



ILC2-driven innate immune checkpoint mechanism antagonizes NK cell antimetastatic function in the lung

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Metastasis constitutes the primary cause of cancer-related deaths, with the lung being a commonly affected organ. We found that activation of lung-resident group 2 innate lymphoid cells (ILC2s) orchestrated suppression of natural killer (NK) cell-mediated innate antitumor immunity, leading to increased lung metastases and mortality. Using multiple models of lung metastasis, we show that interleukin (IL)-33-dependent ILC2 activation in the lung is involved centrally in promoting tumor burden. ILC2-driven innate type 2 inflammation is accompanied by profound local suppression of interferon- γ production and cytotoxic function of lung NK cells. ILC2-dependent suppression of NK cells is elaborated via an innate regulatory mechanism, which is reliant on IL-5-induced lung eosinophilia, ultimately limiting the metabolic fitness of NK cells. Therapeutic targeting of IL-33 or IL-5 reversed NK cell suppression and alleviated cancer burden. Thus, we reveal an important function of IL-33 and ILC2s in promoting tumor metastasis via their capacity to suppress innate type 1 immunity.

Many cancers spread to the lung, with grave consequences for patient survival¹. For the formation of lung metastases, circulating tumor cells (CTCs) must extravasate into the tissue interstitium and evade tissue-resident immune cells. Lung NK cells are the innate counterpart to cytotoxic CD8⁺ T cells and comprise approximately 10–20% of all lung-resident lymphocytes in humans and mice. NK cells are critical for antigen-independent recognition and elimination of infiltrating CTCs, as demonstrated by overwhelming metastatic burden in their absence or impairment².

ILC2s are the innate counterpart to adaptive CD4⁺ T_H2 cells; like NK cells, they comprise a tissue-resident population in the naive lung³. Lung ILC2s are activated by the alarmin IL-33 that is released from various immune and nonimmune cell types in response to lung injury, infection or allergen exposure⁴. ILC2s mediate innate and adaptive type 2 inflammation through rapid release of effector cytokines such as IL-5 and IL-13, and expression of costimulatory ligands that influence T_H2 cells⁴. ILC2s also closely interact with regulatory T (T_{reg}) cells, indicating a potential contribution to immunosuppressive functions^{5,6}. Relatedly, several groups described distinct regulatory ILCs that can counter type 1 immunity^{7,8} or ILC2-specific expression of immunosuppressive molecules⁹. Nevertheless, it is unclear if the physiological role of ILC2s extends beyond regulating type 2 immunity.

Although type 2 inflammation is largely associated with tumor progression, via type 2 cytokine-mediated polarization of alternatively activated macrophages and myeloid derived suppressor cells, IL-33 has both pro- and antitumor functions in primary solid and hematological malignancies¹⁰. Similarly, opposing functions for ILC2s have been reported in the primary tumor environment^{11–13}. Nevertheless, type 2 inflammation is associated with metastasis formation^{14,15}. Given the central role of lung ILC2s in directing innate and adaptive type 2 immunity⁴, we hypothesized that ILC2 activation in the premetastatic niche can influence the formation of lung metastases.

In this study, we demonstrate that pre-existing type 2 airway inflammation greatly increases metastatic seeding of the lung in an IL-33- and ILC2-dependent pathway. In addition to orchestrating type 2 inflammation, ILC2s also profoundly suppress NK cell-driven antitumor immunity independent of the adaptive immune system. Mechanistically, ILC2s mediate their immunosuppressive effect via recruitment and activation of eosinophils in an IL-5-dependent manner. Eosinophils, but not ILC2s, can directly suppress NK cell function by modulating the metabolic environment of the inflamed niche. Therapeutic intervention reverses lung NK cell metabolic restraint, effector molecule production and antitumor function. As such, we demonstrate the important function of ILC2s in cancer

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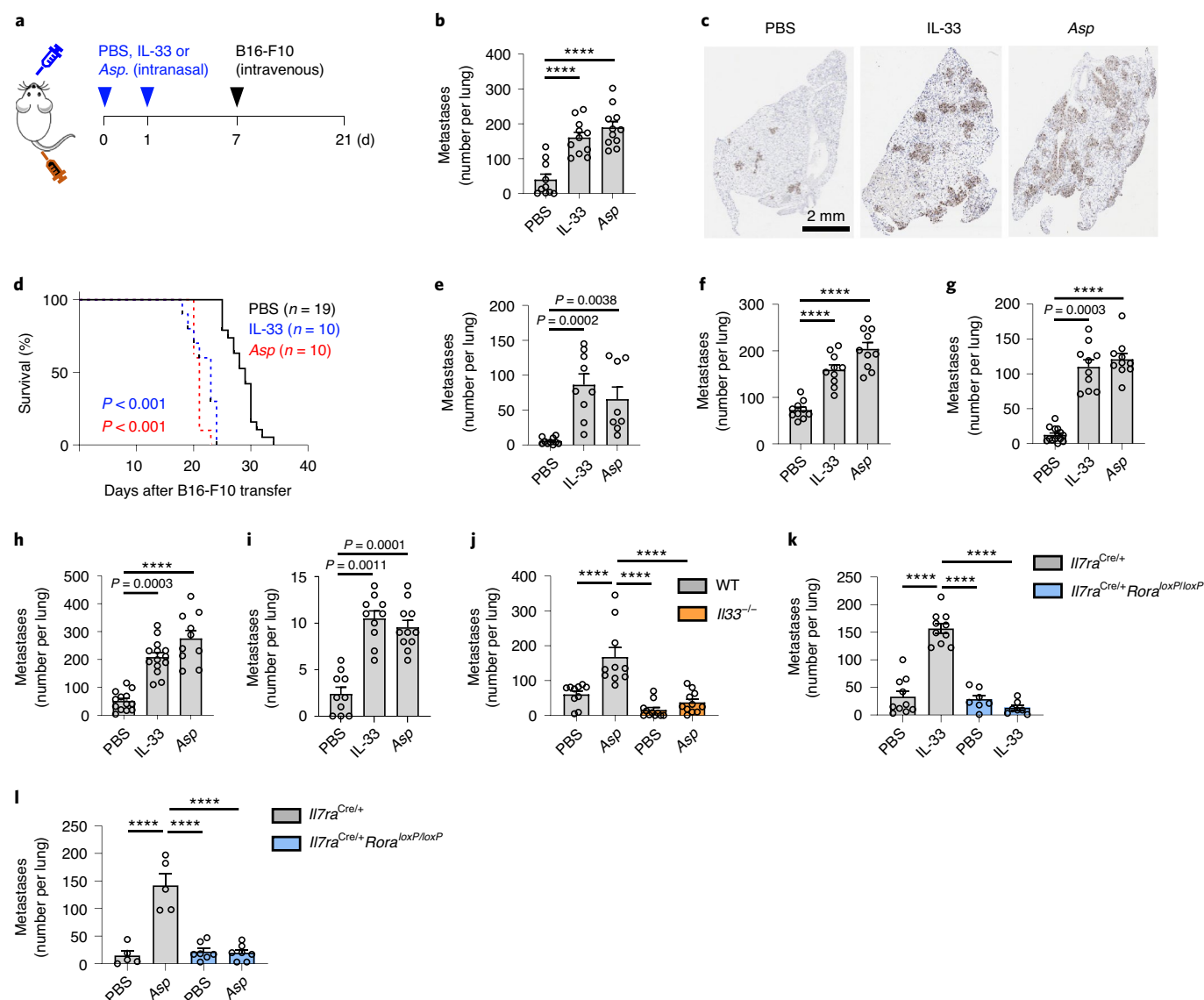


Fig. 1 | IL-33-driven activation of ILC2s is critical for promoting lung metastases. **a**, WT mice were treated intranasally with IL-33, Asp or PBS. This was followed by intravenous transfer of metastatic B16-F10 cells. Mice were killed on day 21. **b**, Visual quantification of lung metastases ($n = 11$). **c**, Histological staining for Ki-67⁺ tumor cells. **d**, Mice were kept alive to determine lung metastases-related mortality. **e, f**, Lung metastases were visually quantified in mice treated as in Fig. 1a with LL/2 ($n = 10, 9, 8$) (**e**) or 4T1 cells ($n = 10$) (**f**). **g, h**, 4T1 ($n = 10$) (**g**) or 4T1-T ($n = 13, 14, 10$) (**h**) breast cancer cells were implanted in the mammary fat pad of BALB/c mice on day 0. This was followed by intranasal treatment with IL-33, Asp or PBS on days 7 and 14 and visual quantification of lung metastases on day 21. **i**, Twelve-week-old female B6-MMTV-PyMT mice were randomized and treated intranasally with IL-33, Asp or PBS once a week and killed at 20 weeks of age. This was followed by visual quantification of lung metastases ($n = 10, 10, 11$). **j–l**, WT and IL33^{-/-} mice ($n = 10$) (**j**) or IL7ra^{Cre/+} and IL7ra^{Cre/+} Rora^{loxP/loxP} mice were treated as in Fig. 1a with IL-33 ($n = 10, 10, 7, 7$) (**k**) or Asp ($n = 5, 5, 7, 7$) (**l**). This was followed by visual quantification of lung metastases. The bar graphs indicate the mean ($\pm$ s.e.m.) and show the combined data of two (**d–h, j–k**) or three (**b, i**) experiments or are representative of three independent experiments (**c** and **l**). Statistical analyses were calculated using a one-way ANOVA or log-rank (Mantel-Cox) test (**d**) with **** $P \leq 0.0001$.

dissemination to the lung and further reveal an immunoregulatory collaboration between ILC2s and eosinophils that antagonizes innate type 1 immunity.

Results

IL-33-driven activation of ILC2s is critical for promoting lung metastases. To assess the effect of airway innate type 2 inflammation on lung metastasis formation, we employed a model of IL-33 or *Aspergillus* protease allergen (Asp)-induced airway inflammation before adoptive transfer of metastatic B16-F10 melanoma cells (Fig. 1a). We noted a significant increase in metastatic burden by

day 21 and increased mortality in both IL-33 or Asp-conditioned mice (Fig. 1b–d and Extended Data Fig. 1a). Protease allergens can activate lung-resident ILC2s via IL-33 release¹⁶, which is produced primarily by epithelial cell adhesion molecule (EpCAM)⁺ cells (Extended Data Fig. 1b). Resolution of acute type 2 inflammation after 14 d, as measured by lung eosinophilia, diminished the prometastatic effect of IL-33 (Extended Data Fig. 1c,d). Moreover, administration of IL-33 after metastatic engraftment, or intranasal sensitization with non-type 2-inducing inflammatory agents (CpG or lipopolysaccharide (LPS)) had no effect on metastatic seeding (Extended Data Fig. 1e,f). Next, we assessed the role of IL-33 or

Asp sensitization in metastatic models of lung carcinoma (LL/2, C57BL/6J genetic background) and breast cancer (4T1, BALB/c genetic background), which also showed increased tumor burden in mice exposed to innate type 2 inflammation (Fig. 1e,f). Similarly, both orthotopically implanted 4T1 and highly metastatic 4T1-T breast cancer cells developed more lung metastases in mice exposed to intranasal *Asp* or IL-33, without influencing primary tumor size (Fig. 1g,h and Extended Data Fig. 1g). In an autochthonous model of breast cancer (B6-MMTV-PyMT) that metastasizes to the lung, IL-33 or *Asp* treatment similarly promoted lung metastases without affecting primary tumor size (Fig. 1i and Extended Data Fig. 1h). Therefore, we conclude that IL-33- or allergen-driven type 2 airway inflammation significantly increases lung metastatic burden in cancer.

