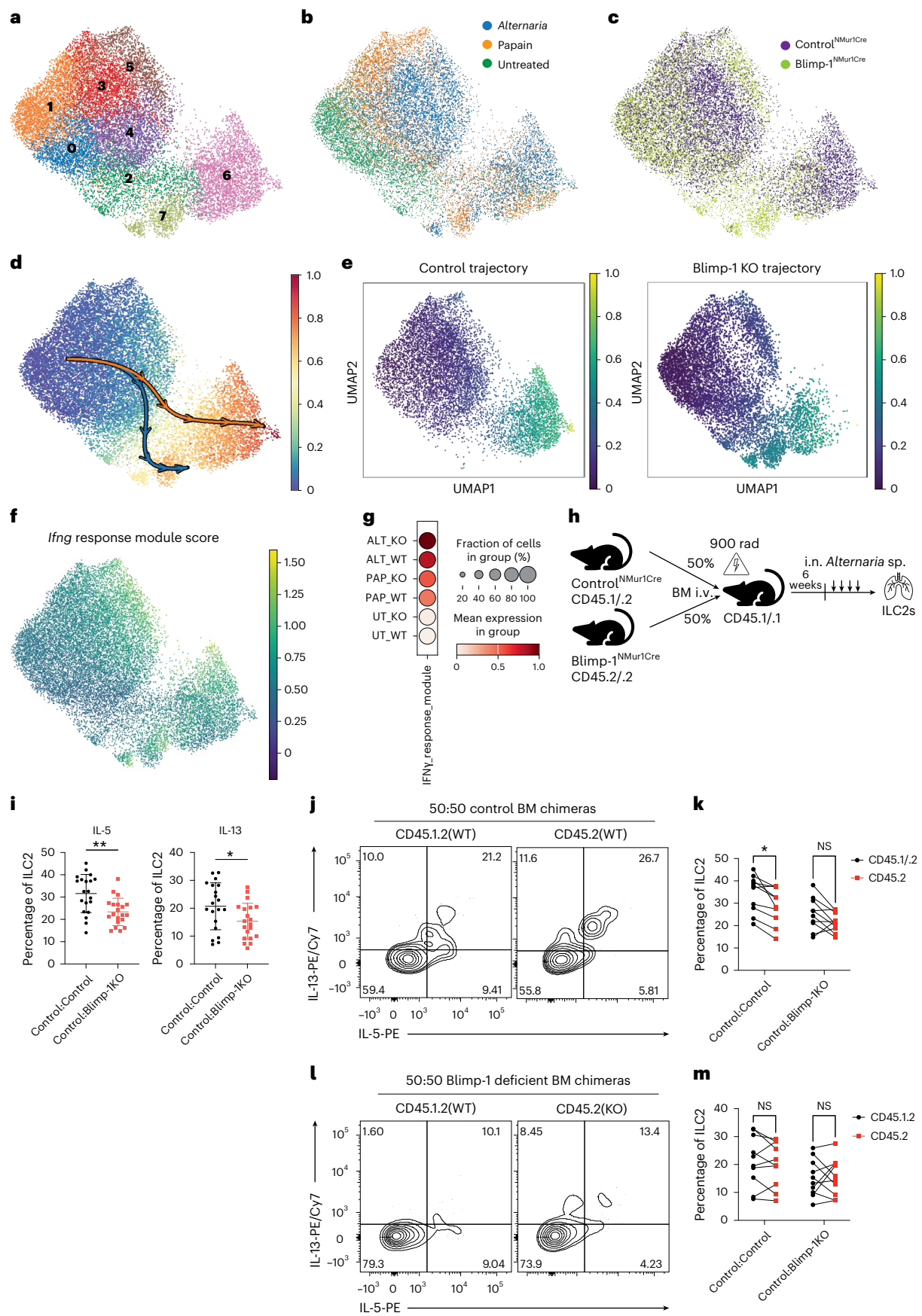
upregulated in ILC2s lacking Blimp-1, in alignment with increases in type 1 cytokine expression in the absence of Blimp-1.

Having established that Blimp-1 regulates the ILC2 cellular state, we next asked whether it controls type 2 cytokine expression intrinsically or through extrinsic mechanisms. We generated mixed BM chimeras by combining congenically mismatched control^{NMuriCre} (CD45.1/2) and Blimp-1^{NMuriCre} (CD45.2) BM at a 1:1 ratio and transferring the cells into irradiated hosts (CD45.1). After reconstitution, mice were immunized with *Alternaria* sp. (Fig. 7h). In control animals, we noted a small increase in one control population (CD45.1/2) over the other (CD45.2); however, ratios were consistent in hosts receiving a mix of control and Blimp-1^{NMuriCre} BM (Extended Data Fig. 8h). As expected, total type 2 cytokine production was significantly reduced in chimeras containing Blimp-1-deficient BM compared to control-only chimeras (Fig. 7i). Comparing within control–control mixed chimeras (control^{NMuriCre} CD45.1/2 mixed with control^{NMuriCre} CD45.2), ILC2s from CD45.1/2 controls produced more IL-5 compared to ILC2s from CD45.2 control BM, in accordance with the increased reconstitution, although the MFI was equal across both groups (Extended Data Fig. 8i). However, control Blimp-1-deficient, mixed chimeras (control^{NMuriCre} CD45.1/2 mixed with Blimp-1^{NMuriCre} CD45.2) produced comparable amounts of IL-5 and IL-13 (Fig. 7j–m), suggesting that Blimp-1 does not intrinsically regulate type 2 cytokine production. Intriguingly, type 1 cytokines were not statistically increased in chimeras with Blimp-1-deficient ILC2s (Extended Data Fig. 8j). These data suggest that, as in T cells, IFN γ from Blimp-1-deficient ILC2s can drive IFN γ production in control ILC2s via extrinsic mechanisms. Together with scRNA-seq, this supports a model in which Blimp-1 regulates ILC2 transcriptional state without intrinsically controlling type 2 cytokines, which are instead reduced through extrinsic effects of elevated type 1 cytokines.

To test whether increased type 1 cytokines drive reductions in type 2 cytokines and eosinophils, we blocked IFN γ and TNF in vivo during ILC2 activation with IL-33 + IL-25 i.n. (Fig. 8a). Blocking IFN γ and TNF reduced IFN γ in ILC2s, which was more pronounced in Blimp-1^{NMuriCre} mice (Fig. 8b), suggesting that IFN γ also plays an extrinsic role in amplifying IFN γ in ILC2 cells, as noted above in chimera studies. Blocking IFN γ and TNF had no impact on eosinophil recruitment in control mice compared to immunoglobulin G (IgG) alone (Fig. 8c,e). In contrast, eosinophils in Blimp-1^{NMuriCre} mice receiving anti-IFN γ and anti-TNF were similar to controls, whereas mice receiving IgG alone had a significant reduction as expected (Fig. 8c,e). IL-5 and IL-13 from ILC2s were reduced as expected in IgG Blimp-1^{NMuriCre}-treated mice, which recovered slightly on anti-IFN γ and anti-TNF blockade (Fig. 8d,f). These data suggest that

Fig. 7 | Blimp-1 maintains the transcriptional state of ILC2s and extrinsically regulates type 2 cytokines. **a–c**, A UMAP visualization of ILC2 cells from scRNA-seq colored by Leiden clustering labels 0–7 (**a**), allergen treatments (**b**) and control^{NMuriCre} and Blimp-1^{NMuriCre} ILC2s (**c**). **d**, Palantir-inferred ILC2 differentiation trajectory on UMAP embedding. **e**, DPT-inferred ILC2 differentiation pseudotime separated by control^{NMuriCre} (left) and Blimp-1^{NMuriCre} (right) mice on UMAP embedding. **f**, UMAP embedding of *Ifng* response module score. **g**, Dot plot of *Ifng* response module score within each sample. **h**, Schematic for mixed BM chimera experiment. **i**, Quantification of total IL-5 (left) or IL-13 (right) expression in ILC2s from the BM chimera model ($n = 10$). **j,l**, Representative flow plots

of IL-5 $^{+}$ IL-13 $^{+}$ ILC2s from control (j) or Blimp-1-deficient (l) mixed chimeras. **k,m**, Quantification of IL-5 (k) or IL-13 (m) from j and l. Each point represents one individual sample. The data are shown as mean $\pm$ s.d. and represent two independent experiments. A two-tailed, unpaired Student's *t*-test was performed for i and a two-tailed, paired Student's *t*-test corrected with the Holm–Šidák method for k and m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. The specific *P* values are as follows: for i: IL-5, $P = 0.0012$, IL-13, $P = 0.0294$; for k, control–control, $P = 0.0053$, control–Blimp-1KO, $P = 0.0769$; and, for m, control–control, $P = 0.4211$, control–Blimp-1KO, $P = 0.9357$. i.v., intravenously.



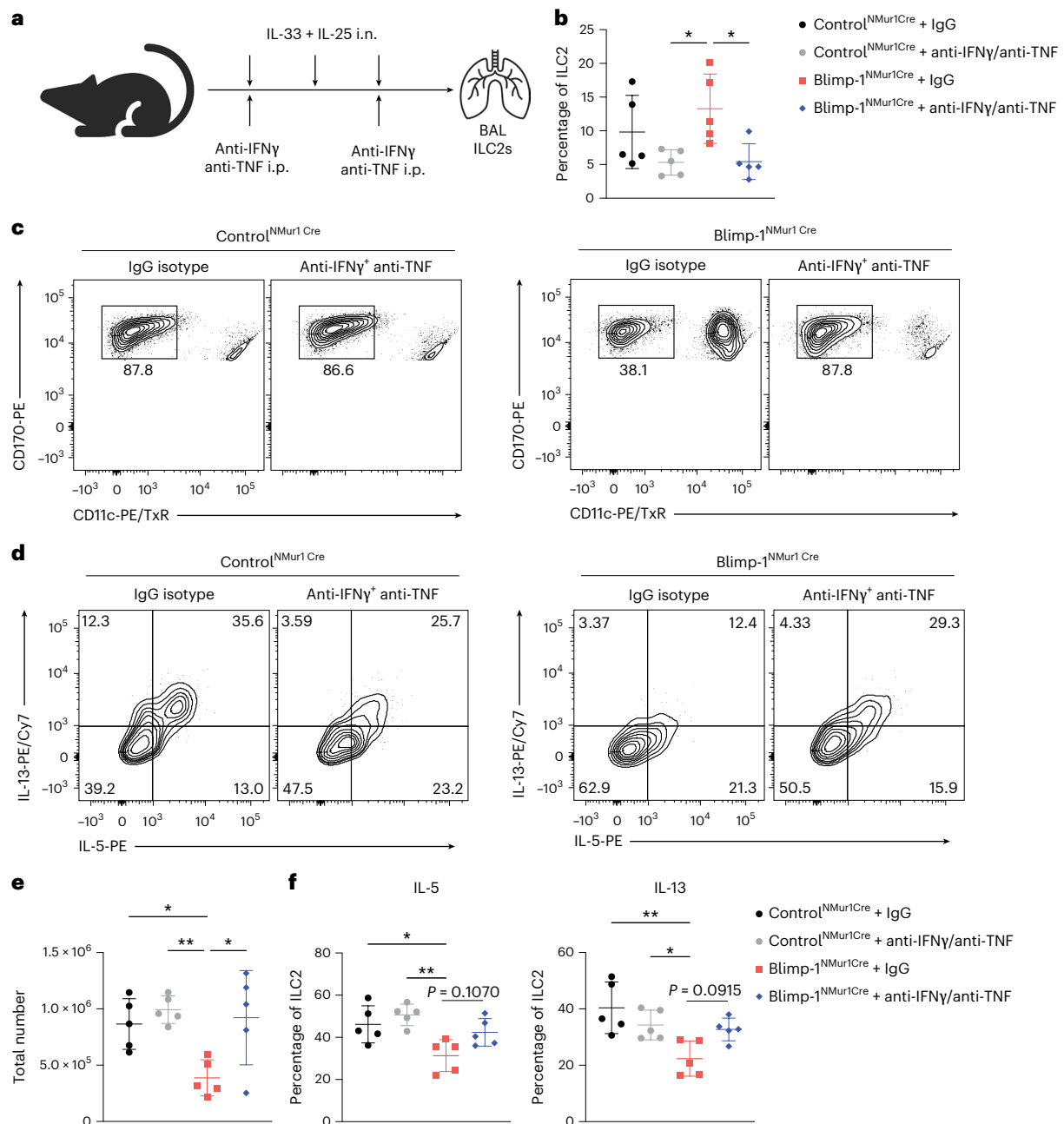


Fig. 8 | Blockade of type 1 cytokines in ILC2-conditional, Blimp-1-deficient mice restores type 2 responses. **a**, Experimental schematic. **b**, Quantification of IFN γ expression in ILC2s after antibody blockade with anti-IFN γ and anti-TNF ($n = 5$). **c, d**, Representative flow plot for eosinophils (**c**) and IL-5+ and IL-13+ ILC2 cells (**d**) in indicated mice with or without antibody blockade with anti-IFN γ and anti-TNF. **e**, Quantification of eosinophil numbers from **c**. **f**, Quantification of **d**. Each point represents one individual sample. The data are shown as mean $\pm$ s.d. and represent two independent experiments. Tukey's multiple comparisons test was performed for **b**, **d** and **f**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

The specific P values are as follows: for **b**: control^{NMuriCre} + anti-IFN γ or anti-TNF with Blimp-1^{NMuriCre} + IgG, $P = 0.0324$, Blimp-1^{NMuriCre} + IgG with Blimp-1^{NMuriCre} + anti-IFN γ or anti-TNF, $P = 0.0353$; for **d**: control^{NMuriCre} + IgG with Blimp-1^{NMuriCre} + IgG, $P = 0.0440$, control^{NMuriCre} + anti-IFN γ or anti-TNF with Blimp-1^{NMuriCre} + IgG, $P = 0.0093$, Blimp-1^{NMuriCre} + IgG with Blimp-1^{NMuriCre} + anti-IFN γ or anti-TNF, $P = 0.0223$; and, for **f**, left (IL-5), control^{NMuriCre} + IgG with Blimp-1^{NMuriCre} + IgG, $P = 0.0214$, control^{NMuriCre} + anti-IFN γ or anti-TNF with Blimp-1^{NMuriCre} + IgG, $P = 0.0029$; right (IL-13), control^{NMuriCre} + IgG with Blimp-1^{NMuriCre} + IgG, $P = 0.0022$, control^{NMuriCre} + anti-IFN γ or anti-TNF with Blimp-1^{NMuriCre} + IgG, $P = 0.0438$.

increased type 1 cytokines driven by loss of Blimp-1 in ILC2 cells can extrinsically limit type 2 responses. Collectively, our study identifies Blimp-1 as a critical cell-intrinsic regulator of ILC2 fate that sustains a type 2 inflammatory environment during allergen challenge.