Allergen exposure leads to IL-33 release, resulting in the rapid activation of ILC2s which subsequently contribute to innate and adaptive type 2 inflammation⁴. We confirm that intranasal IL-33 induced ILC2 expansion and that *Asp* induced IL-33-dependent acute type 2 inflammation, as measured by eosinophilia (Extended Data Fig. 1i–k). To investigate whether *Asp*-driven increases in lung metastases depend on IL-33, we primed C57BL/6J wild-type (WT) and *Il33*^{−/−} mice with *Asp* before adoptively transferring B16-F10 cells. While WT mice treated with *Asp* had significantly more lung metastases on day 21, we observed no effect of allergen treatment in *Il33*^{−/−} mice (Fig. 1j). Since ILC2s are the primary lung cells that respond to acute IL-33 release¹⁶, we hypothesized that ILC2s may promote metastatic seeding after IL-33 or allergen exposure. To test this, we exposed *Il7ra*^{Cre/+}*Rora*^{loxP/loxP} ILC2-deficient⁵ or control (Ctrl) mice to IL-33 or *Asp* allergen, followed by adoptive transfer of B16-F10 cells and assessment of metastatic burden. Similar to *Il33*^{−/−} mice, ILC2-deficient mice produced significantly fewer lung metastases after exposure to either IL-33 or *Asp* allergen compared to Ctrl mice (Fig. 1k,l). Hence, we propose that allergen-induced promotion of lung metastasis formation is reliant on IL-33-driven activation of ILC2s.

IL-33 does not promote early seeding of the lung by CTCs. To determine whether IL-33-ILC2-dependent airway inflammation influences the early arrest of CTCs in the capillaries of the inflamed lung, we quantified either B16-F10-mCherry fluorescent or B16-F10-AkaLuc bioluminescent cells at early time points in IL-33-primed or Ctrl mice. IL-33-primed lungs were not seeded with more CTCs (Fig. 2a,b), arguing that another mechanism may promote metastatic burden. NK cells are essential for detecting and eliminating both CTCs and early metastatic lesions². To investigate if IL-33 functions in a parallel pathway with NK cells, we asked if IL-33 priming synergizes with NK cell depletion (Extended Data Fig. 2a). We first confirmed that NK cell depletion resulted in a substantial increase in lung metastasis formation (Fig. 3a). Moreover, we observed no additive effect of IL-33 priming in NK cell-depleted mice; Thus, while IL-33 does not promote early arrest of CTCs in the lung, we hypothesized that IL-33 influences NK cell-driven anti-tumor function.

IL-33 suppresses lung NK cell function. NK cells are the major ILC population and the predominant source of interferon- γ (IFN- γ) in the lungs of naive mice, which is involved in the antimetastatic function of lung NK cells (Extended Data Fig. 2b) (ref. ¹⁷). IL-33 administration moderately increased total lung NK cell numbers (Extended Data Fig. 2c). We confirmed that IL-33 administration induced the expansion of ST2⁺ ILC2s, while ST2[−] lung NK cells did not increase Ki-67 expression (Fig. 3b and Extended Data Fig. 2d), suggesting a potential indirect effect of IL-33 on NK cells. We next assessed the functional capacity of lung NK cells in PBS- or IL-33-treated mice by detection of intracellular IFN- γ , granzyme B (GzmB) and tumor necrosis factor (TNF). In IL-33-treated mice,

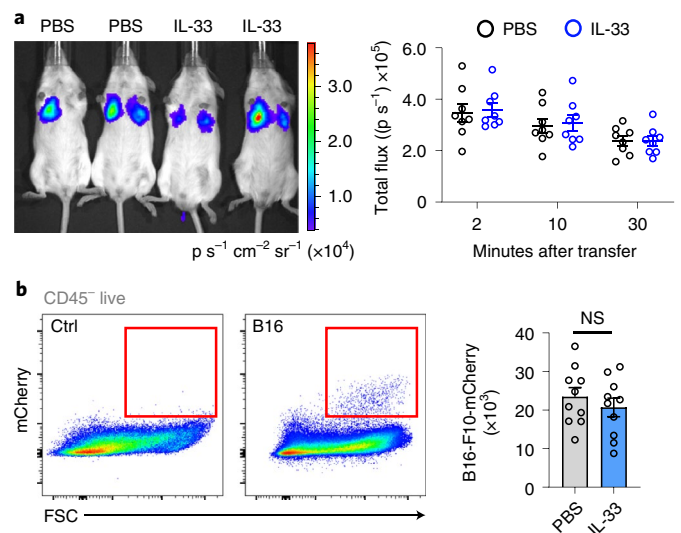


Fig. 2 | IL-33 does not promote early seeding of the lung by CTCs.

a, b, Albino C57BL/6 mice were treated intranasally with IL-33 or PBS on days 0 and 1. This was followed by intravenous transfer on day 7 of substrate-conditioned B16-F10-AkaLuc cells, followed by IVIS imaging at the indicated times (representative image at 10 min) and quantification of signal in the chest of mice (**a**; $n=8$), or B16-F10-mCherry cells after quantification of total B16-F10 tumor cells in the lung 24 h after injection (**b**; $n=10$). Representative gating shown for noninjected Ctrl and B16-F10-mCherry cell-injected (B16) mice. FSC, forward scatter. **a, b,** The bar graphs indicate the mean ($\pm$ s.e.m.) and show the combined data of two independent experiments; the IVIS and flow cytometry plots show representative images of two independent experiments. **b,** Statistical analyses were calculated using an unpaired two-tailed Student's *t*-test. NS, not significant.

we observed a significant reduction in IFN- γ production after ex vivo stimulation with either phorbol 12-myristate 13-acetate (PMA) plus ionomycin (PI) or anti-NK1.1 antibody (Fig. 3c and Extended Data Fig. 2e,f,g), and substantial reductions in total lung IFN- γ ⁺ NK cell numbers. Additionally, we observed a significant reduction in GzmB and TNF production (Fig. 3d). Similar results were obtained after ex vivo stimulation with a combination of IL-12 and IL-18 (Extended Data Fig. 2h). Intranasal IL-33 administration had no or minimal effect on circulating or splenic NK cell function (Fig. 3e), although pericardiac adipose NK cells were suppressed (Extended Data Fig. 2i).

We investigated the kinetics of lung NK cell suppression in time course experiments and found that IFN- γ production was suppressed for about 2 weeks after intranasal IL-33 administration (blue), while mice treated with intranasal LPS (gray) did not differ from baseline (black, day 0) (Fig. 3f). Suppression was inversely correlated with type 2 inflammation (Extended Data Fig. 1c). Notably, the capacity of lung CD4⁺ and CD8⁺ T cells to produce IFN- γ was unaffected by IL-33 treatment (Extended Data Fig. 2j). Moreover, IFN- γ and GzmB production by purified NK cells from naive mouse lungs was effectively suppressed on coculture with IL-33-treated lung cells, indicating that the lung inflammatory milieu has potent immunosuppressive properties (Fig. 3g and Extended Data Fig. 2k). Conversely, purified NK cells from IL-33-treated lungs regained IFN- γ and GzmB expression on coculture with PBS-treated lung cells, indicating that suppression is reversible. Next, by in vitro cytotoxicity assay, we found that lung NK cells from IL-33-treated mice were impaired in their ability to eliminate tumor cells (Fig. 3h).

To assess if allergen-induced IL-33 release could affect lung NK cells, we treated both WT and *Il33*^{−/−} mice with PBS or *Asp*.

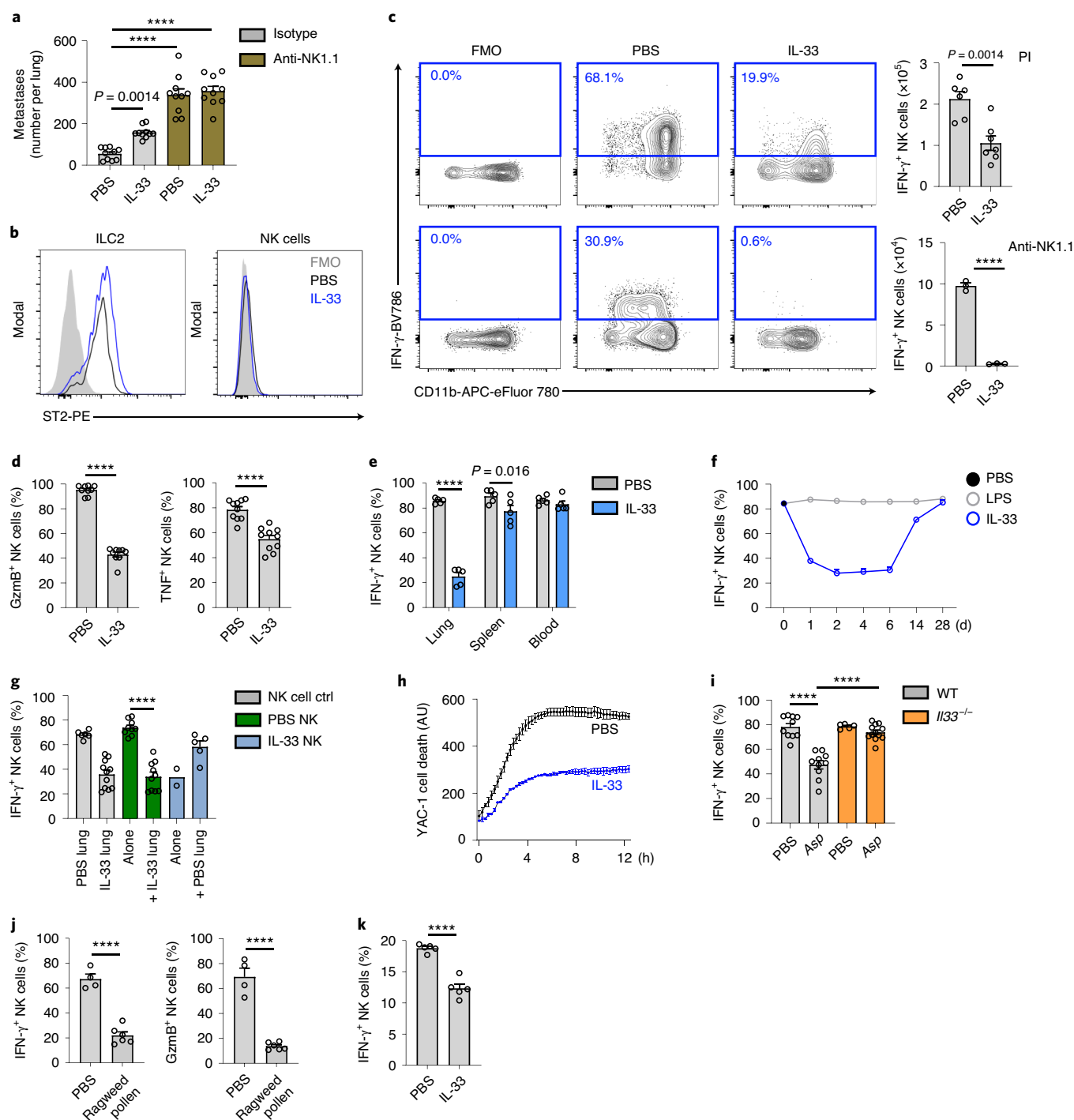


Fig. 3 | IL-33 suppresses lung NK cell function. **a**, WT mice treated as in Fig. 1a were given anti-NK1.1 or Ctrl antibody. This was followed by visual quantification of lung metastases on day 21 ($n=10$). **b–e**, WT mice were treated with IL-33 or PBS on day 0 and 1 and were killed on day 3. **b**, ST2 expression by lung ILC2s and NK cells was measured. FMO, fluorescence minus one. **c**, Identification (left) and quantification (right) of total IFN- γ + lung NK cells after PI (top; $n=6, 7$) or anti-NK1.1 (bottom; $n=3$) stimulation. **d**, Quantification of GzmB+ and TNF+ lung NK cells ($n=10$). **e**, Quantification of IFN- γ + NK cells from the anatomical sites shown ($n=5$). **f**, Quantification of IFN- γ + lung NK cells at the indicated time points after intranasal treatment with IL-33 (blue) or LPS (gray) on days 0 and 1, compared to PBS-treated WT animals (day 0, black) ($n=6$). **g**, Lung NK cells from PBS- or IL-33-treated WT mice were cocultured for 12 h with CFSE-labeled whole-lung cell homogenates from PBS- or IL-33-treated WT mice. CFSE+ endogenous NK (gray) or purified NK (green, blue) cells were subsequently assessed for IFN- γ production ($n=6, 11, 10, 10, 2, 5$). **h**, Killing assay of YAC-1 target cells by lung NK cells from PBS- or IL-33-treated WT mice ($n=3$). **i**, WT and Il33^{-/-} mice were treated with Asp on days 0 and 1. This was followed by quantification of IFN- γ + lung NK cells on day 3 ($n=10, 10, 5, 10$). **j**, WT mice were treated with ragweed pollen, which was followed by quantification of IFN- γ + and GzmB+ lung NK cells ($n=4, 6$). **k**, BALB/c mice were treated as in Fig. 3b. This was followed by quantification of IFN- γ + lung NK cells ($n=5$). Unless otherwise indicated, PI restimulation was used to measure NK cell cytokine production. The bar graphs indicate the mean ($\pm$ s.e.m.) and show the combined data of two (**d,i**) or three (**a**) independent experiments or are representative of three independent experiments (**b,c,e–h,j,k**). Statistical analyses were calculated using a one-way ANOVA, unpaired two-tailed Student's *t*-test (**c**) or Wilcoxon matched-pairs signed-rank test (**h**), with **** $P \leq 0.0001$.

Intranasal *Asp* administration led to reduced lung NK cell function in WT but not *Il33*^{-/-} mice on day 3 (Fig. 3i). Administration of ragweed pollen similarly impaired lung NK cell function (Fig. 3j). We also observed suppression of CD49b⁺ NK cell function in BALB/c mice treated with IL-33 (Fig. 3k). Moreover, parallel experiments conducted on C57BL/6 mice in a different animal facility produced comparable results in terms of NK cell suppression and increased metastatic seeding with IL-33 treatment (Extended Data Fig. 2l,m). Taken together, these data suggest that allergen-induced IL-33 release suppresses lung NK cell function.

ILC2s mediate an innate immune checkpoint on lung NK cell function. As the main IL-33-responsive cells in naive lungs, ILC2s may orchestrate suppression of NK cells. Indeed, IL-33- or *Asp*-treated ILC2-deficient mice failed to suppress production of IFN- γ and GzmB by lung NK cells, or induce type 2 inflammation (Fig. 4a and Extended Data Fig. 3a). ILC2-deficient mice that were also depleted of NK cells were now susceptible to increased metastatic seeding (Fig. 4b), supporting the hypothesis that ILC2-mediated suppression of NK cells is important in promoting lung metastasis formation. Relatedly, IL-33 sensitization did not influence metastatic seeding in NK cell-depleted ILC2-deficient mice (Fig. 4b). While these data suggest that ILC2s can influence lung NK cell function, possibly via a direct AMP-mediated pathway¹⁸, we found that both in vitro lung ILC2 and NK cell coculture, and small molecule inhibition of A2AR in vivo, failed to suppress or rescue NK cell function, respectively, indicating that another indirect mechanism may elaborate the NK cell-suppressing function of ILC2s (Extended Data Fig. 3b–d).

ILC2s can promote both conventional and regulatory CD4⁺ T cell expansion in the lung^{5,6,19,20}. To ascertain if ILC2-mediated suppression of NK cells relies on concomitant CD4⁺ T cell function, we used a CD4-depleting antibody together with IL-33 treatment. However, neither lack of CD4⁺ T cells nor neutralization of IL-10 or transforming growth factor- β (TGF- β) could rescue IL-33-induced suppression of NK cells (Fig. 4c and Extended Data Fig. 3e). Similarly, depletion of CD4⁺ T_{reg} cells (B6-*Foxp3*^{DTT}) did not impair the effect of IL-33 on lung NK cell suppression (Extended Data Fig. 3f). To ask if other adaptive immune cells contribute to this phenotype, we administered IL-33 to WT, μ MT and *Rag2*^{-/-} mice, followed by measurement of IFN- γ production by lung NK cells. We found that lung NK cells in WT, μ MT and *Rag2*^{-/-} mice were similarly impaired on IL-33 treatment (Fig. 4d,e, left panels), suggesting that ILC2s collaborate with nonadaptive immune cells to suppress NK cell function. Moreover, IL-33 priming of *Rag2*^{-/-} mice followed by B16-F10 or LL/2 adoptive transfer resulted in a similar increase in metastatic burden compared to IL-33-treated WT mice (Fig. 4f, left panel, and Extended Data Fig. 3g). B16-F10 adoptive transfer into mice lacking both adaptive and innate lymphocytes confirmed the important role of NK cells in preventing metastatic seeding of the lung and demonstrated no additive effect of IL-33 sensitization in the absence of ILC (Fig. 4f, right panel). Lastly, we created ILC2-deficient mice on the *Rag2*^{-/-} background (*Rag2*^{-/-}*Il7*^{ra^{Cre/+}Rora^{loxP/loxP}), which also failed to suppress NK cells in response to IL-33 (Fig. 4g and Extended Data Fig. 3h). Thus, activated ILC2s can suppress NK cells via an innate immunoregulatory mechanism.}

ILC2-derived IL-5 promotes eosinophil-mediated suppression of lung NK cells. Myeloid cells are implicated in promoting lung metastasis formation²¹, can suppress NK cells² and are induced or stimulated by ILC2s, advancing the hypothesis that a myeloid-ILC2 interaction elaborates the IL-33-driven suppression of NK cells. We first characterized the myeloid compartment in the lungs of PBS- and IL-33-treated mice on day 3, which revealed an expected influx of eosinophils, monocyte-derived inflammatory macrophages and dendritic cells, as well as neutrophils and monocytes

(Extended Data Fig. 4a,b). Given the known immunosuppressive functions of Gr-1⁺ (Ly-6G⁺ or Ly-6C⁺) myeloid cells^{22,23}, we used antibody-mediated depletion to assess their role in IL-33-driven suppression of NK cells. Anti-Gr-1 treatment reversed the effect of IL-33 on suppression of IFN- γ and GzmB production by NK cells (Fig. 5a and Extended Data Fig. 5a). However, parallel assessment of the myeloid compartment revealed that multiple immune cells were affected by anti-Gr-1 treatment in IL-33-sensitized mice, including eosinophils, highlighting broad direct or indirect effects of this reagent (Fig. 5a and Extended Data Fig. 5a). Targeted depletion of Ly-6G⁺ neutrophils or impairment of CCR2-CCL2-mediated influx of monocytes was largely unsuccessful in reverting IL-33-driven suppression of NK cells (Extended Data Fig. 5b–d). Similarly, clodronate-liposome-mediated depletion of alveolar macrophages failed to rescue NK cell suppression (Extended Data Fig. 5e). This suggested that neutrophils, despite their prometastatic function in other models^{22,24,25} are not involved in IL-33-mediated suppression of lung NK cells.

We found lung eosinophilia to be most strongly correlated with suppression of IFN- γ production by NK cells (Fig. 5b and Extended Data Fig. 4d). Although negative for surface Ly-6G, eosinophils express Ly-6C (Extended Data Fig. 4c), and numbers were reduced in IL-33-sensitized lungs on anti-Gr-1 treatment (Fig. 5a). To specifically assess the role of eosinophils on NK cell suppression, we neutralized IL-5, a cytokine essential for eosinophilic inflammation²⁶, which is primarily secreted by ILC2s during innate type 2 lung inflammation (Extended Data Fig. 6a). Lung eosinophil numbers were significantly reduced by anti-IL-5 treatment in IL-33-sensitized mice, while neutrophil numbers were unaffected (Fig. 5c and Extended Data Fig. 6b,c). Moreover, anti-IL-5 treatment also did not impair other inflammatory effects of IL-33, such as alternative activation of alveolar macrophages and monocyte-derived inflammatory macrophages as assessed by RELM α expression (Fig. 5d,e). Anti-IL-5 treatment protected against the suppressive effect of IL-33 on lung NK cell production of IFN- γ and GzmB, after restimulation with both PI and anti-NK1.1 (Fig. 5c and Extended Data Fig. 6d).

Intranasal administration of recombinant IL-4, IL-13, IL-5 or granulocyte-macrophage colony-stimulating factor did not induce lung eosinophilia or NK cell suppression, suggesting that IL-5 is essential but not sufficient for NK cell suppression (Extended Data Fig. 6e). Indeed, IL-5 operates in concert with other inflammatory mediators to promote lung eosinophilia²⁶. Relatedly, *Il5*^{-/-} mice developed fewer lung metastases²⁷ and we found prolonged survival in PBS- or *Asp*-treated *Il33*^{-/-} mice after B16-F10 transfer (Extended Data Fig. 6f). Moreover, adoptive transfer of eosinophils to naive mice or coculture of NK cells with ex vivo-derived or purified lung eosinophils from IL-33-treated mice effectively suppressed lung NK cell function, whereas type 2 conventional dendritic cells (cDC2), monocyte-derived inflammatory macrophages and alveolar macrophages did not (Fig. 5f,g and Extended Data Fig. 6g). Notably, both naive and inflamed lung eosinophils did not express ST2, and no additive effect of IL-33 on NK cell-eosinophil cocultures was observed (Fig. 5h,i). These data indicate that ILC2-derived IL-5 is important for eosinophil-mediated suppression of NK cell function.

IL-33-induced suppression of lung NK cell effector molecules is not transcriptionally regulated. To investigate how lung NK cells were affected in terms of gene regulation, we performed both single-cell RNA sequencing (scRNA-seq) and bulk RNA-seq analysis on flow cytometry-purified lung NK cells from PBS- or IL-33-treated WT mice at different time points (Extended Data Fig. 7a,b). On day 3, we identified 7 clusters (Methods) of NK cells in PBS- and IL-33-treated lungs in scRNA-seq data (Fig. 6a), which were annotated by their gene expression patterns and assessed for maturation and proliferation markers. Cluster 7 exhibited signatures indicative of CD27^{hi}CD11b^{low} immature NK cells, whose