Discussion

Blimp-1 is a transcriptional repressor expressed across immune cell types, where it binds DNA to repress alternative gene programs through heterochromatin formation³⁶. We previously identified a role for

Blimp-1 in T_H2 cell differentiation in response to inhaled allergens^{11,12}. Here we extend Blimp-1's function in type 2 responses to allergens to ILC2 cells. ILCs are helper-like innate cells that lack antigen specificity but share transcriptional programs with CD4 T cells⁴. Accordingly, ILC2s have similar transcriptional profiles to T_H2 cells but respond to tissue alarmins to produce type 2 cytokines³⁰. Lung ILC2s reside in adventitial niches adjacent to the airways, where they sense tissue damage, recruit inflammatory cells and influence local allergen-specific T cell responses³⁷. Together, T_H2 cells and ILC2s recruit eosinophils and

mast cells that drive the hallmark features of asthma, including mucus production and airway remodeling³⁸.

We found that Blimp-1 plays a multifaceted role in shaping ILC2-driven airway responses during allergic inflammation. Loss of Blimp-1 in ILC2s shifts the cytokine milieu away from a canonical type 2 program toward increased type 1 cytokine production, coinciding with reduced lung inflammation and diminished mucus production. Mechanistically, Blimp-1 intrinsically suppresses type 1 cytokines such as IFN γ and TNF in ILC2s, thereby limiting type 1 polarization of surrounding immune cells, including T cells and ILC2s themselves. In its absence, this restraint is lost, leading to extrinsic amplification of type 1 inflammation and reduced production of ILC2-derived IL-5 and IL-13.

Notably, reduced type 2 inflammation was accompanied by increased IL-9 production in Blimp-1-deficient ILC2s, uncoupling the type 2 effector program. Elevated IL-9 coincided with increases in mast cells and a subtle increase in airway hyperresponsiveness, consistent with prior work implicating the IL-9–mast cell axis as a driver of airway dysfunction^{32,33}. These observations highlight the complexity of Blimp-1 regulation in ILC2s, constraining both type 1 inflammation and IL-9-dependent airway pathology. Rather than acting as a unidirectional regulator of type 2 immunity, Blimp-1 functions as a stabilizing factor that enforces transcriptional fidelity while constraining divergent inflammatory programs with distinct pathological consequences.

ILC2 plasticity toward a type 1 state occurs during viral infection or in response to IL-1 β and toward an ILC3-like state during fungal infection or culture with transforming growth factor (TGF) β and IL-6 (ref. 39,40). Thus, ILC2s can respond to the tissue environment, producing effector responses outside of traditional type 2 responses. Although plasticity may be advantageous, ILC2s must also maintain their developmentally programmed identity. Our study identifies Blimp-1 as a mechanism that maintains ILC2 transcriptional identity through repression of type 1 gene programs. Whether Blimp-1 is reduced in settings such as viral infection to enable ILC2 plasticity remains an area of active investigation.

In T cells, Blimp-1 is driven by cytokine–JAK–STAT pathways such as IL-12–STAT4 in T_H1 cells and IL-10–STAT3 in T_H2 cells^{23,24}. Unlike T_H2 cells, IL-10 was not able to induce Blimp-1 in ILC2s. Rather, IL-9 was necessary and sufficient for Blimp-1 in ILC2s. IL-9 is a common γ -chain cytokine that signals via STAT5 and STAT3 (ref. 41). STAT3 plays a role in promoting allergic lung inflammation in pre-clinical models^{42–44}. As STAT3 promotes Blimp-1 in T_H2 cells downstream of IL-10, STAT3 may regulate Blimp-1 in ILC2s. IL-33 drives noncanonical STAT3 activation through serine 727 phosphorylation, leading to mitochondrial localization impacting ILC2 metabolism^{42,45}. IL-9-mediated STAT3 activation in ILC2s is less well understood, although we speculate that Blimp-1 may be important for the effects of IL-9 on ILC2s.

We initially observed Blimp-1 upregulation in response to alarmin stimulation using Blimp-1 YFP reporter mice. We consistently found YFP⁺ and YFP⁺ populations of ILC2s; however, we were unable to identify unique features of Blimp-1⁺ ILC2s. scRNA-seq demonstrated expression of Blimp-1 throughout all ILC2 clusters with a slight increase after papain or *Alternaria* treatment. This suggested that Blimp-1 is expressed in both resting and activated ILC2 cells, increasing with stimulation, perhaps nonuniformly in vivo compared to in vitro activation. Blimp-1 YFP mice may also under-report YFP expression in ILC2 cells. Collectively, these data support a broad role for Blimp-1 across the ILC2 compartment rather than defining a discrete functional subset. Trajectory inference analysis supports this, because Blimp-1-deficient ILC2s had an alternative fate rather than control ILC2s. Blimp-1 likely acts in concert with additional transcription factors and is reported to interact with several transcription factors including IRF4, cMaf, Batf and Bach2. Future studies that explore the gene-regulatory network of ILC2 cells and Blimp-1's contribution will provide a more complete understanding of ILC2 effector biology.

Clinically, allergic asthma is associated with type 2 inflammation; however, a subset of patients lacks canonical type 2 mediators and instead present with severe, corticosteroid-refractory disease^{46–49}. Severe asthma is characterized by mixed inflammatory phenotypes, including elevated type 1 cytokines and, in some cases, increased T_H17 cells and neutrophilic inflammation. In parallel, mast cells are well-established contributors to airway pathology in allergic asthma. The inflammatory profile observed in ILC2-specific, Blimp-1-deficient mice, including increased type 1 cytokines, enhanced IL-9 and mast cell recruitment, recapitulates some features of this heterogeneous, mixed inflammatory state. This uncoupling of discrete type 2 effector programs provides a unique experimental framework to dissect how individual cytokine axes independently contribute to airway inflammation, mucus production and airway hyperresponsiveness in response to allergen exposure. We propose that ILC2-specific, Blimp-1-deficient mice represent a tractable model of mixed inflammation that may inform therapeutic strategies for severe asthma by enabling targeted interrogation of type 1–type 2 cytokine crosstalk and identification of inflammatory pathways that persist despite suppression of canonical type 2 responses.

In summary, our findings establish Blimp-1 as a key regulator of ILC2 transcriptional identity during allergic lung inflammation, acting to suppress type 1 inflammatory programs and stabilize the ILC2 state. The mixed inflammatory environment unmasked by Blimp-1 loss provides a useful experimental context to dissect how distinct immune pathways contribute to airway pathology. Leveraging this model may help clarify the relative roles of mast cell-driven and eosinophil-driven inflammation and inform strategies to more precisely target heterogeneous inflammatory mechanisms in asthma.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41590-026-02509-3>.

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Methods

Mice

C57BL/6J (000664), B6.Cg-Tg(Prdm1-EYFP)1Mnz/J (008828), B6.129-Prdm1^{tm1Clme}/J (008100), Il1rl1^{tm1Anjm} and Il7r^{tm1L1}(icre)Hrr mice were either purchased from Jackson Laboratories or maintained separately. Il7r^{tm1L1}(icre)Hrr mice were obtained from Hans-Reimer Rodewald⁵⁰. Il1rl1^{tm1Anjm} mice were obtained from Heth Turnquist (University of Pittsburgh). C57BL/6-Tg(Nmur1-iCre,-eGFP)1Dart/J (038197) mice were obtained from David Artis (Weill Cornell University), now available from Jackson Laboratories⁵¹. Blimp-1^{Nmur1Cre} mice were generated by crossing with B6.129-Prdm1^{tm1Clme}/J (008100). Mice were housed in specific pathogen-free enclosures at the John G. Rangos Sr. Research Center of University of Pittsburgh Medical Center (UPMC) Children's Hospital of Pittsburgh or the Assembly Building under the following conditions: 12 h light–dark cycle, temperature of 19–23 °C and humidity of 40–60%. All experiments were approved by the University of Pittsburgh Animal Care Committee and the American Veterinary Medical Association. All mice were aged >6 weeks and either matched male or matched female mice were used for each experiment. All in vivo experiments were performed independently at least 2–3×, with at least three mice per group for one independent experiment. Randomization and blinding were not used. The sample size was determined by power analysis and prior experience. No datapoints were excluded from the analyses.

Airway inflammation models

A total of 25 µg of lipopolysaccharide-low HDM (Stallergenes Greer) in phosphate-buffered saline (PBS) was given i.n. to mice anesthetized with isoflurane daily for up to 10 d (priming). To induce allergic lung inflammation, mice were immunized i.n. with HDM for 10 d and rechallenged twice for 2–3 d. For papain, 25 µg or 40 µg of papain (Sigma-Aldrich) was used daily for 3 d. For acute *Alternaria* sp., 5 µg of *Alternaria* sp. (Stallergenes Greer) was diluted in 100 µl of PBS and given i.n. daily for 4 d. For chronic *Alternaria* sp., 5 µg was diluted in PBS and given i.n. twice a week (Monday and Friday) for 3 weeks consecutively. For cytokine intranasal stimulation, recombinant IL-33 (BioLegend) and IL-25 (BioLegend) or IL-9 (PeproTech) was used. Of each cytokine, 0.5 µg was diluted in 25 µl of sterile PBS and given i.n. to isoflurane-anesthetized mice daily for 3 d. For FTY720 (Enzo life science, cat. no. BML-SL233-0025), the mice treated with HDM were given 25 µg of FTY720 every other day for 10 d (i.p.). Lungs were harvested for evaluation 1 d after treatment.

Skin model

For sensitization, 0.5 µg of recombinant IL-33 and IL-25 in 100 µl of sterile PBS was subcutaneously injected to shaved flank skin of mice every day for 3 d. Control mice received 100 µl of sterile PBS. Draining lymph nodes (LNs) were isolated on day 4 for evaluation.

Small intestinal lamina propria model

Recombinant IL-33 and IL-25, 0.5 µg, in 100 µl of sterile PBS was injected i.p. to mice every day for 3 d. Control mice received 100 µl of sterile PBS. Mesenteric LNs (mLNs) were isolated on day 4 for evaluation.

Inflammatory ILC2 induction

To induce iILC2s in the lung, 0.5 µg of recombinant IL-25 in 100 µl of sterile PBS was injected i.p. into mice every day for 3 d. Control mice received 100 µl of sterile PBS. Lungs were harvested for evaluation on day 4.

Helminth infection

Infective L3 larvae, 300–500, from *Nippostrongylus brasiliensis* were injected subcutaneously as previously described⁵². Lung cells were harvested on day 5 after infection for flow cytometric analysis.

Tissue processing

Lung. Mice were anesthetized and sacrificed with an injection i.p. of ketamine or xylazine. To collect BAL fluid, a catheter was inserted into the trachea and 0.9 ml of PBS was instilled and retracted. BAL was later centrifuged at 2,000 rpm and 4 °C for 10 min, the cell pellet resuspended with 200 µl of PBS and counted with automated cell counters (Nexcelom Bioscience LLC). Around 4×10^5 BAL cells were stained for flow cytometry. The lung was perfused with PBS from the right ventricle through the pulmonary artery. In some experiments, the left lung was tied and removed for flow cytometry. The right lung was inflated and submerged with Safefix II (Thermo Fisher Scientific) for histology or directly digested for flow cytometry. For sorting experiments, the entire lung was used. For single-cell suspensions cells, lung tissue was digested with 5 ml of 0.7 mg ml⁻¹ of collagenase A (Sigma-Aldrich) and 0.03 mg ml⁻¹ of DNase I (Sigma-Aldrich) in Roswell Park Memorial Institute (RPMI) medium using a GentleMACS Octo dissociator with heaters and the lung tissue program from Miltenyi. A solution of 2% fetal bovine serum (FBS), 2 mM EDTA PBS buffer was used to stop digestion. Red blood cells were lysed with ammonium–chloride–potassium (ACK) lysing buffer (Thermo Fisher Scientific). Cells were filtered through a 70-µm filter (CELLTREAT).