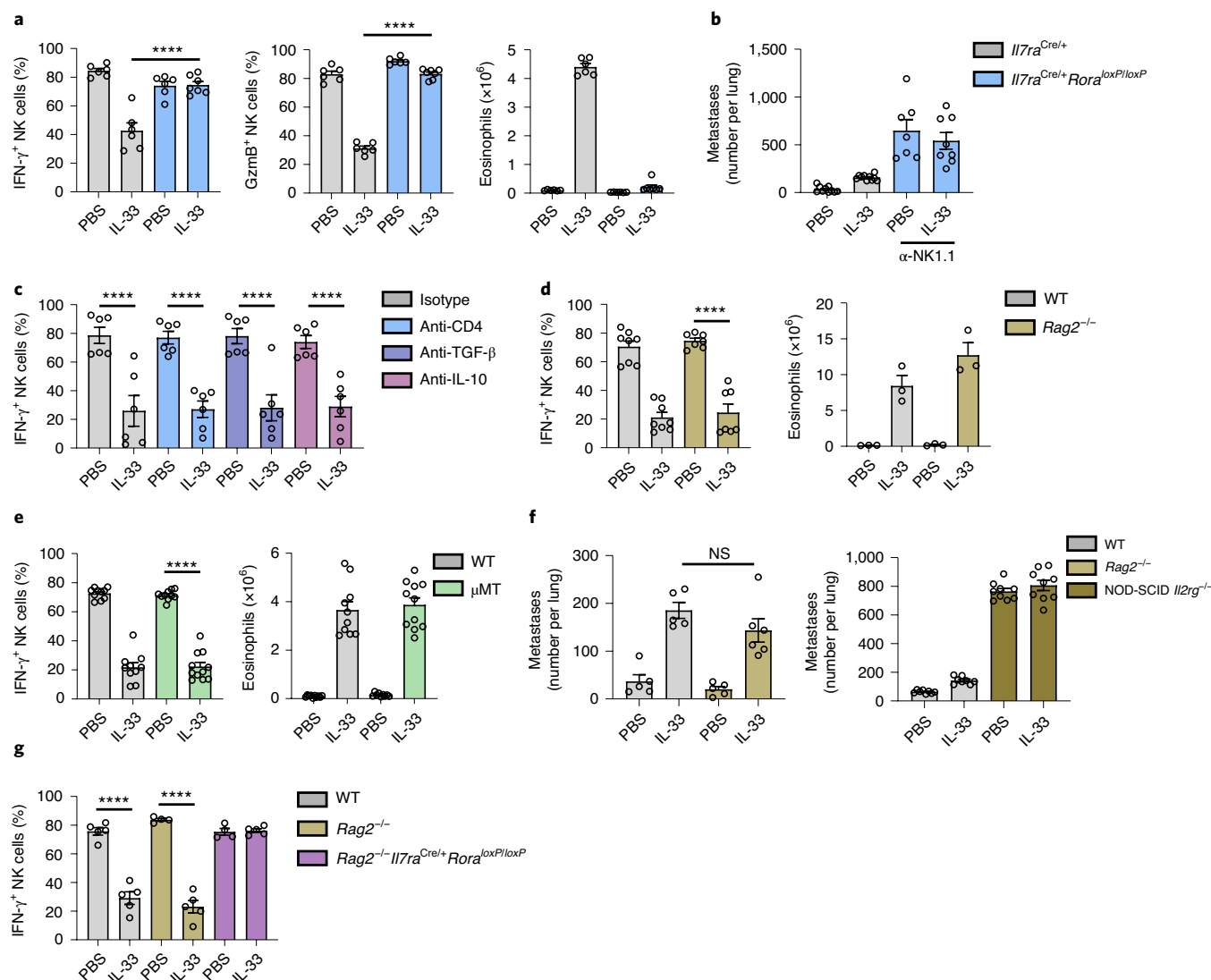


Fig. 4 | ILC2s mediate an innate immune checkpoint on lung NK cell function. **a,b**, *Il7ra*^{Cre/+} and *Il7ra*^{Cre/+} *Rora*^{loxP/loxP} mice were treated with PBS or IL-33 on days 0 and 1. This was followed by quantification of IFN- γ ⁺ and GzmB⁺ lung NK cells, and lung eosinophilia on day 3 ($n=6, 6, 6, 7$) (**a**), or treated as in Extended Data Fig. 2a, followed by visual quantification of lung metastases ($n=10, 10, 7, 8$) (**b**). **c**, WT mice were treated with PBS or IL-33 on days 0 and 1 and the indicated mAb on days -1 and 1. This was followed by quantification of IFN- γ ⁺ lung NK cells on day 3 ($n=6$). **d**, WT and *Rag2*^{-/-} mice were treated with PBS or IL-33 on days 0 and 1. This was followed by quantification of IFN- γ ⁺ lung NK cells (left, $n=8, 8, 7, 7$) and lung eosinophilia (right, $n=3$) on day 5. **e**, WT and μ MT mice were treated with PBS or IL-33 on days 0 and 1. This was followed by quantification of IFN- γ ⁺ lung NK cells (left, $n=10, 10, 10, 11$) and lung eosinophilia (right, $n=10, 10, 10, 11$) on day 3. **f**, WT, *Rag2*^{-/-} (left, $n=5, 5, 5, 6$) and NOD-SCID *Il2rg*^{-/-} (right, $n=9$) mice were treated as in Fig. 1a. This was followed by visual quantification of lung metastases. **g**, Mice of the indicated genotypes were treated with PBS or IL-33 on days 0 and 1. This was followed by quantification of IFN- γ ⁺ lung NK cells on day 3 ($n=5, 5, 4, 5, 4, 5$). PI restimulation was used to measure NK cell cytokine production. The bar graphs indicate the mean ($\pm$ s.e.m.). The data shown are combined from two (**c–e**) or three (**b,f**) independent experiments or are representative of three independent experiments (**a**). Statistical analyses were calculated using a one-way ANOVA or unpaired two-tailed Student's *t*-test (**c**) with **** $P \leq 0.0001$.

relative abundance corresponded with our flow cytometry observations (Extended Data Fig. 7c,d). Comparison of treatment arms did not reveal the emergence of new clusters but suggested shifts in relative proportions of clusters and subtle global changes in gene expression across clusters on IL-33 treatment (Fig. 6b,c and Extended Data Fig. 7e). In contrast to protein expression measurements, effector molecule genes such as *Ifng* and *Gzmb* were either unaffected or upregulated by IL-33 treatment, respectively.

We also performed a bulk RNA-seq time course on lung NK cells after IL-33 administration to capture changes in gene transcription over time (Extended Data Fig. 7a). We tested if IL-33 treatment led to changes in expression of NK cell consensus, effector molecule

or activating and inhibitory receptor gene sets. We did not observe substantial modulation in expression at 2, 7 or 14 d after intranasal IL-33 administration ($n=3$) or on day 3 in a higher-powered ($n=6, 7$) bulk RNA-seq experiment (Fig. 6d,e and Extended Data Fig. 7f,g). Altogether, these transcriptomic studies suggest that post-transcriptional regulation may influence the functional impairment of lung NK cells after IL-33 administration.

Activated ILC2s suppress NK cells via an eosinophil-mediated metabolic mechanism. Since posttranscriptional regulation of NK cell function can be controlled by their metabolic state²⁸, we hypothesized that global suppression of lung NK cell function is influenced

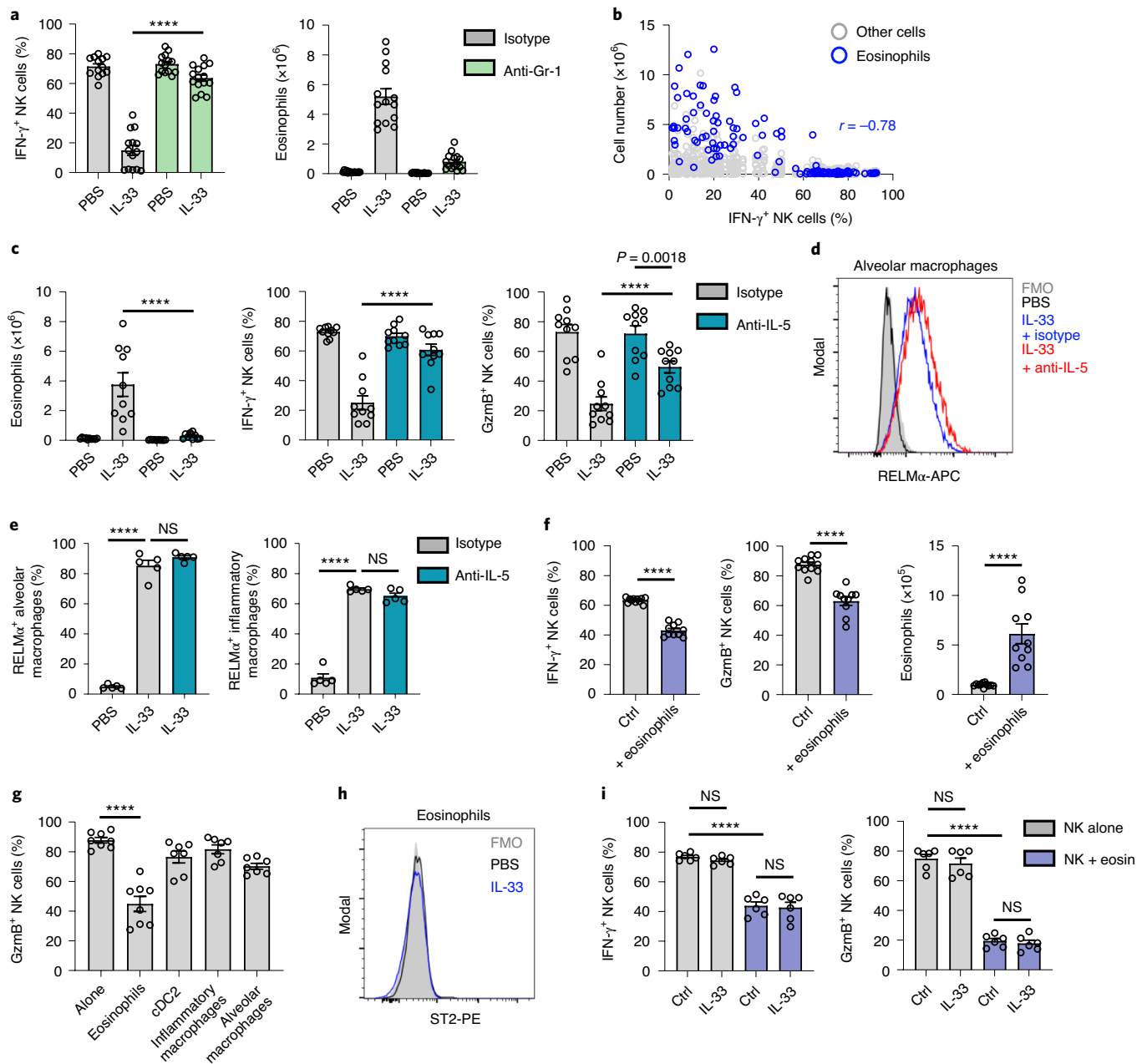


Fig. 5 | ILC2-derived IL-5 promotes eosinophil-mediated suppression of lung NK cells. **a**, WT mice were treated with PBS or IL-33 on days 0 and 1 and anti-Gr-1 or Ctrl antibody on days -1 and 1. This was followed by quantification of IFN- γ ⁺ lung NK cells and eosinophil numbers ($n=15, 14, 14, 15$) on day 3. **b**, Correlation of lung eosinophil numbers and percentage IFN- γ ⁺ NK cells on day 3 from WT mice treated with PBS and IL-33 (days 0 and 1) ($n=125$). **c-e**, WT mice were treated with PBS or IL-33 on days 0 and 1 and anti-IL-5 or Ctrl antibody on days -6, -3 and -1. This was followed by quantification of IFN- γ ⁺ and GzmB⁺ lung NK cells and eosinophilia ($n=10$) (**c**), or flow cytometry identification (**d**) and quantification of RELM α ⁺ alveolar macrophages and monocyte-derived inflammatory macrophages ($n=5$) (**e**) on day 3. **f**, Eosinophils were adoptively transferred to WT mice on day 0. This was followed by quantification of IFN- γ ⁺ and GzmB⁺ lung NK cells and lung eosinophilia on day 1 ($n=10$). **g**, Flow cytometry-sorted immune cells from WT mice were cocultured with purified lung NK cells for 12 h. This was followed by quantification of GzmB⁺ lung NK cells ($n=8, 8, 7, 7, 7$). **h**, ST2 expression by WT mouse lung eosinophils on day 3 after treatment with PBS or IL-33 (days 0 and 1). **i**, WT mouse lung NK cells were cocultured for 12 h with eosinophils (1:10 ratio) with the addition of IL-33 or Ctrl. This was followed by quantification of IFN- γ ⁺ and GzmB⁺ lung NK cells ($n=6$). PI restimulation was used to measure NK cell cytokine production. The bar graphs indicate the mean ($\pm$ s.e.m.). The data shown are combined from two (**c-e-g-i**) or three (**a,f**) independent experiments, with **g** representing Pearson's r ($n=125$). **f**, Statistical analyses were calculated using a one-way ANOVA or unpaired two-tailed Student's t -test with **** $P \leq 0.0001$.

by eosinophil-induced changes in NK cell metabolism. Metabolic stress is sensed by NK cells, resulting in suppressed mammalian target of rapamycin complex 1 activity and reduced phosphorylation of ribosomal subunit S6 (ref. ²⁹). We observed reduced phospho-S6 levels in IL-33- or *Asp*-treated lung NK cells on stimulation (Fig. 7a,b).

Phospho-S6 levels were unchanged in *Asp*-treated ILC2-deficient mice and restored on IL-5 neutralization in IL-33-treated WT mice (Fig. 7c,d). Moreover, metabolic profiling of lung cultures by nuclear magnetic resonance (NMR) revealed an increase in glucose use on IL-33 treatment (Fig. 7e). Activated eosinophils primarily

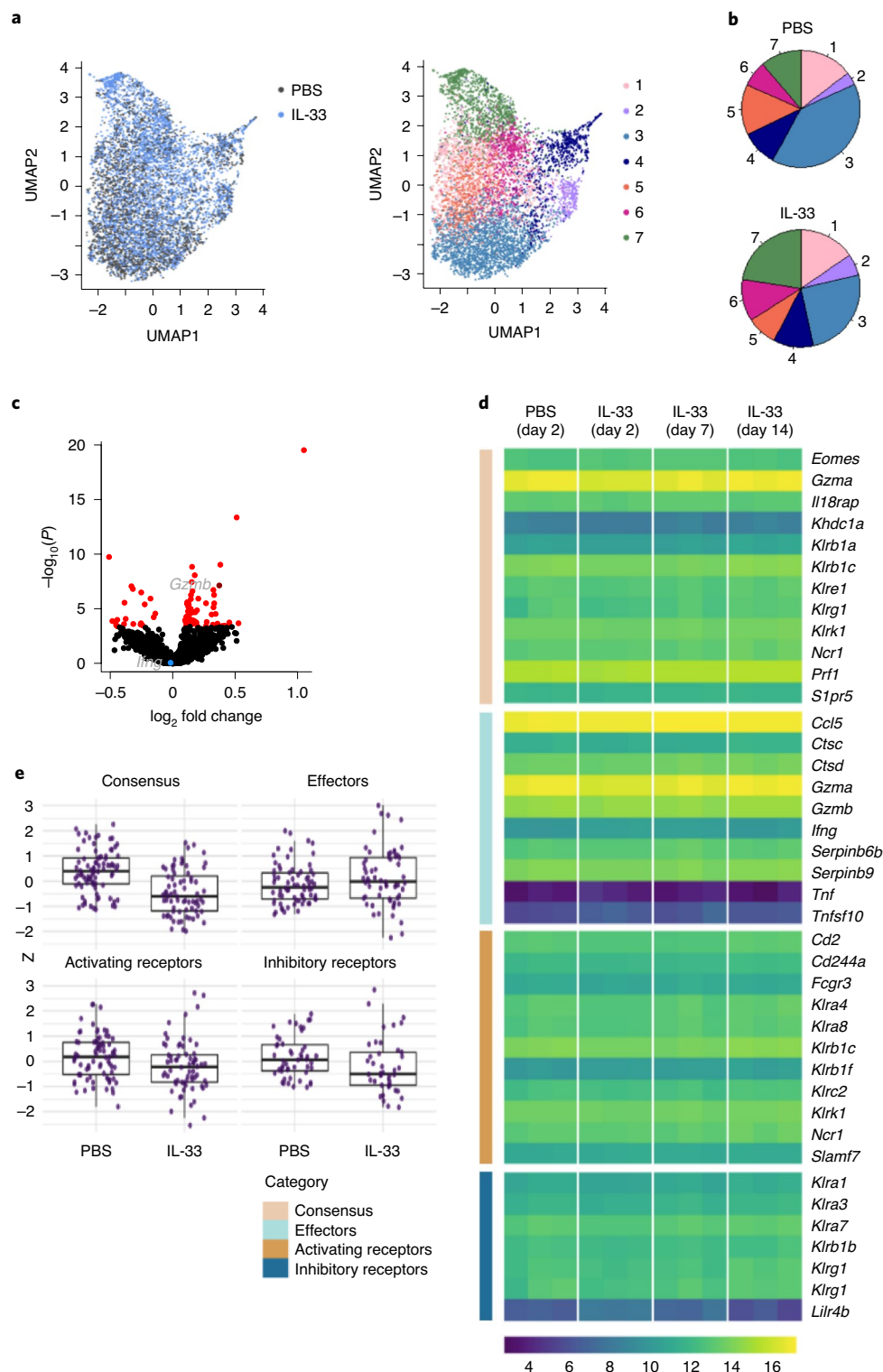


Fig. 6 | IL-33-induced suppression of lung NK cells is not transcriptionally regulated. **a**, WT mice were treated with IL-33 or PBS on days 0 and 1. This was followed by isolation of lung NK cells on day 3 for scRNA-seq analysis. Uniform manifold approximation (UMAP) and projection of NK cell scRNA-seq data from two mice from each treatment colored by treatment (left) and cluster (right). **b**, Average proportions of lung NK cells in each cluster from PBS- or IL-33-treated mice. **c**, Volcano plot of differential expression analysis comparing IL-33- and PBS-treated NK cells. Red indicates significantly differentially expressed genes (false discovery rate (FDR) < 0.05). *Ifng* (not significant, blue) and *Gzmb* (significant, dark red) are labeled. **d,e**, Bulk RNA-seq analysis of sorted lung NK cells from PBS- or IL-33-treated WT mice. Expression of NK cell consensus, effector and both activating and inhibitory receptor transcripts (manually curated) are shown as a heatmap of $\log_2$ -transformed normalized read counts of individual genes at the indicated time points (**d**) or grouped by category for day 3 (z-scaled expression values for genes within the 4 gene lists) (**e**). Each point represents the expression value obtained by one replicate for a given gene at a given time point. The box plots represent the mean (black line), 25th and 75th percentiles (box) and range within 1.5 times the interquartile range from the box (whiskers).

use glycolysis³⁰ and express associated metabolic pathway genes, including the glucose transporters Glut1 and Glut3 (encoded by the genes *Slc2a1*, *Slc2a3*) (ref.³¹). Correspondingly, we observed uptake of the 2-NBDG glucose analog by lung eosinophils, which increased in IL-33-treated mice (Fig. 7f). Importantly, IL-5-neutralization reversed IL-33-mediated changes in glucose use and lactate production in both C57BL/6J and BALB/c mice (Fig. 7g and Extended Data Fig. 8a). Additionally, mass spectrometry imaging (MSI) of mice infused with [¹³C]glucose was performed to assess in vivo glycolysis in inflamed lungs (Fig. 7h and Extended Data Fig. 8b–d). We observed increased [¹³C] and [¹²C]lactate/glucose ratios on IL-33 treatment in WT but not ILC2-deficient mice (Fig. 7i and Extended Data Fig. 8b–d). These data support the hypothesis that ILC2-induced eosinophilia modulates glucose use in the lung environment.

We further tested if depletion of glucose or increased concentrations of lactate were responsible for impaired NK cell function. We found that glucose restriction impaired naive lung NK cell function, while high-glucose media rescued IFN- γ and GzmB production, as well as phospho-S6 expression, by IL-33-treated lung NK cells on activation (Fig. 7j,k). Moreover, ex vivo activated NK cells from IL-33-treated mouse lungs exhibited an increased extracellular acidification rate (ECAR) after glucose administration (Fig. 7l). Gene set enrichment analysis (GSEA) identified enrichment of genes associated with glycolysis in IL-33-treated lung NK cells compared to PBS Ctrl (Fig. 7m). Interestingly, culture of naive lung NK cells in lactic acid suppressed IFN- γ but not GzmB production (Fig. 7n). These data suggest that ILC2-induced eosinophilia orchestrates suppression of NK cell function by restraining NK cell glucose metabolism.

Therapeutic targeting of the ILC2–eosinophil axis restores NK cell-mediated tumor control. Since we observed that IL-33-driven activation of ILC2s represents a central node in a mechanism of type 1 immunity regulation, we postulated that this axis may present a potential therapeutic target. Given that IL-5- and IL-33-targeted therapeutics are already in the clinic or undergoing phase 2 clinical trials, respectively³², we first used an IL-33 trap (IL-33R-Fc) to block *Asp* allergen-induced airway inflammation. Prophylactic dosing prevented NK cell suppression in WT mice on *Asp* administration, while also reducing markers of type 2 inflammation, including eosinophilic inflammation (Fig. 8a). Similarly, the *Asp*-induced metastatic burden of B16-F10 melanoma was alleviated on IL-33 neutralization (Fig. 8b,c). In addition to anti-IL-5 restoring NK cell function (Fig. 5c), we found that prophylactic administration significantly reduced the metastatic burden in both *Asp*- and IL-33-sensitized mice on transfer

of B16-F10 cells (Fig. 8b–d). We further administered anti-IL-5 to BALB/c mice bearing 4T1 or 4T1-T orthotopically implanted breast cancer cells and found a reduction in lung metastasis formation in *Asp*-treated groups, but no effect on primary tumor burden (Fig. 8e and Extended Data Fig. 8e,f). Moreover, given that BALB/c mice are predisposed toward both innate and adaptive type 2 inflammation³³, we investigated if prophylactic dosing may reduce metastasis formation in naive mice; we observed that both IL-5 and IL-33 neutralization reduced lung metastases in a model of metastasis from primary breast cancer (Fig. 8f). Thus, targeting of the ILC2–eosinophil axis can restore the antimetastatic function of lung NK cells (Extended Data Fig. 8g).

Discussion

In summary, our data implicate ILC2-driven innate type 2 inflammation in promoting metastatic seeding of the airways. We find that ILC2s can locally antagonize lung NK cell function via an eosinophil-mediated metabolic checkpoint. Therapeutic or genetic interference in this pathway restores NK cell functionality, with benefit on tumor burden.

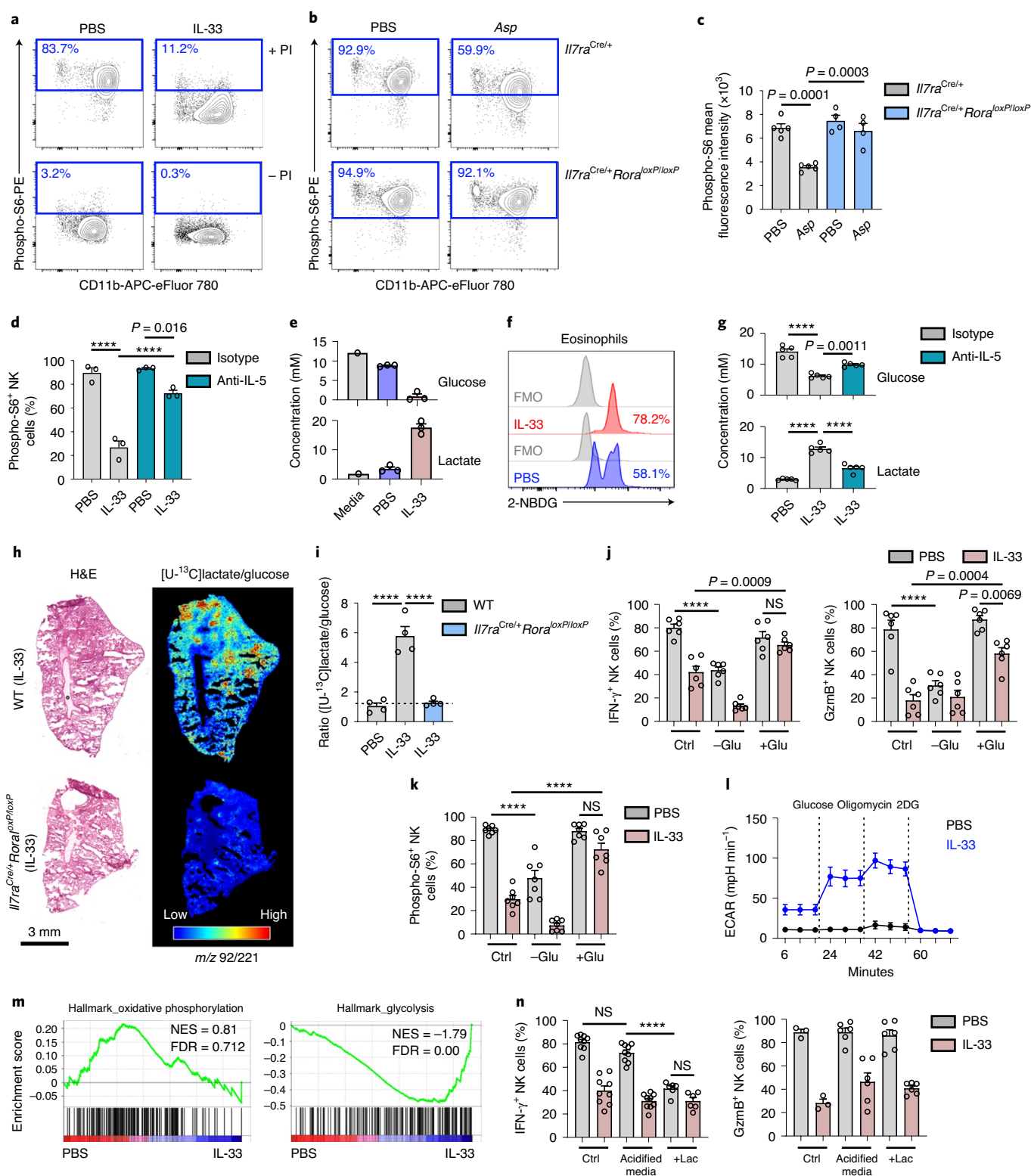
Both primary tumor-derived and exogenous factors that promote inflammation can influence CTC arrest in the premetastatic niche^{1,21}. We found that ILC2-driven inflammation had no effect on mechanical trapping of CTCs, but acutely suppressed lung NK cell function. Against our expectations, adaptive immune cells did not play a major role, while targeting of eosinophils was effective in reversing the effect of IL-33 on NK cells. Although neutrophils are also known to enhance CTC extravasation and lung metastasis formation^{22,34}, we found that specific ablation was ineffective at blocking IL-33-mediated NK cell suppression. Conversely, anti-IL-5 monoclonal antibody (mAb) treatment targeted eosinophils specifically and reduced both NK cell suppression and metastatic seeding of the lung. Nevertheless, eosinophils are also reported to have antimetastatic function in the lung³⁵. However, our study used 10- to 20 fold less IL-33 or used physiological stimuli in conjunction with *IL33*^{−/−} mice to reveal a prometastatic role in six different tumor models on different genetic backgrounds and in different animal facilities. While eosinophils are primarily associated with type 2 inflammatory diseases, little is known about their ability to suppress type 1 immunity²⁶.