Small intestinal lamina propria. To harvest siLP ILC2s, the small intestine was isolated, the associated mesenteric tissue removed and the contents emptied. The tissue was opened with scissors and Peyer's patches were removed for further digestion. Small intestinal tissue was rinsed with PBS and cut into pieces, followed by immersion in medium containing 3% FBS, 5 mM EDTA and 0.145 mg ml⁻¹ of dithiothreitol and incubated at 37 °C while stirring at 800 rpm for 20 min. After incubation, the tissue and medium were filtered, collected and shaken with buffer (2 mM EDTA in RPMI-1640) for 30 s, then strained again and repeated 3×. The tissue was collected and digested in digestion buffer with 0.5 mg ml⁻¹ of DNase I and 0.1 mg ml⁻¹ of Liberase TL (Roche) at 37 °C for 25 min while stirring. Then, 3% FBS medium was used to stop the digestion and the single-cell suspension was collected through a 70-µm filter. For in vivo gut ILC2 activation experiments, mLNs were isolated and processed for flow cytometry.

Skin. To isolate cells from flank skin, mice were shaved and the desired sample was cut and spread with the inner side up on a 10-cm cell-culture plate. The blunt end of the forceps was used to remove the fat and cut the tissue into small pieces. Tissue was digested with digestion buffer (collagenase XI (2.5 mg ml⁻¹, Sigma-Aldrich), hyaluronidase (0.25 mg ml⁻¹, Sigma-Aldrich) and DNase I (0.05 mg ml⁻¹) in RPMI-1640) at 37 °C while shaking at 300 rpm for 1 h. After digestion, a GentleMACS Octo dissociator (Miltenyi) was used to dissociate cells to a single-cell suspension and filtered through a 70-µm filter. For in vivo skin ILC2 activation experiments, draining LNs were isolated and processed for flow cytometry.

Spleen and LNs. Spleen and LNs were isolated in RPMI-1640 (BioWhittaker) and crushed through 40-µm filters. For the spleen, red blood cells were lysed with ACK lysing buffer.

Flow cytometry

Single-cell suspensions were collected as above. The cell number was determined using an automated cell counter (Nexcelom Bioscience LLC) and cells were resuspended to 1×10^7 cell ml⁻¹ and plated on to 96-well plates for staining. Cells were washed with PBS twice before staining. Cell pellets were resuspended with 40 µl of Hank's balanced salt solution containing 0.5% Fc block and an antibody cocktail for 30 min at 4 °C. Lineage antibodies included NK1.1, Ter119, CD11b, CD11c, Ly6G and CD45R/B220. For intracellular staining, cells were stimulated with 50 ng ml⁻¹ of phorbol myristate acetate, 1 µg ml⁻¹ of ionomycin, brefeldin A (GolgiPlug, BD) and monensin (eBioscience) at 37 °C for

2 h. The same concentrations of brefeldin A and monensin were used for unstimulated control. Cells were stained with antibodies for surface markers and live or dead, fixed at 4 °C with BD Cytofix or Cytoperm (BD Bioscience) and stained for cytokines and transcriptional factors with BD Perm or Wash buffer (BD Bioscience). For YFP staining on fixed samples, anti-GFP-488 was used to enhance brightness. A BD LSRFortessa flow cytometer was used for flow analysis and data were further analyzed with FlowJo (v10.8.1 and v10.10.0).

FACS

Cells were resuspended with 2% FBS and 2 mM EDTA PBS buffer (EasySep buffer) and lineage-negative cells were enriched using EasySep Mouse Hematopoietic Progenitor Cell Isolation Kit (STEMCELL Technologies) with the recommended protocol. Cells were then stained for surface markers and ILC2s (live, lineage negative, CD90.2⁺, CD3⁻) were sorted on a Sony MA900 Cell Sorter. Purity was enhanced by double sorting. Gating pre-sort and post-sort is found in Supplementary Fig. 2.

In vitro ILC2 culture and stimulation

ILC2 cells were isolated by FACS from naive or treated mouse lungs as described above. Cells, 15,000, were plated per well in a 96-well plate and cultured with cRPMI (RPMI-1640 containing 10% FBS 1% penicillin–streptomycin, 2 mM L-glutamate, 50 mM Hepes, pH 7.2–7.4) plus 20 ng ml⁻¹ of recombinant mouse IL-2. Additional cytokines were added at the same concentration for further stimulation. Cells were collected after the indicated days of culture for analysis by flow cytometry.

RNA-seq and analysis

For bulk RNA-seq, ILC2s were flow sorted ex vivo from lungs and 15,000 ILC2s from each sample were cultured in complete RPMI medium with or without IL-33 for 24 h or IL-33 + IL-25 + IL-2 for 72 h. RNA was isolated with RNeasy Plus Micro Kit (QIAGEN, cat. no. 74034). Complementary DNA was prepared using the SMART-seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech Laboratories). Sequencing libraries were prepared using the Nextera XT DNA Library Preparation kit (Illumina). Cluster generation and 75-bp paired-end, dual-indexed sequencing was performed on an Illumina NextSeq 2000. FastQC was used to perform a quality assessment on all fastq files. The mouse reference genome (GRCm38) was downloaded from Ensembl. Adapters were trimmed using cutadapt v1.18 and reads were aligned using HISAT2 v2.1.0. Raw count values were generated using Subread and gene expression values were normalized using TPM. DESeq2 was applied to raw count transcriptome data in a pairwise manner to determine differentially expressed genes. These gene lists were compiled to make a master list of all differentially expressed genes. The log₂(transformed normalized counts) were then displayed as a heatmap to display expression of differentially expressed genes. Metascape⁵³ and GSEA⁵⁴ were used to identify pathways associated with gene sets.

ATAC-seq and analysis

For ATAC-seq, ILC2s were flow sorted and 15,000 cells cultured in complete RPMI complete with IL-33 + IL-25 + IL-2 for 72 h. ILC2s were collected for the OMNIATAC-seq protocol⁵⁵. Sequencing libraries were prepared using the Nextera XT DNA Library Preparation kit (Illumina). Cluster generation and 101-bp paired-end, dual-indexed sequencing was performed on an Illumina NextSeq2000. FastQC was used to perform a quality assessment on all fastq files. The mouse reference genome (GRCm38) was downloaded from Ensembl. Adapters were trimmed using cutadapt v1.18. Raw count values were generated using Subread and reads were aligned to the reference genome using Bowtie2 v2.3.4.2. Duplicates were removed using Picard and regions from the ENCODE Blacklist were removed using bedtools intersect. Peaks (narrow) were called using MACS2 v2.1.1 with a *P*-value cutoff of 0.05. The irreproducible discovery rate used a threshold of 0.05 to identify reproducible peaks. Irreproducible discovery rate peak

files of all samples were merged to create a master list of peaks that, together with alignment files, were used to generate tag counts for each replicate (bedtools coverage). DESeq2 was applied to raw tag count data for called peaks in a pairwise manner to determine differential peaks. These peak lists were compiled to make a master list of all differential peaks for each mark. Peaks were annotated to the nearest gene using ChIPpeakAnno⁵⁶. Heatmaps were generated using deepTools2 (v3.3.0)⁵⁷. Bam files were combined across sample replicates. Samples were normalized by bins per million (BPM) and converted to bigwig files. Heatmaps were peak centered in a 2,000-bp region by computing matrix from normalized and merged bigwig files for all peaks of each mark (computeMatrix reference point –referencePoint center -a 1000 -b 1000) and visualized (plotHeatmap). Genome browser tracks are visualized from bigwig files using the integrative genomics viewer⁵⁸.

scRNA-seq and analysis

Sample preparation and sequencing. Mice were treated with PBS, papain or *Alternaria* sp. i.n. as described above. ILC2s were flow sorted from lungs as described above and 5,000 ILC2s from each sample were loaded to GEM-X OCM Chip (10x Genomics). RNA barcoding, on-chip multiplexing and library generation were performed with GEM-X Universal 3' Gene Expression v4 4-plex kit (10x Genomics) according to the protocol, where three pooled libraries were generated for sequencing on an Illumina NovaseqXPlus paired-end 150 bp with approximately 250 million reads per sample.

Alignment and pre-processing. Raw sequencing data were processed using the Cell Ranger multi pipeline (v9.0.1; 10x Genomics) for alignment, filtering and quantification. Reads were aligned to the mouse reference genome (GRCm39). Demultiplexing of pooled samples was performed based on assignment to specific oligonucleotide tags. This resulted in filtered unique molecular identifier, feature-barcode matrices for each individual sample, which were used for downstream analysis.

Quality control and dimensionality reduction. Downstream analysis was performed using the Scanpy toolkit (v1.10.3)⁵⁹. After importing raw gene expression matrices, cells expressing <200 genes or exhibiting high mitochondrial content (>20%) were excluded. To remove potential multiplets, cells with abnormally high gene counts (>6,000) were filtered out. Doublet detection was performed using Scrublet⁶⁰. Detected doublets were removed with a high-confidence doublet score threshold of 0.3. After quality control, raw counts were normalized to the median total counts per cell and log(transformed). The top 2,000 highly variable genes were identified and used for principal component analysis. Dimensionality reduction was performed using UMAP based on the nearest neighbor graph and cells were clustered using the Leiden algorithm⁶¹. Non-ILC2 contamination was identified via canonical marker expression (for example, Epcam for epithelial cells, Col1a1 for fibroblasts and Cd79a for B cells) and excluded from downstream analysis.

Trajectory analysis. After excluding low-quality cells and contamination, 18,414 ILC2 cells were identified from the scRNA-seq dataset. These cells underwent standard normalization, log(transformation), principal component analysis and UMAP processing as described above. ILC2 subsets (corresponding to those in Fig. 7a) were identified using Leiden clustering with a resolution of 0.7. Differentiation trajectories were modeled using Palantir (v1.4.1)⁶². A starting cell with a representative transcriptomic profile was selected from cluster 1, which contained most of the untreated naive ILC2s. Pseudotime values were calculated to reconstruct the developmental timeline. To verify the robustness of the inferred trajectory, DPT³⁴ analysis was independently performed using Scanpy (v1.10.3). Both analyses were conducted on the same combined dataset (including both wild-type and knockout

conditions) and rooted in the same starting cluster (cluster 1) to ensure a unified and directly comparable pseudotime axis across conditions.

***Ifng* response module score.** An *Ifng* response module (L7) of 309 genes identified in the lung after infection was downloaded from ref. 35. The top 50 most highly expressed genes in our ILC2 dataset plus *Cxcl10* and *Cxcl9* was used to compute the module score with function score_genes from scanpy.

Luminex assay

ILC2s were sorted as above and stimulated with IL-33 and IL-25 for 3 d. Cells were stimulated with 50 ng ml⁻¹ of phorbol myristate acetate and 1 µg ml⁻¹ of ionomycin for 2 h and supernatants collected. Bio-Plex Pro Mouse Cytokine 23-plex Assay Kit (BioRad, cat. no. M60009RDPD) was performed according to the manufacturer's protocol and data were collected by Bio-Plex MAGPIX Multiplex Reader.

Histology

Lung tissue was fixed with Safefix II (Thermo Fisher Scientific) and embedded with paraffin. H&E and PAS were used to stain the slides at Pitt Biospecimen Core at UPMC Shadyside or at the Rangos Histology Core at UPMC Children's Hospital of Pittsburgh. Photos of slides were taken using an Olympus IX83 microscope or Leica ImageScopex64 slide scanner. Quantification was performed using QuPath⁶³, an open-source software used to analyze whole-image slides. QuPath has built-in algorithms to detect cells and tissues, which can be classified using machine learning to identify objects from training datasets. Both control and treated groups were used to train QuPath to identify healthy parenchyma, immune cell infiltration, airways and blood vessels. For PAS, QuPath tools for color deconvolution were used to isolate magenta PAS signal from other stains. Lung sections were manually traced in software and cell detection was applied to identify nucleated cells. The trained ML algorithm was then applied to all sections to assign and quantify nucleated cells per region type or intensity and area for PAS-positive structures. These values were used to identify changes in the number of cells per area or percentage of tissue with mucus in control versus experimental lung sections.

Analysis of pSTAT3, pSTAT4, pSTAT5 and pSTAT6 expression

For pSTAT3, pSTAT4, pSTAT5 and pSTAT6 detection in vitro, the cells were fixed using BD Phosflow Lyse/fix buffer (BD Biosciences, cat. no. 558049), followed by permeabilization with BD Perm buffer III (BD Biosciences, cat. no. 558050) and stained with phosphoantibodies. Naive T cells stimulated with cytokines for 30 min were used as positive controls.

In vivo blockade

Mice were injected i.p. with 0.2 mg of anti-IL9 monoclonal antibody (BioXCell, cat. no. BE0181-9C1) or anti-IFNγ (BioXCell, cat. no. BE0055) and anti-TNF (BioXCell, cat. no. BE0058) on days 1 and 3 during IL-33 and IL-25 immunization i.n. The same amount of IgG1 isotype control antibody (BioXCell, cat. no. BE0083-MOPC-21 or BE0088) was injected i.p. into control mice.

In vitro JAK inhibitor treatment

Sorted ILC2s from Blimp-1 YFP mice were cultured in cRPMI with 20 ng ml⁻¹ of recombinant mouse IL-33 with or without ruxolitinib (5 µM) (medchemexpress, cat. no. HY-50856).