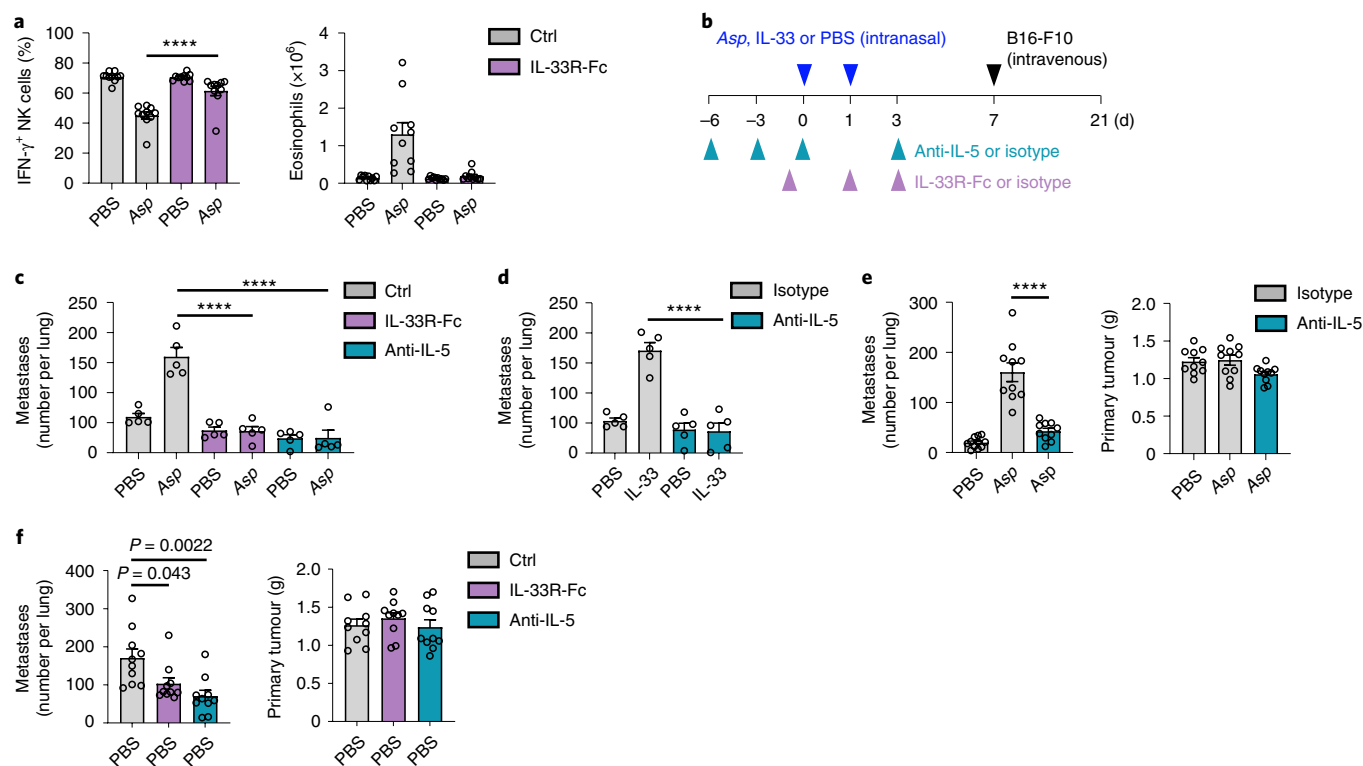
Type 1 cytokines can directly antagonize the effector functions of adaptive and innate type 2 lymphocytes^{6,36}. Reciprocal type 2 cytokine-driven regulatory mechanisms are less well defined, although IL-4 can suppress IFN- γ production at the very early stages of T cell activation³⁷. Moreover, T_{reg} cells and recently identified ILC_{reg} cells are known to restrain type 1 immune cells via production of IL-10 or TGF- β ^{8,38,39}; however, we found that

Fig. 7 | Activated ILC2s suppress NK cells via an eosinophil-mediated metabolic mechanism. **a**, WT mice were treated with IL-33 or PBS on days 0 and 1 and killed on day 3. This was followed by intracellular detection of phospho-S6⁺ lung NK cells after culture in media $\pm$ PI. **b,c**, Mice of the indicated genotypes were treated as in **a** with *Asp* or PBS, followed by intracellular detection of phospho-S6 in lung NK cells. **b**, Representative data. **c**, Mean fluorescence intensity ($n=5$, 5, 4, 4). **d**, WT mice were treated with PBS or IL-33 on days 0 and 1 and anti-IL-5 or Ctrl antibody on days −6, −3 and −1, followed by quantification of phospho-S6⁺ lung NK cells on day 3 ($n=3$). **e,f**, Lung cells from WT mice treated as in **a**. **e**, Lung cells were cultured for 18 h followed by quantification of glucose or lactate in the media by NMR ($n=1$, 3, 3). **f**, Quantification of ex vivo 2-NBDG uptake by lung eosinophils. **g**, Lung cells from WT mice treated as in **d** were cultured for 18 h. This was followed by quantification of glucose or lactate in the media by NMR ($n=5$). **h,i**, *IL7ra*^{Cre/+}/*Rora*^{loxP/loxP} or WT mice were treated intranasally with PBS or IL-33 on days 0 and 1. This was followed by [¹³C]glucose infusion and MSI on day 3. **h**, H&E and corresponding MSI analysis of [¹³C]lactate over [¹³C]glucose ratio (pixel per pixel) as shown by the heatmap. **i**, Mean relative abundances ($n=4$). **j,k**, Lung cells from WT mice treated as in **a** were assessed for NK cell expression of IFN- γ and GzmB ($n=6$) (**j**), or phospho-S6 ($n=7$) (**k**) after being cultured (with PI) for 3 h in normal (Ctrl), no glucose (−Glu) or high glucose (+Glu) media. **l**, Lung homogenates from mice treated as in **a** were cultured with PI for 3 h, after which NK cells were isolated and assessed for ECAR after the addition of glucose, oligomycin and 2DG ($n=5$). **m**, GSEA of PBS- and IL-33-treated lung NK cell bulk RNA-seq data (day 3). The normalized enrichment score (NES) and FDR are shown for each plot. **n**, Lung cells from WT mice treated as in **a** were cultured with PI for 3 h in normal (Ctrl) or acidified media or in media containing 15 mM lactic acid (+Lac) and assessed for NK cell expression of IFN- γ ($n=9$, 9, 9, 6, 6) and GzmB ($n=3$, 3, 6, 6, 6). The bar graphs indicate the mean ($\pm$ s.e.m.) and show the combined data of two (**c,g,i,n**) or three (**j,k**) independent experiments. Data are representative of two (**b,d,f,h,i,m**) or three (**a,e**) independent experiments. Statistical analyses were calculated using a one-way ANOVA with **** $P \leq 0.0001$.

these cytokines, or adaptive immunity, did not play a significant role in ILC2-mediated suppression of NK cells. While the ILC2–eosinophil-driven innate immune checkpoint suppresses the anti-metastatic function of NK cells, its effect on adaptive antitumor immunity is unknown. B16-F10 and other cancer cell lines are susceptible to mono-immunotherapy and combination immunotherapy¹⁰, prompting future studies to assess the role of ILC2-driven suppression on adaptive antitumor immunity.

IL-33 primarily promotes both innate and adaptive type 2 immunity, although there are reports of IL-33 directly stimulating NK and CD8⁺ T cells^{41–43}. However, our results in ILC2-deficient or anti-IL-33-treated mice reveal indirect suppression by IL-33 of innate type 1 immunity. Nevertheless, forced overexpression of *Il33* by tumors, or prolonged administration of high-dose IL-33, can promote anti-tumor immunity via indirect mechanisms that involve ILC2s in the primary tumor environment^{13,44}. Our data reveal a critical role of





ILC2s and IL-33 in the premetastatic niche, which may stimulate further studies that disentangle physiological and therapy-induced effects of IL-33 at both the primary tumor and peripheral sites. Moreover, since our IL-33 neutralization and *Il33*^{-/-} mouse experiments targeted the endogenous release of IL-33, we speculate that ILC2-mediated innate type 1 immunosuppression may influence other physiological roles such as lipid metabolism and wound healing; indeed, IL-33 can also suppress adipose tissue NK cells.

IL-5 is primarily known for its importance in eosinophil development and function⁴⁵. Our neutralization experiments revealed an essential role for IL-5 in mediating ILC2-dependent NK cell suppression. Importantly, IL-5 alone is not sufficient to exert this function and likely works in concert with other IL-33-induced factors that promote eosinophilic inflammation such as IL-13 and eotaxins^{26,46}. By neutralizing endogenously produced IL-5 (and IL-33), we targeted a physiological pathway that complements experiments using ILC2-deficient mice, which lack the primary innate source of IL-5 in the airways. Similarly, these approaches identify eosinophils as the critical myeloid cell type that collaborates with ILC2s to suppress NK cell function. It remains to be determined whether ILC2s, or other sources of IL-5 such as T_H2 or mast cells, exert similar control over NK cells in other organs.

Unexpectedly, we found that suppression of lung NK cell effector function did not coincide with major changes in the transcription of core NK cell genes or shifts in lung NK cell clusters after IL-33 administration. However, posttranscriptional regulation of

NK cell function can be governed by activation-induced metabolic demand and extracellular metabolite concentrations²⁸. Although functionally and metabolically quiescent at rest, ex vivo activation resulted in increased glycolysis of NK cells from IL-33-treated lungs. Consistent with this, naive NK cells are initially more reliant on oxidative phosphorylation for effector function⁴⁷, raising the possibility that the inflammatory environment provides signals that increase their reliance on glycolysis²⁹. Increased reliance on glycolysis of lung NK cells coincides with altered metabolite bioavailability in the IL-33-treated lung milieu. Low concentrations of glucose and lactate acidosis impair effector functions of NK and other lymphoid cells^{28,48}. Lung eosinophils are probably responsible for local glucose depletion and production of lactic acid in the IL-33-inflamed setting; eosinophils are the most prevalent myeloid population in the inflamed lungs and readily take up 2-NBDG, while ex vivo cultures of inflamed lung use glucose and secrete lactate more than uninflamed lungs, which is reversed by anti-IL-5-mediated prevention of eosinophilia. The implications of glucose depletion or lactic acidosis on naive lung NK cell function is confirmed by our observed reductions in phospho-S6 and downstream translation of effector molecules. This mirrors the phenotype of NK cells from inflamed lungs, while reduced phospho-S6 and effector molecule production can be rescued by the genetic or therapeutic blockade of eosinophilia. Similarly, suppressed lung NK cells can be partially rescued by culture in high-glucose conditions. Lastly, using MSI of [U-¹³C]glucose-infused mice, we demonstrated that IL-33 increases

glucose flux in the inflamed lung environment. These findings support the concept of nutrient competition in the immune microenvironment⁴⁹. It is unknown what NK cell-intrinsic mechanisms are responsible for sensing metabolite availability, although in CD8⁺ T cells AMPK α 1 can sense glucose availability to modulate mammalian target of rapamycin complex 1 and S6K⁵⁰. Thus, we provide compelling evidence that ILC2-driven lung eosinophilia modulates the extracellular availability of metabolites, which impairs efficient glycolysis-dependent effector functions of lung NK cells.

Online content

Any methods, additional references, Nature Research 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/s41590-020-0745-y>.