Cell mixing and transwell co-culture

Sorted ILC2s from Il1rl1^{tm1Anjm} mice were mixed with ILC2s from control mice in a 1:1 ratio and plated in a 96-well plate. ILC2s were treated with 20 ng ml⁻¹ of recombinant mouse IL-33 in cRPMI for 3 d. Cells were collected from the plate for flow analysis on day 4. ST2 surface staining was used to distinguish the genetic source of cells. For transwell co-culture,

a 96-well plate with a 0.4-µm polycarbonate membrane insert (Corning, cat. no. 3391) was used. Sorted ILC2s from Il1rl1^{tm1Anjm} mice were plated in the bottom wells (receiver plate) and an equal number of sorted control ILC2s were plated in the upper wells (insert plate). ILC2s were treated with 20 ng ml⁻¹ of recombinant mouse IL-33 in cRPMI for 3 d. Cells were collected for flow analysis on day 4.

ELISA

BAL samples were centrifuged at 2,000 rpm and 4 °C for 10 min. The cell pellet was processed for flow analysis and the supernatant was collected for ELISA. Cell-culture supernatants were generated by stimulating sorted ILC2s with IL-33 and IL-25 for 3 d. Cells were stimulated with 50 ng ml⁻¹ of phorbol myristate acetate and 1 µg ml⁻¹ of ionomycin for 2 h and supernatants collected. Sandwich ELISA kits (Thermo Fisher Scientific) were used for TNF, IFNγ and IL-9 ELISAs following the corresponding protocols. The samples underwent a 1:2 dilution before loading on to the plates and technical duplicates were performed for each biological replicate. Samples were read using an Epoch Microplate Spectrophotometer.

BM chimera

BM cells were isolated from the tibias and femurs of mice and T cells were depleted using Thy1.2-positive selection and magnetic separation kit (BioLegend). Cells were counted and mixed in a ratio of 1:1 and injected intravenously into lethally irradiated hosts (900 rad, single dose, cesium irradiator). The total cells, 20 × 10⁶, were injected per host mouse. Then, 2 weeks after injection, mice were bled to check reconstitution and immunized with *Alternaria* sp. i.n. at 6 weeks post-injection as described above.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

RNA-seq, ATAC-seq and scRNA-seq described in the paper are available at the National Center for Biotechnology Information's Gene Expression Omnibus: SuperSeries (accession no. GSE328307) RNA-seq (accession no. [GSE291231](#)), ATAC-seq (accession no. [GSE291230](#)) and scRNA-seq (accession no. [GSE316969](#)). Source data are provided with this paper.

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Acknowledgements

We are grateful to X. Si, T. Hand, T. Edwards, A. Liu and D. Kaplan for experimental advice on cell isolation from skin and siLP tissue. We thank R. Lee Reinhardt for advice on detection of iILC2s. We thank H. R. Rodewald, D. Artis and H. Turnquist for IL7R α -Cre, NMur1Cre and ST2-deficient strains, respectively. We thank T. Hand and all members of A.C.P.'s lab for critical reading of the manuscript, technical advice and experimental input. We thank the UPMC Children's Hospital of Pittsburgh Flow Cytometry Core, the Unified Flow Core, the Hillman Cancer Center Flow Core, the Health Sciences Sequencing Core, the Hillman Cancer Center Histology Core and the Rangos Histology Core. This research was supported in part by the University of Pittsburgh Center for Research Computing through the resources provided. Specifically, this work used the HTC cluster, which is supported by the National Institutes of Health (NIH, award no. S10OD028483). This work was supported by the RK Mellon Foundation Pilot Award (to A.C.P.), American Lung Association Innovation Award (to A.C.P.), the US NIH (grant nos. AI153104 and AI156093 to A.C.P.) and Tsinghua University School of Medicine Education Foundation (to Y.Z. and J.Z.).

Author contributions

Y.Z. designed and performed experiments, analyzed the data and wrote the paper. S.G. designed and performed experiments for revisions. J.Z., S.L.K., H.B. A.P., A.D.M., A.C., K.H. and N.B. designed and performed experiments and analyzed the data. Y.C. and A.Y. performed bioinformatics analysis. J.S.T., Y.B., A.R.C. and A.R. provided crucial experimental and technical support. R.A.G and D.M.S. provided crucial reagents and scientific insights. A.C.P. conceived the project, designed experiments, analyzed the data and co-wrote the paper, provided overall direction and acquired funding.

Competing interests

D.M.S. consults for Sobi and received grants from Sobi and Eli Lilly. The other authors declare no competing interests.

Additional information

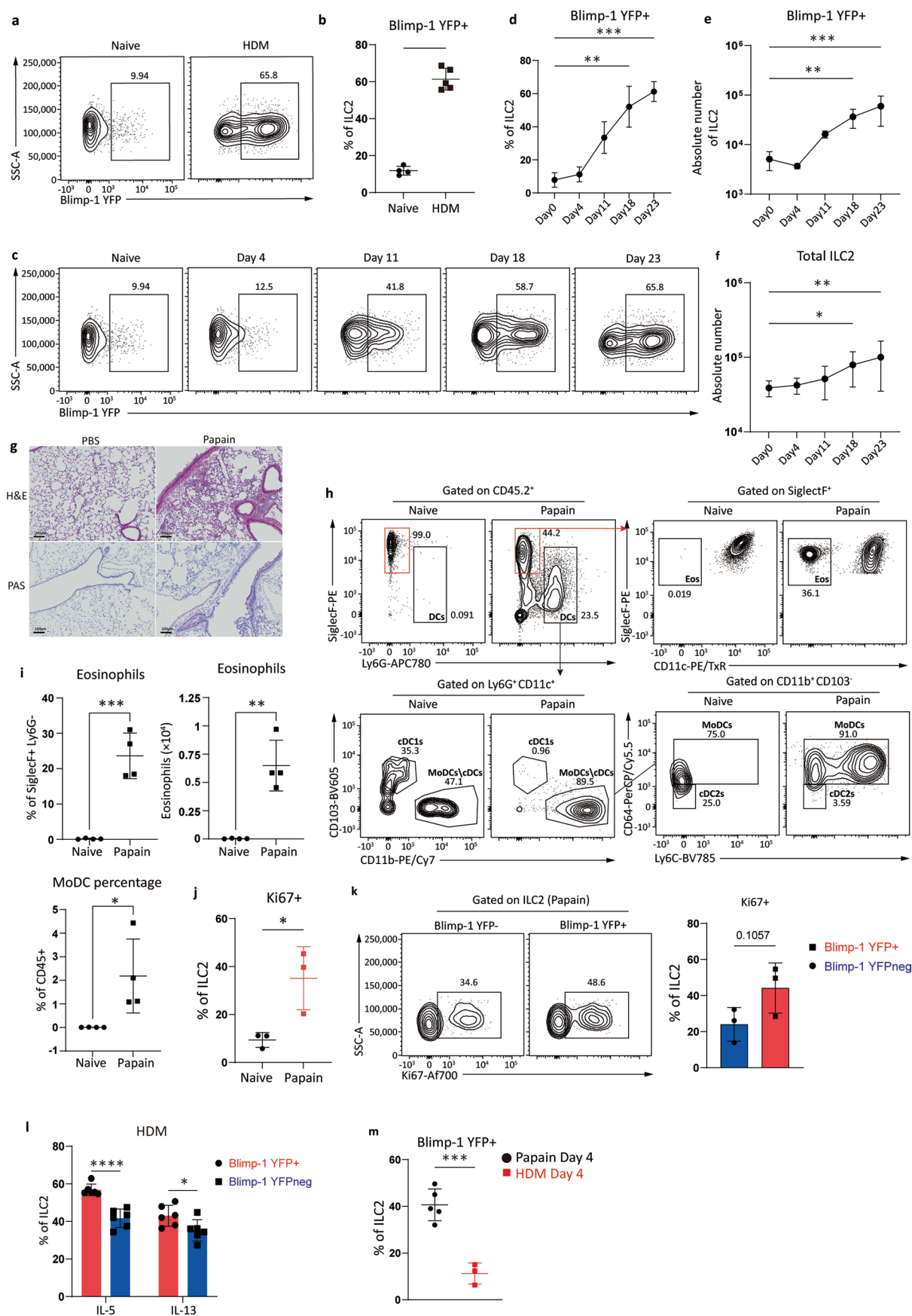
Extended data is available for this paper at <https://doi.org/10.1038/s41590-026-02509-3>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41590-026-02509-3>.

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Peer review information *Nature Immunology* thanks Roma Sehmi and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: L. A. Dempsey in collaboration with the *Nature Immunology* team.

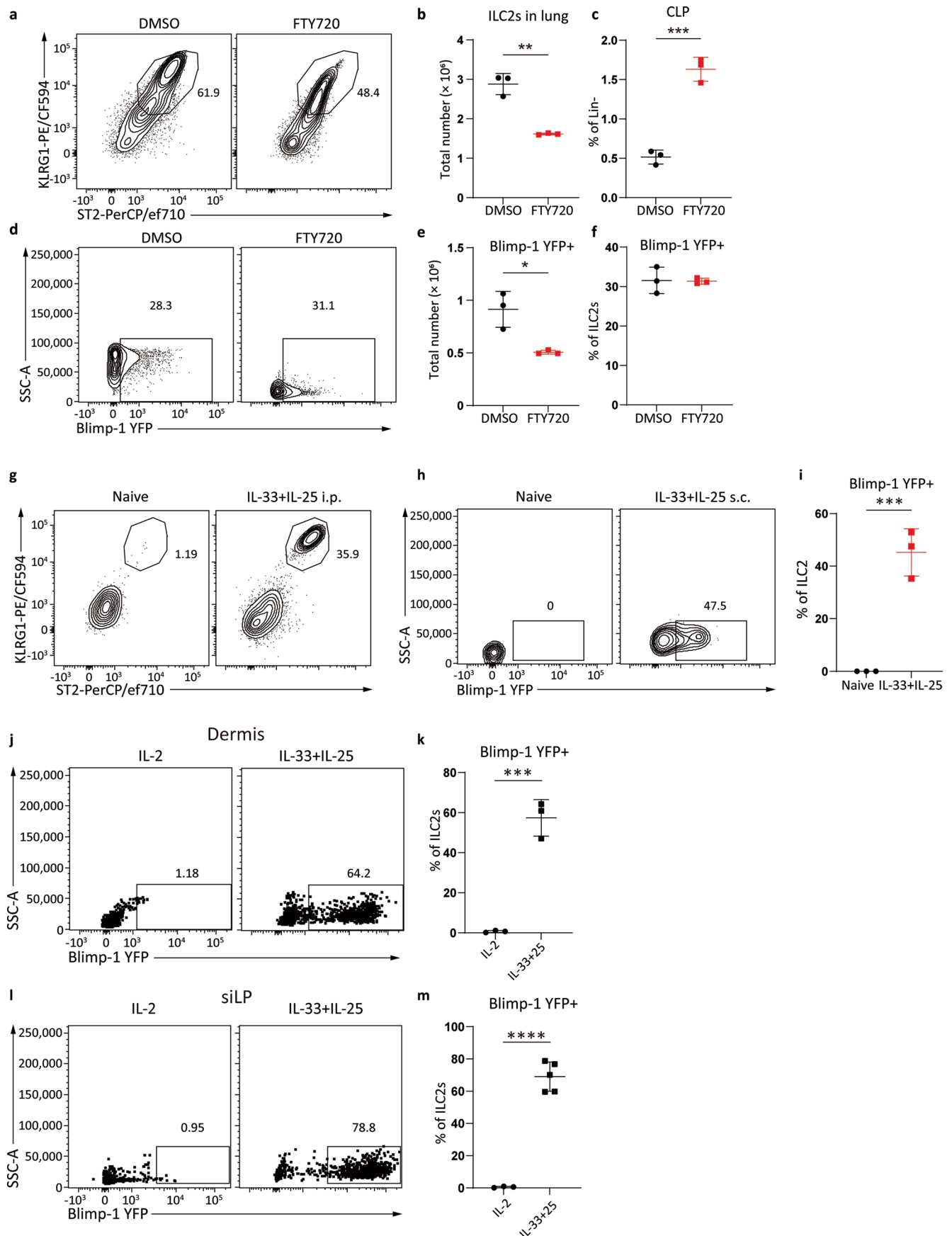
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Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Allergen specific activation of Blimp-1 in ILC2 cells in the lung. **a**, Percentage of Blimp-1 YFP in ILC2s after HDM. **b**, Quantification of **(a)** ($n = 5$) **c**, Percentage of Blimp-1 YFP in ILC2s at indicated timepoints by HDM model. ($n = 3$ –5 mice per group) **d–f**, Percentage **(d)**, absolute number **(e)** of Blimp-1 YFP in ILC2s, or absolute number of ILC2s **(f)** at indicated timepoints. ($n = 4$ –6 mice per group) **g**, Representative H&E and PAS staining of lungs from indicated groups. Lung samples were fixed for histology ($n = 5$) **h**, Gating strategy for eosinophils, cDC1s, cDC2s and MoDCs. **i**, Quantification of eosinophil percentage, eosinophil number and MoDC number in BAL after papain. ($n = 4$) **j**, Quantification of Ki67 + ILC2s after papain. ($n = 3$) **k**, Percentage of Ki67 expression in Blimp-1 YFP + ILC2 or Blimp-1 YFP- ILC2. **l**, Percentage of IL-5 and IL-13 expression in Blimp-1 YFP + ILC2 or Blimp-1 YFP- ILC2 after HDM. ($n = 6$)

m, Quantification of Blimp-1 YFP percentage after papain or HDM. ($n = 3$ –5 mice per group) Each point represents one individual sample. The data are shown as means $\pm$ s.d., and present two or three independent experiments. A two-tailed unpaired *t* test was performed for **b**, **i**, **j**, **k** and **m**. Šidák's multiple comparisons test was performed for **l**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The specific *P* values are as follows for **b**, $P < 0.0001$; for **d**, day0 vs. day18, $P = 0.0016$, day0 vs. day23, $P = 0.0001$; for **e**, day0 vs. day18, $P = 0.0054$, day0 vs. day23, $P = 0.001$; for **f**, day0 vs. day18, $P = 0.0385$, day0 vs. day23, $P = 0.0084$; for **i**: comparison between eosinophil percentage, $P = 0.0003$; comparison between total eosinophils, $P = 0.0012$; for **E**, $P = 0.0323$; for **j**, $P = 0.0299$; for **k**, $P = 0.1057$; for **l**, comparison between IL-5, $P < 0.0001$, comparison between IL-13, $P = 0.0255$; for **m**, $P = 0.0006$.