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Methods

In vivo animal studies. BALB/c, NOD-SCID *Il2rg*^{-/-}, C57BL/6J (B6), B6-MMTV-PyMT, B6-*Tyr*^{-/-}, B6-*Il33*^{cit/cit} (*Il33*^{-/-}), B6-*Il7ra*^{Cre/+} (provided by H.-R. Rodewald⁵¹), B6-*Il7ra*^{Cre/+}*Rora*^{loxP/loxP}, B6-*Foxp3*^{3TR}, B6- μ MT, B6-*Rag2*^{-/-} and B6-*Rag2*^{-/-}*Il7ra*^{Cre/+}*Rora*^{loxP/loxP} mice were maintained in the Cancer Research UK Cambridge Institute (CRUK-CI) animal facility under specific pathogen-free conditions. Mice housed at the CRUK-CI were kept in individually ventilated cages, between 19 and 23 °C with 45–65% humidity and a 12 h dark–light cycle. Some studies were performed at the Medical Research Council ARES facility. Mice were sex- and age-matched whenever possible; most mice were used at 8–12 weeks of age. All animal work was conducted under project license no. PD7484FB9 at the CRUK-CI (with approval from the CRUK-CI Animal Welfare and Ethical Review Body) or at the Medical Research Council ARES facility (with approval from Babraham Institute's Animal Welfare and Ethical Review Body), all in accordance with Home Office regulations.

In vivo experiments. Mice were anesthetized by isoflurane inhalation, followed by the intranasal administration of mouse recombinant IL-33 (0.2 μ g; BioLegend), IL-4 (0.5 μ g), IL-13 (0.5 μ g), GM-CSF (0.5 μ g) and IL-5 (0.5 μ g), LPS (1 μ g; Sigma-Aldrich), CpG (10 μ g; InvivoGen), *Asp* (0.01 U; Sigma-Aldrich), ragweed pollen extract (300 μ g; Greer) or clodronate liposome (30% clodronate liposome/PBS; Liposoma) in 40 μ l of PBS. Diphtheria toxin (10 ng g⁻¹; Sigma-Aldrich), IL-33R-Fc (10 mg kg⁻¹; AstraZeneca), mAb to anti-NK1.1 (50 μ g, clone PK136; Bio X Cell), anti-CCR2 (20 μ g, MC-21; provided by M. Mack⁵²), anti-CCL2 (200 μ g, MCP-1; Bio X Cell), anti-Ly-6C/G (200 μ g, GR-1; Bio X Cell), anti-Ly-6G (200 μ g, clone 1A8; Bio X Cell), anti-CD4 (100 μ g, clone GK1.5; Bio X Cell), anti-IL-10 (300 μ g, clone JES5-2A5; Bio X Cell), anti-TGF- β (400 μ g, clone 1D11.16.8; Bio X Cell), anti-IL-5 (100 μ g, clone TRFK5; Bio X Cell), rat IgG1, κ (Bio X Cell), rat IgG2a, κ (Bio X Cell) or mouse recombinant IL-33 (0.5 μ g; BioLegend) were administered by intraperitoneal injection in 100 μ l of PBS. A2AR antagonist (20 μ g, SCH 58261; Sigma-Aldrich) was administered in dimethylsulfoxide/PBS (v/v).

Tumor cells and experimental lung metastasis models. All cell lines used in this study tested negative for *Mycoplasma* and were authenticated by short tandem repeat profiling. B16-F10 (J. Shields), B16-F10-mCherry, LL/2 (M. de la Roche), B16-F10-AkaLuc, as well as 4T1 and 4T1-T (G. Hannon⁵³) tumor cells were grown in DMEM with 10% FCS. Tumor cells were detached using 0.05% Trypsin-EDTA (Gibco) and washed twice in PBS before injection. Unless otherwise stated, 1×10^5 B16-F10, 4T1 or 4T1-T, and 2×10^6 LL/2 cells were used for intravenous injection. All orthotopic injections were performed using 1×10^5 4T1 or 3×10^4 4T1-T mouse mammary tumor cells resuspended in 30 μ l of PBS. For the early seeding studies, 1×10^5 B16-F10-mCherry cells were injected intravenously and lung cells were collected 24 h postinjection. In the autochthonous breast cancer model, 12-week-old female B6-MMTV-PyMT mice were randomized and treated intranasally with IL-33, *Asp* or PBS once a week and killed at 20 weeks of age; this was followed by visual quantification of lung metastases and weighing of primary tumors. Tumors on the lung surface were quantified by counting the metastatic foci under a dissection microscope, after which lungs were collected for further processing. For survival studies, mice were treated as described in the figures and injected with B16-F10 tumor cells on day 0; mice were monitored daily and were killed when reaching the humane end point.

Live imaging studies. Third-generation lentiviral particles were produced by transient cotransfection of 293FT cells with AkaLuc plasmid⁵⁴ (provided by G. Hannon) and the three packaging constructs pMDL, CMV-Rev and VSV-G. Lentivirus-containing particles were concentrated on Centricon Plus-70 filters (Merck Millipore); B16-F10 cells were infected with concentrated lentivirus in the presence of 8 mg ml⁻¹ polybrene and selected 3 d after infection with neomycin (Geneticin 1 mg ml⁻¹; Thermo Fisher Scientific). IVIS bioluminescent imaging was performed by in vitro exposure of B16-F10-AkaLuc cells to 250 μ M of AkaLumine substrate for 30 min at 37 °C in a humidified, 5% CO₂ incubator. Cells were washed with PBS twice and 1×10^5 cells were injected intravenously into B6 (*Tyr*^{-/-}) mice and imaging was performed 2, 10 and 30 min after. Total photon emission from the thorax of each mouse was quantified with the Living Image software package (v4.3.1.0.15880) (Xenogen).

Single-cell preparation. Cell suspensions were prepared from the lung by mechanical dissociation, followed by digestion in 5 ml of Roswell Park Memorial Institute (RPMI) 1640 (Gibco) medium containing collagenase I (500 U ml⁻¹) and DNase I (0.2 mg ml⁻¹) for 45 min at 37 °C on a shaker (220 r.p.m.), followed by filtration through a 70- μ m strainer, 25% Percoll gradient enrichment of leukocytes and red blood cell (RBC) lysis. Tumor cells were recovered without Percoll enrichment. Blood cells were lysed in 5 ml RBC lysis buffer 3 times for 5 min and spleens were strained through a 70- μ m filter in RPMI 1640 before lysing erythrocytes with RBC lysis buffer for 5 min. Single cells were restimulated and stained for surface and intracellular markers (see Flow cytometry section).

Primary cell culture. Mouse primary cells were cultured in RPMI 1640 supplemented with 10% FCS, 100 U ml⁻¹ penicillin (Invitrogen) and 100 μ g ml⁻¹

streptomycin (Invitrogen). Cells were cultured at 37 °C in a humidified, 5% CO₂ incubator.

NK cell isolation and coculture. Single-cell suspensions from PBS or IL-33-sensitized lung were used to isolate NK cells by magnetic bead negative selection strategy (EasySep; STEMCELL Technologies), according to the manufacturer's protocol. NK cell purity was assessed by flow cytometry and cells were used when purity exceeded 90%. Purified NK cells were cultured overnight, alone or with carboxyfluorescein succinimidyl ester (CFSE)-labeled PBS or IL-33 lung single-cell suspensions at different ratios. Alternatively, NK cells were cocultured with sort-purified ILC2 cells overnight in equal concentrations.

Eosinophil culture and adoptive transfer. Bone marrow-derived eosinophils were cultured as described elsewhere⁵⁵. Briefly, bone marrow-derived progenitor cells were differentiated for 4 d in IMDM with GlutaMAX-1 (Gibco) with 10% FCS, 100 U ml⁻¹ penicillin and 100 μ g ml⁻¹ streptomycin supplemented with 100 ng ml⁻¹ Stem Cell Factor (PeproTech) and 100 ng ml⁻¹ Flt3 ligand (PeproTech) at a concentration of 1×10^6 per ml. Thereafter, expanded progenitor cells were differentiated into eosinophils using 10 ng ml⁻¹ recombinant mouse IL-5 (PeproTech) for 10 d; the derived eosinophils were subsequently cocultured with purified NK cells at different ratios or adoptively transferred into recipient mice (3×10^6 per mouse).

Glucose and lactate cultures. For the in vitro glucose uptake experiments, mouse primary cells were cultured in glucose-free RPMI 1640 supplemented with 10% FCS and 2-NBDG (50 μ M; Thermo Fisher Scientific). Cells were cultured for 1 h at 37 °C in a humidified, 5% CO₂ incubator, followed by collection, processing and flow cytometry analysis at 4 °C. For the glucose-deficient or glucose-high experiments, mouse primary lung cells were cultured in glucose-free RPMI 1640 supplemented with 10% FCS $\pm$ glucose (2.0 or 4.5 mM), 100 U ml⁻¹ penicillin and 100 μ g ml⁻¹ streptomycin. Cells were cultured for 3 h at 37 °C in a humidified, 5% CO₂ incubator, followed by collection, processing and flow cytometry analysis at 4 °C. For the lactic acid experiments, mouse primary lung cells were cultured in RPMI 1640 supplemented with 10% FCS, 100 U ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin and either lactic acid (15 mM, pH 6.4; Sigma-Aldrich), HCl (pH 6.4) or normal pH (7.4). Cells were cultured for 3 h at 37 °C in a humidified, 5% CO₂ incubator, followed by collection, processing and flow cytometry analysis at 4 °C. For intracellular cytokine detection, PMA + ionomycin and protein transport inhibitor (Thermo Fisher Scientific) was added during the 3-h culture.

Sample preparation and ¹H-NMR analysis. For metabolic profiling of lung cultures, WT mice were treated with IL-33 or PBS on days 0 and 1, followed by culture of 1×10^6 cells (whole lung) for 18 h in 200 μ l of RPMI 1640 supplemented with 10% FCS, 100 U ml⁻¹ penicillin and 100 μ g ml⁻¹ streptomycin. Cells were cultured at 37 °C in a humidified, 5% CO₂ incubator. Three technical replicates were set up for each mouse and combined (600 μ l) for NMR analysis. Then, 590 μ l of sample was placed in a 5-mm Wilmad standard NMR tube and 10 μ l of 60 mM of 3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid sodium salt (TSP) was added as the chemical shift and internal quantitation standard. ¹H-NMR spectra were acquired on a 600 MHz AVANCE NMR spectrometer (Bruker) using a water presaturation sequence with 1,024 averages, 5 s repetition time, 2.28 s acquisition time (repetition time = 7.28 s) and 32 K time domain data points. Time domain data were preprocessed by 0.5 Hz line broadening, Fourier transformation, zero and first-order phase correction. Chemical shifts were assigned from the human metabolomic data base (<http://www.hmdb.ca/>) and from our own two-dimensional NMR spectral (correlation spectroscopy and total correlation spectroscopy) data. The absolute metabolite concentrations of the media were estimated using the NMR suite v.8.3 (Chenomx) using the internal standard TSP concentration.

Cytotoxicity assays. To determine their ex vivo cytotoxic capacity, we purified PBS- or IL33-exposed NK cells from mouse lungs using a magnetic bead negative selection strategy according to the manufacturer's protocol. Purified NK cells (>85%) were subsequently CFSE-stained (5 μ M final concentration in PBS) and cocultured with >1.5 week-passaged YAC-1 cells at a 10:1 (NK:YAC-1) ratio in RPMI 1640. The addition of annexin V red reagent (Essen Bioscience) allowed us to follow target cell killing using the IncuCyte platform (IncuCyte software v.2018A; Essen Bioscience).