Extended Data Fig. 2 | See next page for caption.

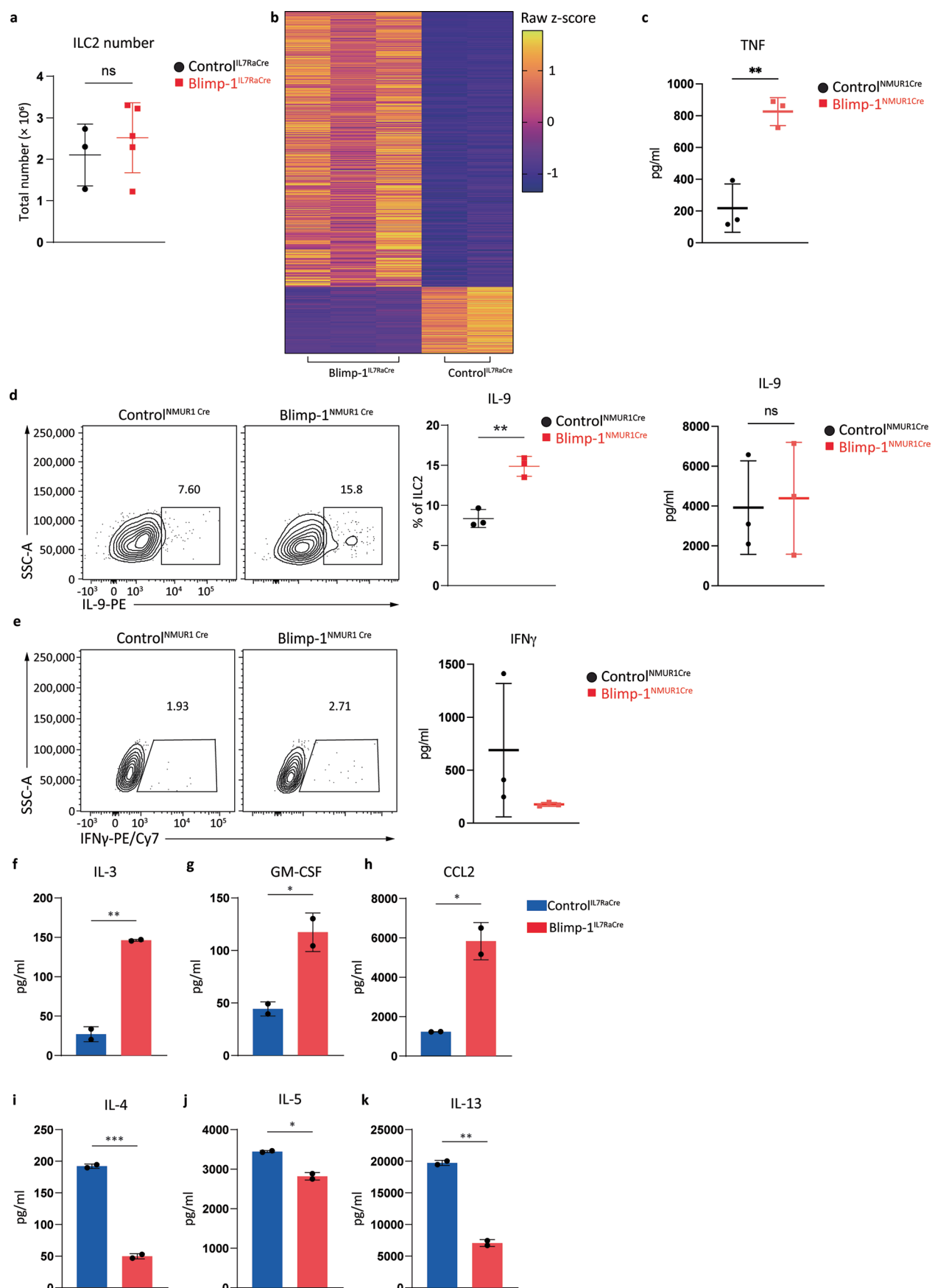
Extended Data Fig. 2 | Blimp-1 expression in ILC2s is a general feature across ILC2 subsets and tissue sites. **a** and **d**, Percentage of ILC2s in the lung (**a**) and Blimp-1 YFP in ILC2s (**d**) after FTY720 treatment. **b–c** and **e–f**, Quantification of CLP in bone marrow (**c**), ILC2s number in lung (**b**), Blimp-1 YFP+ ILC2s number (**e**) and percentage of Blimp-1 YFP+ ILC2s (**f**). ($n = 3$) **g**, Percentage of ILC2s from mesenteric lymph nodes with IL-33 + IL-25 i.p. **h**, Percentage of Blimp-1 YFP in ILC2s from skin draining lymph nodes after IL-33 + IL-25 s.c. **i**, Quantification of (**h**). ($n = 3$) **j**, Percentage of Blimp-1 YFP in dermis ILC2s after IL-33 + IL-25.

k, Quantification of (**j**). ($n = 3$) **l**, Percentage of Blimp-1 YFP in siLP ILC2s after IL-33 + IL-25. **m**, Quantification of (**l**). ($n = 3–5$ mice per group) Each point represents one individual sample. The data are shown as means $\pm$ s.d., and present two or three independent experiments. A two-tailed unpaired *t* test was performed for **b**, **c**, **e**, **f**, **i**, **k** and **m**. * $P < 0.05$, * $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. The specific *P* values are as follows: for **b**, $P = 0.0012$; for **c**, $P = 0.0004$; for **e**, $P = 0.0149$; for **f**, $P = 0.9245$; for **i**, $P = 0.001$ for **k**, $P = 0.0004$; for **m**, $P < 0.0001$.



Extended Data Fig. 3 | IL-33 and IL-25 indirectly drives Blimp-1 expression in activated ILC2s. a-b, Percentage of IL-5+ (**a**) or IL-13+ (**b**) ILC2s at indicated timepoints when stimulated with IL-33. (n = 3) **c**, Histogram for Blimp-1 YFP in ILC2s after TSLP stimulation. **d**, Histogram for Blimp-1 YFP in ILC2s after NMU or IL-25 stimulation. **e-g**, Percentage of phosphorylated STAT5 stimulated with IL-33 (**e**) or TSLP (**f**), or percentage of phosphorylated STAT3 stimulated with IL-33 (**g**). (n = 6) **h**, Representative flow plots (left) and quantification (right) of Blimp-1 YFP in ILC2 cells treated with IL-9 +/- Ruxolitinib or DMSO as a control. (n = 6) Each

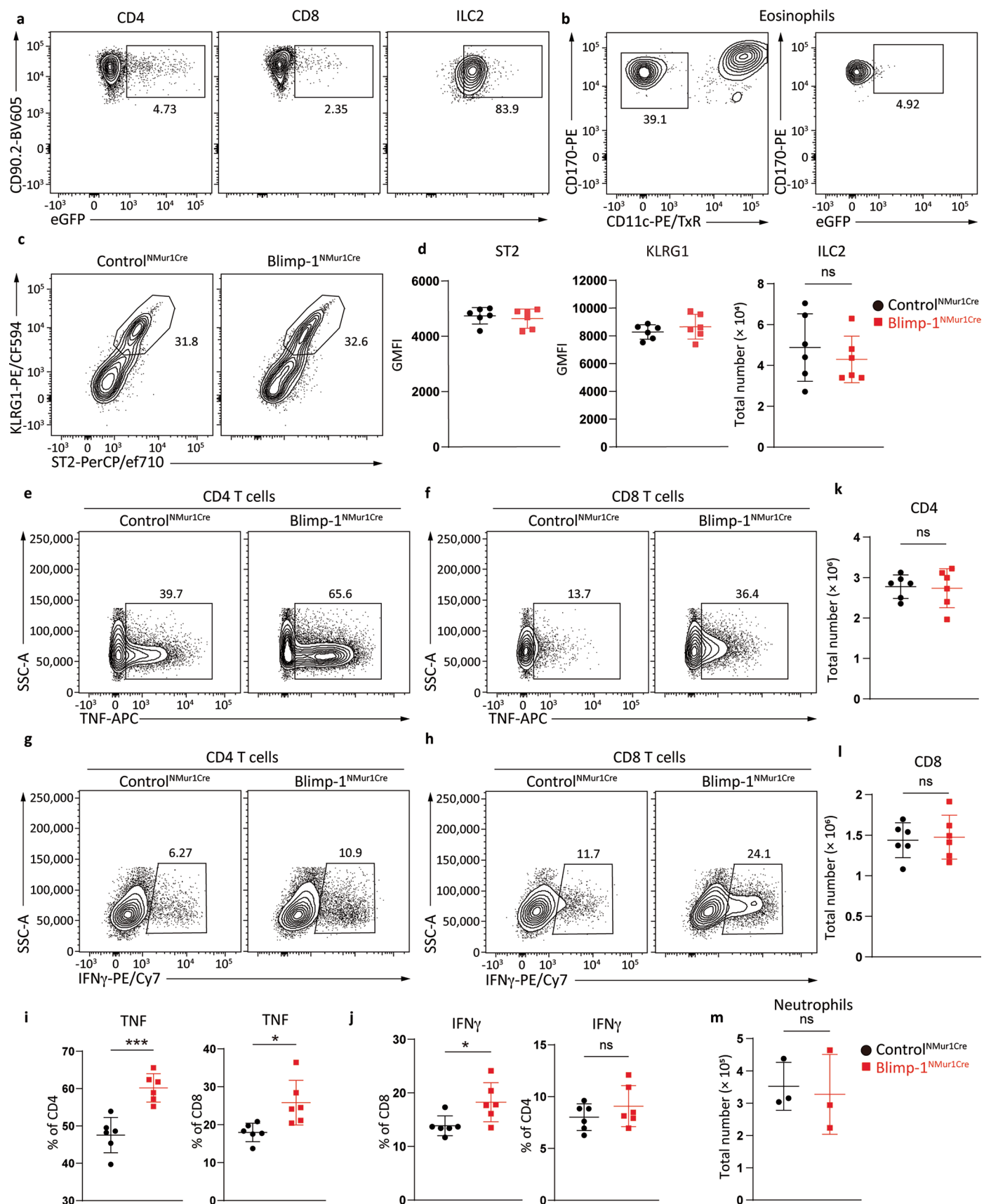
point represents one individual sample. The data are shown as means $\pm$ s.d., and present two or three independent experiments. Dunnett's multiple comparisons test was performed for **a** and **b**. A two-tailed unpaired t test was performed for **h**. The specific P values are as follows: for **a**, 0 h vs. 4 h, $P = 0.0001$, 0 h vs. 8 h, $P < 0.0001$, 0 h vs. 18 h, $P < 0.0001$, 0 h vs. 24 h, $P = 0.0001$; for **b**, 0 h vs. 4 h, $P = 0.0172$, 0 h vs. 8 h, $P < 0.0001$, 0 h vs. 18 h, $P < 0.0001$, 0 h vs. 24 h, $P < 0.0001$; for **h**, $P < 0.0001$.



Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Loss of Blimp-1 in ILC2s alters cytokines and transcriptomes after in vitro activation. **a**, Quantification of ILC2 cell number in the lung in control^{IL7RaCre} or Blimp-1^{IL7RaCre} at steady state. **b**, Heatmap of differentially expressed genes between control^{IL7RaCre} with Blimp-1^{IL7RaCre} ILC2s identified by RNAseq. Z-scores were calculated by TPM. **c**, quantification of TNF found in culture supernatants of stimulated ILC2s (ELISA). **d**, left, Representative flow plots of IL-9 in ILC2s from indicated mice; right, quantification of IL-9 found in culture supernatants of stimulated ILC2s (ELISA) (n = 3) **e**, left, Representative flow plots of IFN γ in ILC2s from indicated mice; right, quantification of IFN γ found in culture supernatants of stimulated ILC2s (ELISA). **f-k**, Quantification of

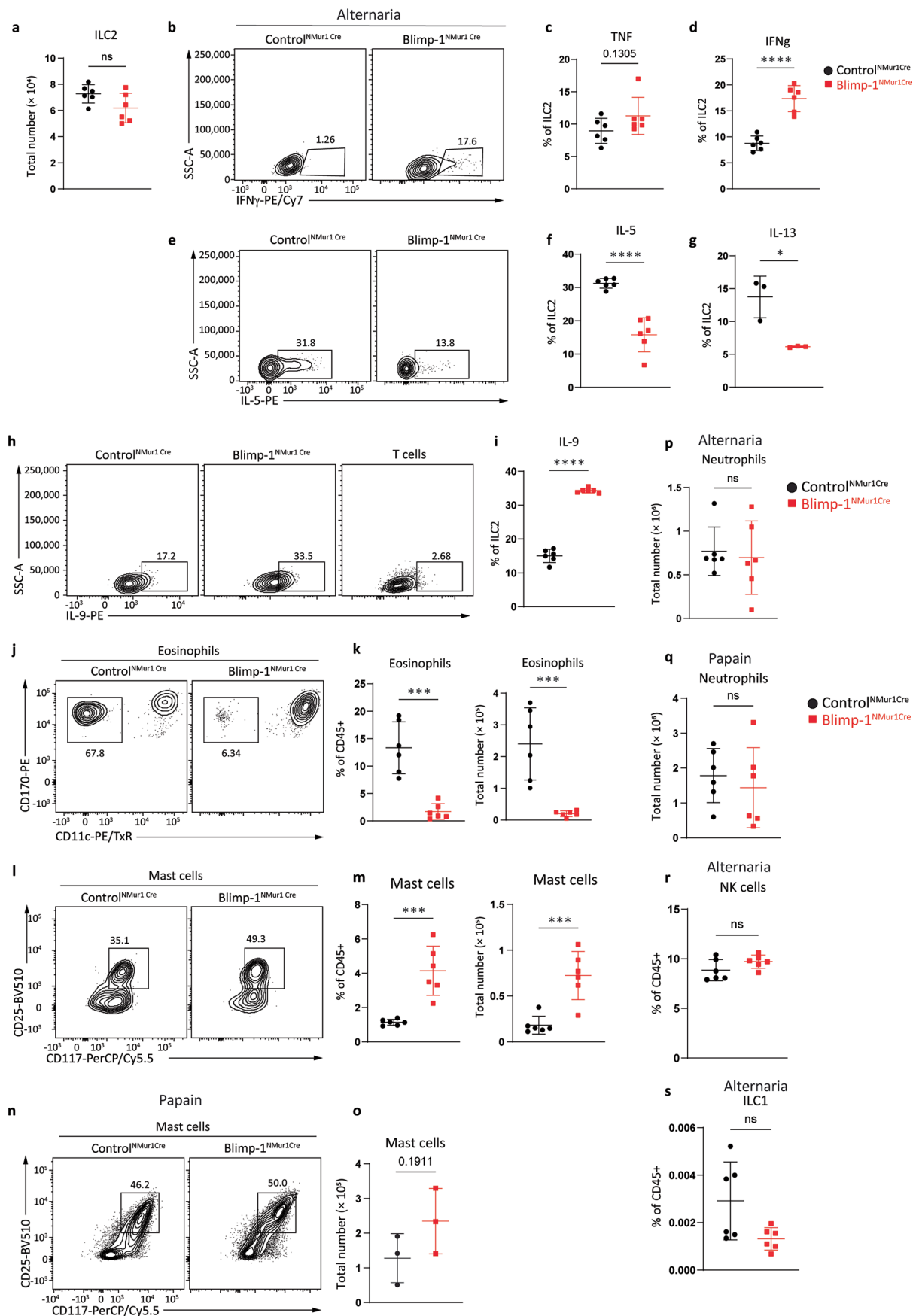
IL-3 (**f**), GM-CSF (**g**), CCL2 (**h**), IL-4 (**i**), IL-5 (**j**) and IL-13 (**k**) in culture supernatants of stimulated ILC2s isolated from indicated mice. (n = 2) Each point represents one individual sorting sample from 2 pooled animals. The data are shown as means $\pm$ s.d., and present two or three independent experiments. A two-tailed unpaired t-test was performed for **a**, **c**, **d**, **e**, **f**, **g**, **h**, **i**, **j** and **k**. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. The specific P values are as follows: for **a**, P = 0.9244; for **c**, P = 0.0039; for **d**, left, P = 0.0025, right, P = 0.8362; for **e**, P = 0.2323; for **f**, P = 0.0071; for **g**, P = 0.0423; for **h**, P = 0.0275; for **i**, P = 0.0038; for **j**, P = 0.0216; for **k**, P = 0.0038.



Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | Mixed inflammatory responses to papain and tissue alarmins in ILC2 specific Blimp-1-deficient mice. **a–b**, Representative flow plots of NMUR1Cre-eGFP⁺ cells in **(a)** CD4, CD8 and ILC2s or **(b)** eosinophils in the lung after IL-33 + IL-25 administration **c**, Representative flow plots of ILC2s from lung in indicated mice after IL-33 + IL-25. (n = 6) **d**, Quantification of ST2 and KLRG1 GMFI in ILC2s, or total ILC2 number. **e–h**, Representative flow plots of TNF + CD4 **(e)**, TNF + CD8 **(f)**, IFN γ + CD4 **(g)** and IFN γ + CD8 **(h)** in lungs of indicated mice after IL-33 + IL-25. (n = 6) **i**, Quantification of **e–f**. **j**, Quantification of **g–h**. **k–l**, Quantification of total CD4 **(k)** or CD8 **(l)** number after IL-33 + IL-25.

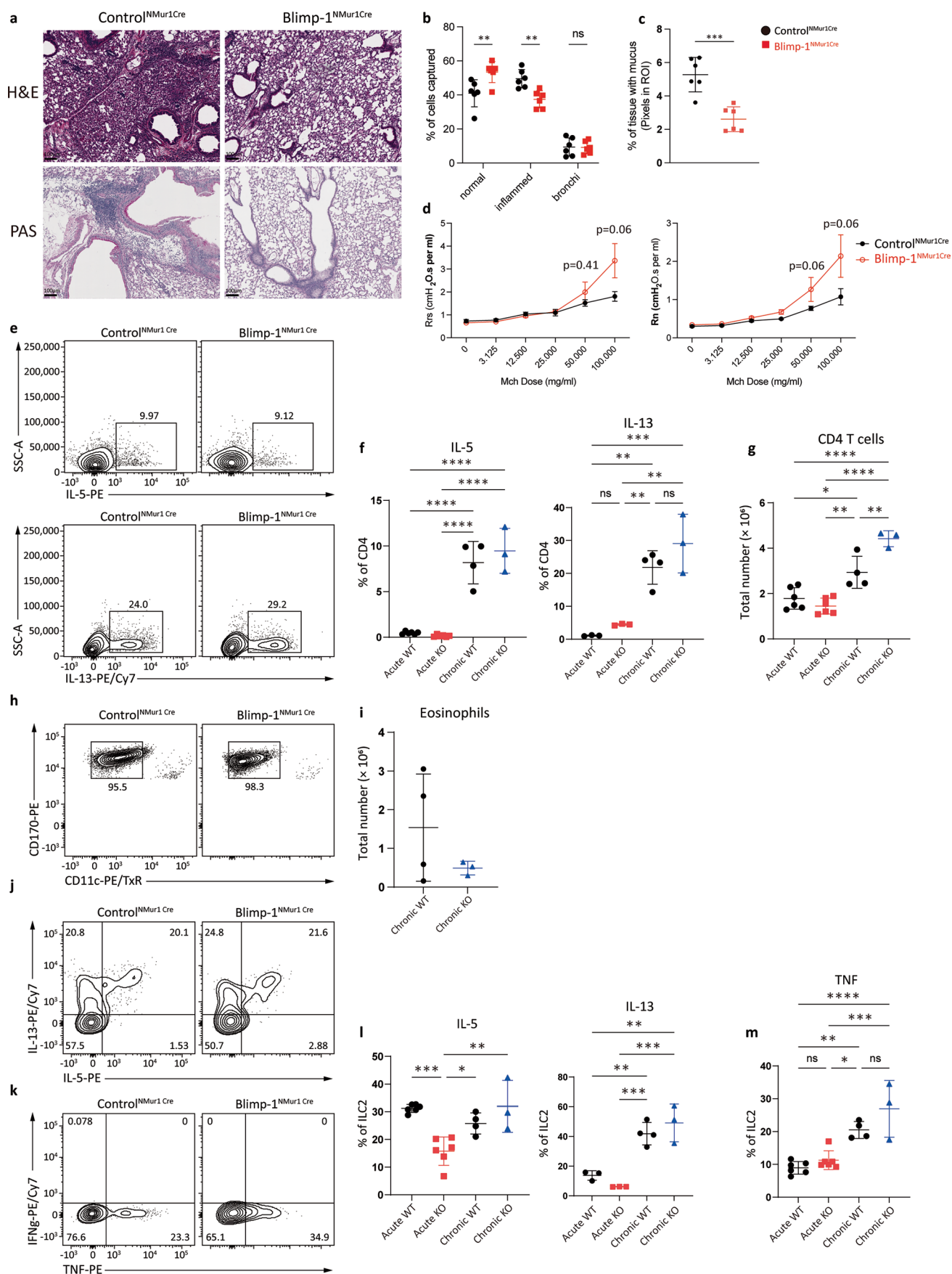
m, Quantification of neutrophils from indicated mice. Each point represents one individual animals. The data are shown as means $\pm$ s.d., and present two or three independent experiments. A two-tailed unpaired t-test was performed for **d**, **i**, **j**, **k**, **l** and **m**. *P < 0.05, *P < 0.01, ***P < 0.001, ****P < 0.0001. The specific P values are as follows: for **d**, comparison for ST2: P = 0.6754, comparison for KLRG1: P = 0.6154, comparison for ILC2 number: P = 0.4955; for **i**, CD4, P = 0.0005, CD8, P = 0.0131; for **j**, CD4 P = 0.2968, CD8, P = 0.0249; for **k**, P = 0.8678; for **l**, P = 0.8018; for **m**, P = 0.7788.



Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | Blimp-1 deficient mice produce more Type1 cytokines in response to the allergen *Alternaria alternata*. **a**, Quantification of total number of ILC2s after *Alternaria*. **b**, Representative flow plots of IFN γ in ILC2s in indicated mice after *Alternaria* i.n. (n = 6) **c-d**, Quantification of TNF (**c**) and IFN γ (**d**) in ILC2s. **e**, Representative flow plots of IL-5 in ILC2s in indicated mice after *Alternaria*. **f,g**, Quantification of IL-5 (**f**) and IL-13 (**g**) in ILC2s. **h**, Representative flow plots of IL-9 in ILC2s and T cells in indicated mice. **i**, Quantification of (**h**). **j**, Representative flow plots of eosinophils from indicated mice after *Alternaria*. **k**, Quantification of (**j**). **l** and **n**, Representative flow plots of mast cells from indicated mice after *Alternaria* (**l**) or papain (**n**). **m** and **o**, Quantification of (**l**) and

(**n**). **p-q**, Quantification of neutrophils from indicated mice after *Alternaria* (**p**) or papain (**q**). **r-s**, Quantification of NK cells (**r**) or ILC1s (**s**) from indicated mice after *Alternaria*. Each point represents one individual animals. The data are shown as means $\pm$ s.d., and present two or three independent experiments. A two-tailed unpaired t-test was performed for **a**, **c**, **d**, **f**, **g**, **i**, **k**, **m**, **o**, **p**, **q** and **r**. Mann Whitney test was performed for **s**. The specific P values are as follows for **a**: P = 0.0737; for **c**, P = 0.1305; for **d**, P < 0.0001; for **f**, P < 0.0001; for **g**, P = 0.0142; for **i**, P < 0.0001; for **k**, percentage, P = 0.0002, total number, P = 0.0008; for **m**, percentage, P = 0.0005, total number, P = 0.0008; for **o**, P = 0.1911; for **p**, P = 0.7307; for **q**, P = 0.5568; for **r**, P = 0.1241; for **s**, P = 0.1017.

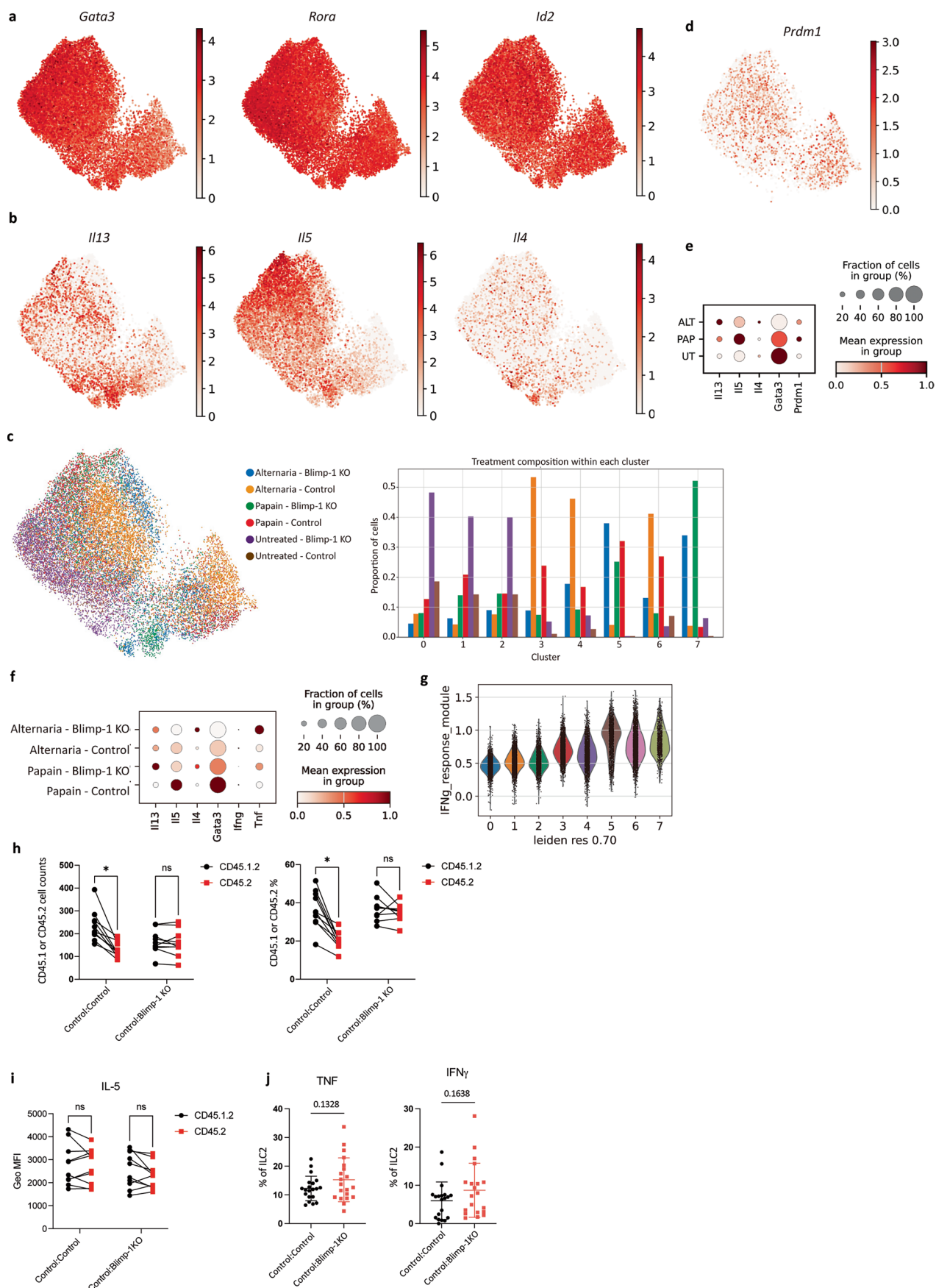


Extended Data Fig. 7 | See next page for caption.

Extended Data Fig. 7 | Blimp-1 is required to maintain the type 2

transcriptional identity of ILC2s. **a**, Representative H&E and PAS staining of control^{1^{NMUR1Cre}} or Blimp-1^{1^{NMUR1Cre}} animals after *Alternaria*. **b–c**, Quantification of inflammation (**b**) and mucus (**c**) from (**a**). ($n = 6$) **d**, Quantification of airway resistance to methacholine challenge after *Alternaria* sensitization. ($n = 4$ –5 mice per group) **e–f**, Representative flow plots (**e**) and quantification (**f**) of IL-5 and IL-13 expression in lung CD4 T cells after chronic *Alternaria*. **g**, Total number of CD4 T cells in lung. **h–i**, Representative flow plots (**h**) and quantification (**i**) of eosinophils in the BAL. **j** and **l**, Representative flow plots (**j**) and quantification (**l**) of IL-5 and IL-13 in lung ILC2s. **k** and **m**, Representative flow plots (**j**) and quantification (**l**) of TNF in lung ILC2s. The data are shown as means $\pm$ s.d., and present two or three independent experiments. A two-tailed unpaired t-test was performed for **c** and **i**. Šidák's multiple comparisons test was performed for **b**. Multiple Mann-Whitney test was performed for **d**. Tukey's multiple comparisons test was performed for **f**, **g**, **l**, and **m**. The specific P values are as follows for **b**,

normal, $P = 0.0187$, inflamed, $P = 0.2616$, bronchi, $P = 0.4843$; for **c**, $P = 0.0004$; for **d**, left, Mch dose 50 mg/ml, $P = 0.412698$, Mch dose 100 mg/ml, $P = 0.063492$, right, Mch dose 50 mg/ml, $P = 0.063492$, Mch dose 100 mg/ml, $P = 0.063492$; for **f**, IL-5, Acute WT vs. Chronic WT, $P < 0.0001$, Acute WT vs. Chronic KO, $P < 0.0001$, Acute KO vs. Chronic WT, $P < 0.0001$, Acute KO vs. Chronic KO, $P < 0.0001$; IL-13, Acute WT vs. Chronic WT, $P = 0.0023$, Acute WT vs. Chronic KO, $P = 0.0004$, Acute KO vs. Chronic WT, $P = 0.0073$, Acute KO vs. Chronic KO, $P = 0.0011$; for **g**, Acute WT vs. Chronic WT, $P = 0.0102$, Acute WT vs. Chronic KO, $P < 0.0001$, Acute KO vs. Chronic WT, $P = 0.0013$, Acute KO vs. Chronic KO, $P < 0.0001$; for **i**, $P = 0.2596$; for **l**, IL-5 Acute WT vs. Acute KO, $P = 0.0004$, Acute KO vs. Chronic WT, $P = 0.0301$, Acute KO vs. Chronic KO, $P = 0.0016$; IL-13, Acute WT vs. Chronic WT, $P = 0.0041$, Acute WT vs. Chronic KO, $P = 0.0013$, Acute KO vs. Chronic WT, $P = 0.0008$, Acute KO vs. Chronic KO, $P = 0.0003$; for **m**, Acute WT vs. Chronic WT, $P = 0.0019$, Acute WT vs. Chronic KO, $P < 0.0001$, Acute KO vs. Chronic WT, $P = 0.0113$, Acute KO vs. Chronic KO, $P = 0.0002$.



Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Blimp-1 is required to maintain the type 2

transcriptional identity of ILC2s. a–b, A UMAP visualization of key ILC2 marker gene expression (**a**) and type 2 cytokine gene expression (**b**) on control^{1^NMur1Cre} and Blimp-1^{N^Mur1Cre} ILC2 cells from scRNA-seq. **c,** A UMAP visualization of ILC2 cells colored by combination of allergens treatments and control^{1^NMur1Cre} and Blimp-1^{N^Mur1Cre} (left). Composition of each combination described above within ILC2 cells sub-clusters identified in Fig. 7a. **d,** A UMAP visualization of Prdm1 gene expression in control^{1^NMur1Cre} ILC2 cells. **e,** Dot plot visualization of key type 2 genes and Prdm1 in control^{1^NMur1Cre} ILC2 cells across treatment groups. **f,** Dot plot visualization of type 1 and type 2 cytokines in control^{1^NMur1Cre} and Blimp-1^{N^Mur1Cre} treated samples. **g,** Violin plot showing IFN γ module score within each cluster

as shown in Fig. 7a. **h,** ILC2 number and percentage across donor chimeric mice. (n = 10) **i,** Geometric mean of IL-5 expression in ILC2s across donor chimeric mice. **j,** Quantification of TNF and IFN γ in ILC2 cells comparing two sets of chimeras. The data are shown as means $\pm$ s.d., and present two or three independent experiments. A two-tailed unpaired t-test was performed for **j**. Multiple paired t-test with Holm-Šidák method was performed for **h, i**. The specific P values are as follows for **h**, left, Control:Control, P = 0.000417, Control:Blimp-1KO, P = 0.510645; right, Control:Control, P = 0.000305, Control:Blimp-1KO, P = 0.359212; for **i** left, Control:Control, P = 0.643284, Control:Blimp-1KO, P = 0.116701; for **j**, TNF, P = 0.1328, IFN γ P = 0.1638.

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Software and code

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Data collection	For flow cytometry data collection, we utilized BD LSRIIFORTESSA with DIVA (10.9) software, and exported the data in fcs3.0 version. For histology imaging, images were collected with Olympus IX83 microscope or Leica ImageScopex64 slide scanner. For RNA-seq and ATAC-seq, the sequencing data was collected on Illumina NextSeq2000 system. For scRNAseq, the sequencing data was collected on Illumina Novaseq XPlus. For luminex assay, sample plate data were collected by Bio-Plex® MAGPIX™ Multiplex Reader. Airway Hyperresponsiveness studies were collected by Flexivent (Scireq)
Data analysis	FlowJo (version 10.10.0 and version 10.8.1 for windows) for flow cytometry data analysis. GraphPad Prism (version 10.3.1 for windows) for statistical analysis. Cutadapt v1.18, HISAT2 v2.1.0, DESeq2, Metascape and Gene set enrichment analysis (GSEA) for RNAseq. Cutadapt v1.18, Bowtie2 v2.3.4.2, MACS2 v2.1.1, DESeq2, ChIPpeakAnno and deepTools2 v.3.3.0 for ATACseq. Histology Quantification was performed using QuPath74 , a open-source software used to analyze whole image slides. QuPath has built-in algorithms to detect cells and tissues, which can be classified using machine learning to identify objects from training datasets. Both control and treated groups were used to train QuPath to identify healthy parenchyma, immune cell infiltration, airways and blood vessels. For PAS, QuPath tools for color deconvolution were used to isolate magenta PAS signal from other stains. Lung sections were manually traced in software and cell detection was applied to identify nucleated cells. The trained ML algorithm was then applied to all sections to assign and quantify nucleated cells per region type or intensity and area for PAS-positive structures. These values were used to identify changes in the number of cells per area or percent of tissue with mucus in control vs experimental lung sections. RNAseq: Sequencing libraries were prepared using the Nextera XT DNA Library Preparation kit (Illumina). Cluster generation and 75-bp paired-

end, dual-indexed sequencing was performed on an Illumina NextSeq 2000. FastQC was used to perform a quality assessment on all fastq files. The mouse reference genome (GRCm38) was downloaded from Ensembl. Adaptors were trimmed using cutadapt v1.18 and reads were aligned using HISAT2 v2.1.0. Raw count values were generated using Subread, and gene expression values were normalized using transcripts per million. DESeq2 was applied to raw counts transcriptome data in a pairwise manner to determine differentially expressed genes. These gene lists were compiled to make a master list of all differentially expressed genes. Log2 transformed normalized counts were then displayed as a heatmap to display expression of differentially expressed genes. Metascape and Gene set enrichment analysis (GSEA) were used to identify pathways associated with gene sets.

ATACseq: Sequencing libraries were prepared using the Nextera XT DNA Library Preparation kit (Illumina). Cluster generation and 101-bp paired-end, dual-indexed sequencing was performed on an Illumina NextSeq2000. FastQC was used to perform a quality assessment on all fastq files. The mouse reference genome (GRCm38) was downloaded from Ensembl. Adaptors were trimmed using cutadapt v1.18. Raw count values were generated using Subread, reads were aligned to the reference genome using Bowtie2 v2.3.4.2. Duplicates were removed using Picard, and regions from the ENCODE Blacklist were removed using bedtools intersect. Peaks (narrow) were called using MACS2 v2.1.1 with a p-value cutoff of 0.05. Irreproducible discovery rate (IDR) using a threshold of 0.05 to identify reproducible peaks. IDR peak files of all samples were merged to create a master list of peaks which together with alignment files were used to generate tag counts for each replicate (bedtools coverage). DESeq2 was applied to raw tag count data for called peaks in a pairwise manner to determine differential peaks. These peak lists were compiled to make a master list of all differential peaks for each mark. Peaks were annotated to the nearest gene using ChIPpeakAnno. Heatmaps were generated using deepTools2 (v.3.3.0). Bam files were combined across sample replicates. Samples were normalized by bins per million (BPM) and converted to bigwig files. Heatmaps were peak centered in a 2,000 bp region by computing matrix from normalized and merged bigwig files for all peaks of each mark. (computeMatrix reference point –referencePoint center -a 1000 -b 1000) and visualized (plotHeatmap). Genome browser tracks are visualized from bigwig files using integrative genomics viewer.

scRNAseq: Raw sequencing data were processed using the Cell Ranger multi pipeline (v9.0.1; 10x Genomics) for alignment, filtering, and quantification. Reads were aligned to the mouse reference genome (GRCm39). Demultiplexing of pooled samples was performed based on assignment to specific oligonucleotide tags. This resulted in filtered unique molecular identifier (UMI) feature-barcode matrices for each individual sample, which were used for downstream analysis.

Quality control and Dimensionality Reduction: Downstream analysis was performed using the Scanpy toolkit (v1.10.3)69. After importing raw gene expression matrices, cells expressing fewer than 200 genes or exhibiting high mitochondrial content (>20%) were excluded. To remove potential multiplets, cells with abnormally high gene counts (>6,000) were filtered out. Doublet detection was performed using Scrublet70. Detected doublets were removed with a high-confidence doublet score threshold of 0.3. Following quality control, raw counts were normalized to the median total counts per cell and log-transformed. The top 2,000 highly variable genes were identified and used for Principal Component Analysis (PCA). Dimensionality reduction was performed using Uniform Manifold Approximation and Projection (UMAP) based on the nearest neighbor graph, and cells were clustered using the Leiden algorithm71. Non-ILC2 contamination was identified via canonical marker expression (e.g., Epcam for epithelial cells, Col1a1 for fibroblasts, Cd79a for B cells) and excluded from downstream analysis.

Trajectory Analysis: After excluding low-quality cells and contamination, 18,414 ILC2 cells were identified from the scRNA-seq dataset. These cells underwent standard normalization, log-transformation, PCA, and UMAP processing as described above. ILC2 subsets (corresponding to those in Fig. 7a) were identified using Leiden clustering with a resolution of 0.7. Differentiation trajectories were modeled using Palantir (v1.4.1)72. A starting cell with a representative transcriptomic profile was selected from Cluster 1, which contained the majority of untreated naive ILC2s. Pseudotime values were calculated to reconstruct the developmental timeline. To verify the robustness of the inferred trajectory, Diffusion Pseudotime (DPT)73 analysis was independently performed using Scanpy (v1.10.3). Both analyses were conducted on the same combined dataset (including both WT and KO conditions) and rooted in the same starting cluster (Cluster 1) to ensure a unified and directly comparable pseudotime axis across conditions.

Ifng response module score: An Ifng response module (L7) of 309 genes identified in the lung after infection was downloaded from Singhania et al41. The top 50 most highly expressed genes in our ILC2 dataset plus Cxcl10 and Cxcl9 was used to compute the module score with function score_genes from scanpy.

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All RNAseq and ATACseq described in the paper are available at NCBI GEO RNAseq [GSE291231] ATACseq [GSE291230]. scRNAseq [GSE316969]

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

NA

Reporting on race, ethnicity, or

NA

other socially relevant groupings

Population characteristics

NA

Recruitment

NA

Ethics oversight

NA

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

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

Sample size

All in vivo experiments were performed independently two or three times (as indicated in figure legends), with at least 2 mice but typically 5 mice per group for one independent experiment.
All in vitro experiments were performed independently two or three times (as indicated in figure legends), with at least 3 sorted ILC2 samples per group for one independent experiment.

Data exclusions

No data were excluded.

Replication

All the experimental findings were reliably reproduced as validated by two or three (as indicated in figure legends) independent experiments. The RNAseq experiments were conducted with three or four replicates each. The ATACseq experiment was conducted with two replicates. scRNAseq were conducted with 2 biological replicates per condition.

Randomization

For all the experiments used in the study, we prepared minimum of 2-4 breeder pairs from which we took litters randomly that were age and sex matched either male or female within one week between the littermate control and mutant groups. For in vivo experiments, all mice were over 6 weeks of age, and either matched male or female mice were assigned randomly to experimental and control groups. For in vitro experiments, all mice were over 6 weeks of age, two sex matched male or female lung cells were pooled and sorted for ILC2s as one sample.

Blinding

For mouse experiments, the investigators were not blind to group allocation during data collection or analysis. The approach is considered standard for experiments of the type performed, as the genetic background of mice must be predetermined prior to analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- | | |
|-------------------------------------|---|
| n/a | Involved in the study |
| ☐ | ☒ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

Methods

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

Antibodies

Antibodies used

Flow cytometry antibodies:
anti-GFP-Alexa Fluor 488 n/a 1:100 Thermo Fisher Scientific A21311
CD16/CD32 2.4G2 1:200 BD Bioscience 553142
CD25-BV510 PC61 1:200 Biolegend 102041
CXCR5-Alexa Fluor 647 L138D7 1:50 Biolegend 145532
CD45.1-APC efluor780 A20 1:200 Thermo Fisher Scientific 47-0453-82
IL-13-PE/Cy7 eBio13A 1:200 Thermo Fisher Scientific 25-7133-82

IL-10-APC JES5-16E3 1:100 Thermo Fisher Scientific 17-7101-82
 Ki67-Alexa Fluor 700 SolA15 1:100 Thermo Fisher Scientific 56-5698-82
 LIVE DEAD™ Fixable Blue n/a 1:1000 Thermo Fisher Scientific L34961
 STAT6(pY641)-PE J71-773.58.11 1:50 BD Bioscience 558252
 STAT3(pY705)-PE/CF594 4/P-STAT3 1:50 BD Bioscience 562673
 STAT4(Y693)-APC 4LURPIE 1:50 Thermo Fisher Scientific 17-9044-42
 STAT5(pY694)-PE/Cy7 47/Stat5 1:50 BD Bioscience 560117
 T-bet-BV421 0446 1:100 BD Bioscience 563318
 TCRβ- BUV737 H57-597 1:200 BD Bioscience 612821
 TCRβ- BV421 H57-597 1:200 BD Bioscience 562839
 B220-eF450 RA3-6B2 1:200 Thermo Fisher Scientific 48-0452-82
 NK1.1-BV421 PK136 1:200 Biolegend 108741
 TER119-BV421 TER-119 1:200 Biolegend 116233
 CD11b-BV421 M1/70 1:200 Biolegend 101251
 CD11c-BV421 HL3 1:200 BD Bioscience 562782
 Ly6G-BV421 1A8 1:200 Biolegend 127628
 CD4-V500 RM4-5 1:100 BD Bioscience 560782
 CD90.2-BV605 53-2.1 1:200 BD Bioscience 563008
 IL-33R-Percp/EF710 RMST2-33 1:200 Thermo Fisher Scientific 46-9333-82
 TCRβ- PE H57-597 1:200 Biolegend 109207
 KLRG1-PE/CF594 2F1 1:200 BD Bioscience 565393
 CD45.2-PE/Cy7 104 1:200 Thermo Fisher Scientific 25-0454-82
 CD3-APC 145-2C11 1:100 Thermo Fisher Scientific 17-0031-82
 CD3-AF700 500A2 1:100 Biolegend 152316
 CD45.2-APC780 104 1:200 Thermo Fisher Scientific 47-0454-82
 CD45.2-BV421 104 1:200 BD Bioscience 562895
 LIVE DEAD™ EF780 n/a 1:1000 Thermo Fisher Scientific 65-0865-14
 CD103-BV605 2E7 1:200 Biolegend 121433
 Ly6C-BV785 HK1.4 1:200 Biolegend 128041
 CD64-Percp5.5 X54-5/7.1 1:200 Biolegend 139308
 CD170-PE S17007L 1:200 Biolegend 155506
 CD11b-PE/Cy7 M1/70 1:200 Thermo Fisher Scientific 25-0112-82
 CD11c-PETxR N418 1:200 Thermo Fisher Scientific MCD11C17
 Ly6G-APC780 1A8-Ly6g 1:200 Thermo Fisher Scientific 47-9668-82
 CD117-Percp5.5 2B8 1:200 Biolegend 105823
 Sca-1-BV785 D7 1:200 Biolegend 108139
 Ly6D-FITC 49-H4 1:200 Biolegend 138605
 Flt3-PE A2F10 1:200 Thermo Fisher Scientific 12-1351-81
 IL-7Ra-PE/Cy7 A7R34 1:200 Biolegend 135013
 CD45.1-APC780 A20 1:200 Thermo Fisher Scientific 47-0453-82
 IFNγ-BUV395 XMG1.2 1:100 Thermo Fisher Scientific 363-7211-82
 IFNγ-PE/Cy7 XMG1.2 1:100 Thermo Fisher Scientific 25-7311-82
 TNFα-APC MP6-XT22 1:100 Thermo Fisher Scientific 17-7321-82
 IL-13-eF660 eBio13A 1:100 Thermo Fisher Scientific 50-7133-80
 IL-17rantib-PE 9B10 1:200 Biolegend 146305
 LPAM-1-APC DATK32 1:200 Biolegend 120607

Blocking antibodies:

Anti-IL-9 BioXCell BE0181
 mouse IgG1 isotype BioXCell BE0083, BE0088
 anti- IFN-γ BE0055, BioXCell
 anti-TNF BE0058, BioXCell
 Anti-CD3 BioXCell BE0002
 Anti-CD28 BioXCell BE0015-1

Validation

All antibodies are commercially available and validated by the vendor for the specificity and application. Additional validation steps were performed by gating on negative controls or cells known to be deficient in expression of the protein of interest.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

C57BL/6J (000664), B6.Cg-Tg(Prdm1-EYFP)1Mnz/J (008828), B6.129-Prdm1 tm1Clme/J (008100), Il1rl1tm1Anjm, Il7rtm1.1(icre)Hrr, animals were either purchased from Jackson Laboratories or maintained separately. Il7rtm1.1(icre)Hrr, animals were obtained from Hans-Reimer Rodewald (DKFZ). Il1rl1tm1Anjm were obtained from Heth Turnquist (U.Pittsburgh). C57BL/6-Tg(Nmur1-iCre,-eGFP)1Dart/J (038197) animals were obtained from David Artis (Weill Cornell University), now available from Jackson Laboratories. Blimp-1INMUR1Cre animals were generated by crossing with B6.129-Prdm1tm1Clme/J (008100). Animals were housed in specific pathogen-free enclosures at the John G. Rangos Sr. Research Center of UPMC Children's Hospital of Pittsburgh or The Assembly Building under conditions: 12 h light–dark cycle, temperature of 19–23°C and humidity of 40–60%. All experiments were approved by the University of Pittsburgh Animal Care Committee and the American Veterinary Medical Association. All animals were over 6 weeks of age, and either matched male or female animals for each experiment.

Wild animals	No wild animals were used in this study.
Reporting on sex	Male and female mice have different level of surface markers expression, resulting difference in gating for ILC2s (Wang et al, Mol. Immunol. 2020) However, there were no differences found in total ILC2 number or ILC2 function (cytokines production) between sex. Therefore, either matched male or female animals were used for each experiment.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Animals were housed in specific pathogen-free enclosures at the John G. Rangos Sr. Research Center of UPMC Children's Hospital of Pittsburgh or The Assembly Building. All experiments were approved by the University of Pittsburgh Animal Care Committee and the American Veterinary Medical Association.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

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

Lung:
Animals were anesthetized and sacrificed with intraperitoneal injection of ketamine/xylazine. To collect bronchoalveolar lavage fluid, a catheter was inserted in the trachea, and 0.9ml of PBS was instilled and retracted. BAL was later centrifuged at 2000rpm at 4°C for 10min, the cell pellet was resuspended with 200µL PBS and counted with automated cell counters (Nexcelom Bioscience LLC). Around 4x10⁵ BAL cells were stained for flow cytometry. The lung was perfused with PBS from right ventricle through pulmonary artery. In some experiments, the left lung was tied and removed for flow cytometry. The right lung was inflated and submerged with Safefix II (Thermo Fisher Scientific) for histology or directly digested for flow cytometry. The whole lung was taken down and processed for sorting experiments. For lung cells, a whole lung was digested with 5ml of 0.7mg/mL collagenase A (Sigma-Aldrich) and 0.03mg/mL DNase I (Sigma-Aldrich) RPMI solution while gently rotating for 45min. And the tissue was processed with gentleMACS dissociator. 2% FBS 2mM EDTA PBS solution was used to stop digestion. RBCs were lysed with ACK lysing buffer (Thermo Fisher Scientific). Cells were filtered through a 70µm filter (CELLTREAT).

Small intestinal lamina propria:
To harvest siLP ILC2s, the small intestine was isolated, the associated mesenteric tissue removed, and the contents emptied. The tissue was opened with scissors, and Peyer's Patches were removed for further digestion. Small intestine tissue was rinsed with PBS and then cut into small pieces. The tissues were immersed in media containing 3%FBS, 5mM EDTA, and 0.145mg/mL DTT and incubated at 37°C while stirring at 800rpm for 20min. After the incubation, the tissue and media were filtered, collected and shaken with buffer (2mM EDTA in RPMI1640) for 30sec, then strained again and repeated 3 times. The tissue was collected and digested in digestion buffer with 0.5mg/mL DNase I (Sigma-Aldrich) and 0.1mg/ml Liberase TL (Roche) at 37°C for 25min while stirring. 3% FBS media was used to stop the digestion, and the single-cell suspension was collected through a 70 µm filter. For in vivo gut ILC2 activation experiments, mLN were isolated and processed for flow cytometry.

Skin:
To isolate cells from flank skin, animals were shaved and the desired sample was cut and spread with the inner side up on a 10 cm cell culture plate. The blunt end of the forceps was used to remove the fat and cut the tissue into small pieces. Tissue was digested with digestion buffer (collagenase XI (2.5mg/mL) (Sigma-Aldrich), hyaluronidase (0.25mg/mL) (Sigma-Aldrich) and DNase I (0.05mg/ml) (Sigma-Aldrich) in RPMI 1640) at 37°C while shaking at 300rpm for 1h. After digestion, a

gentleMACS dissociator was used to dissociate cells to a single-cell suspension and filtered through a 70µm filter. For in vivo skin ILC2 activation experiments, draining LNs were taken down and processed for flow cytometry.

Spleen and lymph node:

Spleen and LNs were isolated in RPMI-1640 (BioWhittaker) and crushed through 40µm filters. For spleen, RBCs were lysed with ACK lysing buffer (Thermo Fisher Scientific).

Staining for flow cytometry:

Single-cell suspensions were collected as above. Cell number was determined by an automated cell counter (Nexcelom Bioscience LLC) and resuspended to 1x10⁷ cell/ml and plated into 96-well plates for staining. Cells were washed with PBS (BioWhittaker) twice before staining. Cell pellets were resuspended with 40µL HBSS containing 0.5% Fc Block and antibody cocktail for 30 min at 4°C. For intracellular staining, cells were stimulated with 50ng/ml PMA, 1 µg/ml ionomycin, brefeldin A (GolgiPlug, BD), and monensin (Ebioscience™) at 37°C for 2h. The same concentration of BFA and monensin was used for unstimulated control. Cells were stained with antibodies for surface markers and live/dead, fixed at 4°C with BD Cytofix/Cytoperm (BD Bioscience) and stained for cytokines and transcriptional factors with BD Perm/Wash buffer (BD Bioscience). BD LSRFortessa flow cytometer was used for flow analysis, and data were further analyzed with FlowJo.

Instrument

BD LSRFORTESSA for flow cytometry analysis.

Software

FlowJo v10.10.0 and v10.8.1 (Tree star), BD FACS DIVA and Graphpad Prism 10.3.1 were used for further analyses.

Cell population abundance

ILC2s were sorted based on widely accepted markers. ILC2 purity was checked by a re-sorted before actual acquisition.

Gating strategy

For all the experiments involving flow cytometry, first, lymphocyte gate was applied on the SSC-A/FSC-A window. Subsequently, sequential singlet/doublet discrimination was applied via FSC-W/FSC-H and SSC-W/SSC-H. From the selected singlets, dead cells were discriminated out by gating on live/dead dye negative population. Subsequent gating depends on the population of interest and is outlined in Supplementary Information.

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