Flow cytometry. Single cells were incubated with anti-mouse CD16/CD32 (Thermo Fisher Scientific) to block Fc receptors and stained as indicated. The lineage cocktail contained the following: $\pm$ CD3 (clone 145-2C11; Thermo Fisher Scientific); $\pm$ NK1.1 (clone PK136; Thermo Fisher Scientific); T cell antigen receptor- β ; CD5 (clone 53-7.3; Thermo Fisher Scientific); CD19 (clone 1D3; Thermo Fisher Scientific); CD11b (clone M1/70; Thermo Fisher Scientific); CD11c (clone N418; Thermo Fisher Scientific); Fc ϵ R1 α (clone MAR-1; Thermo Fisher Scientific); F4/80 (clone BM8; Thermo Fisher Scientific); Ly-6G/Ly-6C (clone Rb6-8C5; Thermo Fisher Scientific); and TER-119 (clone TER-119; Thermo Fisher Scientific) all on eFluor 450 (Thermo Fisher Scientific). For intracellular staining, we used the Foxp3/Transcription Factor Staining Buffer Set (Thermo

Fisher Scientific) or Cytofix/Cytoperm Kit (BD Biosciences) according to the manufacturer's instructions. For intracellular cytokine detection, single cells were stimulated with PMA (60 ng ml^{-1}) and ionomycin (500 ng ml^{-1}) plus $1 \times$ protein transport inhibitor, $1 \times$ cytokine stimulation cocktail (Thermo Fisher Scientific) or plate-bound anti-NK1.1 mAb ($10\text{--}30 \mu\text{g ml}^{-1}$) or recombinant IL-12 (20 ng ml^{-1}) and IL-18 (5 ng ml^{-1}) in culture medium (RPMI 1640, 10% FCS) at 37°C for 3 h before staining. Data were acquired on a BD LSRFortessa or Symphony instrument; cells were quantified using CountBright beads (Thermo Fisher Scientific). Data were analyzed using FlowJo X (FlowJo LLC).

The following antibodies and their conjugates were used: B220 (clone RA3-6B2; APC-eFluor 780; Thermo Fisher Scientific); CD3e (clone 145-2C11; PE-Cy7 and eFluor 450; catalog no. 25-0031-83, lot no. 4304567; Thermo Fisher Scientific); CD4 Alexa Fluor 700 (clone RM4-5; Thermo Fisher Scientific); CD5 (clone 53-7.3; eFluor 450; Thermo Fisher Scientific); CD8a (clone 53-6.7; PerCP-eFluor 710 and Super Bright 645; Thermo Fisher Scientific); CD11b (clone M1/70; eFluor 450, APC-eFluor 780 and BV785; Thermo Fisher Scientific); CD11c (clone N418; eFluor 450 and Alexa Fluor 700; Thermo Fisher Scientific); CD16/CD32 (clone 93; BioLegend); CD19 (clone 1D3; eFluor 450; Thermo Fisher Scientific); CD31 (clone 390; Brilliant Violet 605; BioLegend); CD45 (clone 30-F11; Brilliant Violet 510; BioLegend); CD49b (clone HM α 2; Brilliant Violet 650; BD Biosciences); CD64 (clone X54-5/7.1; Brilliant Violet 711; BioLegend); CD127 (clone SB/199; PE-CF594; BD Biosciences); CD172a (clone P84; Alexa Fluor 488; BioLegend); EP-CAM (clone G8.8; Brilliant Violet 711; BioLegend); Fc ϵ R1 α (clone MAR-1; eFluor 450 and PerCP-eFluor 710); Fixable Viability Dye (eFluor 455UV; Thermo Fisher Scientific); F4/80 (clone BM8; eFluor 450 and APC-eFluor 780); FDXP3 (clone FJK-16s; Alexa Fluor 488; Thermo Fisher Scientific); Gata-3 (clone TWAJ; eFluor 660; Thermo Fisher Scientific); Gzmb (clone NGZB; PerCP-eFluor 710); I-A/I-E (clone 2G9; BUV395; BD Biosciences); CD278 (ICOS) (clone 7E.17G9; PE; Thermo Fisher Scientific); IFN- γ (clone XMGI.2; Brilliant Violet 786; BioLegend); APC IL-5 (clone TRFK5; BD Biosciences); Ki-67 (clone B56; Brilliant Violet 786); Ly-6G/Ly-C (clone Rb6-8C5; eFluor 450); Ly-6G (clone 1A8-Ly6g; PE-eFluor 610; Thermo Fisher Scientific); Ly-6C (clone HK1.4; PE-Cy7; Thermo Fisher Scientific); NK1.1 (clone PK136; BUV395; BD Biosciences; eFluor 450, Thermo Fisher Scientific); RELM α (DS8RELM; APC; Thermo Fisher Scientific); Phospho-S6 (clone cupk43k; PE; Thermo Fisher Scientific); Podoplanin (clone 8.1.1; PE-Cy7; Thermo Fisher Scientific); RORyt (clone Q31.378; PerCP-eFluor 710); CD170 (Siglec F) (clone 1RN44N; Super Bright 600; Thermo Fisher Scientific); IL-33R (ST2) (RMST2-2; PE; Thermo Fisher Scientific); TER-119 (TER-119; eFluor 450).

Metabolic analysis. ECAR was measured using the Seahorse XF96 Extracellular Flux Analyzer (Agilent Technologies). PBS or IL-33 lung homogenates were stimulated for 3 h with PMA (60 ng ml^{-1}) and ionomycin (500 ng ml^{-1}) at 37°C in a 5% CO_2 incubator. Cells were collected and NK cells were purified using a magnetic bead negative selection strategy according to the manufacturer's protocol. Cells were suspended in XF base minimal RPMI (pH 7.4) with L-glutamine (2 mM) and were plated into a culture microplate (5×10^5 cells per well; Agilent Technologies). Before real-time measurement of the ECAR, glucose (20 mM), oligomycin (1.5 μM) and 2DG (50 mM) were added.

MSI sample preparation, acquisition and data analysis. Mice were anesthetized with isoflurane, maintained at 37°C and administered with [^{13}C]glucose (Cambridge Isotope Laboratories) as a bolus at 0.4 mg g^{-1} , followed by continuous infusion of $0.012 \text{ mg g min}^{-1}$ at $300 \mu\text{l h}^{-1}$ for 120 min⁶⁰. The lungs were rapidly removed and snap-frozen in isopentane chilled with dry ice and stored at -80°C until sectioning. Lungs were sectioned to a 10- μm thickness using a CM3050 cryomicrotome (Leica Biosystems) and thaw-mounted onto Superfrost slides (Thermo Fisher Scientific) for desorption electrospray ionization (DESI) MSI and histological examination. Tissue section slides were vacuum-packed and stored at -80°C until analysis.

DESI-MSI was carried out using an automated two-dimensional DESI source (Prosolia) with home-built sprayer assembly⁵⁷ mounted to a Q Exactive Fourier transform mass spectrometer (Thermo Fisher Scientific). Analyses were performed at a spatial resolution of 40 μm in negative ion mode; mass spectra were collected in the 80–900 Da range with the mass resolving power set to 70,000 at m/z 200 and an S-lens setting of 100. Methanol/water (95:5 v/v) was used as the electrospray solvent at a flow rate of $1.0 \mu\text{l min}^{-1}$ and a spray voltage of -4.5 kV . The distance between DESI sprayer and the MSI inlet was 7 mm, while the distance between sprayer tip and sample surface was 1.5 mm at an angle of 75° . Nitrogen N4.8 was used as the nebulizing gas at a pressure of 6.5 bar. The Omni Spray 2D (Prosolia, v2.0.1) and Xcalibur (Thermo Fisher Scientific, v4.1.31.9) software was used for mass spectrometry data acquisition. Individual line scans were converted into centroids (mzML format) using MSConvert (ProteoWizard toolbox v3.0.4043) and subsequently into imzML format with imzML converter v1.3. Hematoxylin and eosin (H&E) staining was performed post-analysis on the same lung sections; the stained sections were imaged at 40 $\times$ with an Aperio CS2 digital pathology scanner (Aperio Tech) and visualized with the ImageScope software (Aperio Tech, v12.4.0.5043).

MSI data were visualized and analyzed using the SCiLS Lab MVS 2019b software (SCiLS). MSI images were normalized to the root mean square to

compensate for signal instabilities and allow comparisons between multiple experiments. Exact mass measurements only were used for metabolite identification with mass deviations <8 parts per million. For labeled metabolites, no mass interferences were found on noninfused sample MSI images (metabolite; ion cluster; m/z theoretical; m/z measures; error; lactate [M-H] $^-$: 89.024418, 89.02386; 6.3 [U- ^{13}C]lactate [M-H] $^-$: 92.033379, 92.03269; 7.5 glucose [M+Cl] $^-$: 215.032789, 215.03241; 1.8 [U- ^{13}C]glucose [M+Cl] $^-$: 221.05291, 221.05359, 3.1).

A bisecting k -means algorithm using a weak pixel denoising and distance correlation as parameters was applied to provide unsupervised clustering of MSI images to create regions corresponding to each lung sample. Then, the mean relative abundance of each molecular species for the pixels of the image was extracted from each sample and expressed in arbitrary units (AUs).

Histology. Lung lobes were fixed in 10% neutral buffered formalin in PBS for 24 h, followed by transfer to 70% ethanol in PBS for another 24 h and embedded into paraffin; 3- μm sections were cut and stained with Ki-67. The CRUK-CI Histology Core performed tissue embedding, sectioning and staining. Image quantification was performed using the HALO software (Indica Labs, v2.3).

Bulk RNA-seq. Mouse lungs were dissected and made into a single-cell suspension as described earlier, followed by staining with anti-mouse CD16/CD32 to block Fc receptors. The lineage cocktail consisted of: anti-CD5; anti-CD19; anti-CD11c; anti-Fc ϵ R1 α ; anti-F4/80; anti-Ly-6G/Ly-6C; anti-TER-119 eFluor 450; anti-CD45 Brilliant Violet 510; anti-CD49b Brilliant Violet 605; anti-NK1.1 BUV395; anti-CD3 PE-Cy7; anti-B220 APC-eFluor 780; and anti-CD4 Alexa Fluor 700. Dead cells were excluded by 4,6-diamidino-2-phenylindole staining, which was followed by electronic gating of live CD45 $^{\text{int}}$ Lineage $^{\text{int}}$ CD3 $^{\text{CD4}}$ B220 $^{\text{NK1.1}}$ CD4 9b $^+$ NK cells that were purified by fluorescence-activated cell sorting (FACS) (BD Aria II; Becton Dickinson). Purity checks were performed after each sorting, with all used samples being $>95\%$ pure; 1 μg of total RNA was used as the input material for library preparation. The NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs) was used to isolate poly(A) RNAs. Libraries were generated with the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (New England Biolabs) according to the manufacturer's instructions. The pooled libraries were quantified with the KAPA Library Quantification Kit for Illumina (KAPA Biosystems) and sequenced (single end 50 nt) on an Illumina HiSeq 4000 system.

Bulk RNA-seq analysis. Sequence data were aligned to the GRCh38 mouse reference genome using STAR v2.7.3 (ref. ⁵⁸). Quality control metrics were computed using Picard's CollectAlignmentStatistics and CollectRnaSeqMetrics (<https://broadinstitute.github.io/picard>, v2.20.8). The assignment and quantification of reads to transcripts from Ensembl release 98 (<https://www.ensembl.org>) were performed using featureCounts from the Subread package v1.5.2 (ref. ⁵⁹). DESeq2 v1.26.0 (ref. ⁶⁰) was used to estimate size factors for normalization, estimate dispersions, fit a negative binomial generalized linear model (GLM) and calculate the Wald statistics for differential expression between sample groups. Gene set enrichment analysis was performed using the GSEA (v4.0.3) software and gene set collection 7.1 (Hallmark signatures). (<http://software.broadinstitute.org/gsea/index.jsp>).

A power calculation was carried out with PROPER⁶¹ using estimates of the variance from the first bulk RNA-seq analysis where there were three replicates for each group. The second RNA-seq analysis had 7 replicate samples taken from mice on day 2 after treatment with PBS and IL-33. The data for one replicate in the IL-33 group failed quality control based on depth of coverage and clustering in a principal components analysis and was discarded.

scRNA-seq processing and quality control. WT mouse lung NK cell FACS purification was performed identical to the bulk RNA-seq experiments. Mapping and counting of 10x Genomics scRNA-seq reads was performed using Cell Ranger v3.1.0 without filtering droplets. Reads with swapped barcodes and empty droplets were identified for filtering using the swappedDrops and emptyDrops functions from the DropletUtils v1.4.3 Bioconductor package^{62,63}. Data were further processed using the scan v1.12.1, scatter v1.12.2 and SingleCellExperiment v1.6.0 Bioconductor packages^{64,65}. Poor quality cells with $\log_{10}$ total unique molecular identifiers more than 3 median absolute deviations (MADs) below the median, total genes detected more than 3 MADs below the median or a $\log_{10}$ ratio of mitochondrial to nonmitochondrial gene unique molecular identifiers deviating more than 3 MADs from the median were removed. Normalization was carried out by deconvolving cell pools as implemented in the computeSumFactors function from the scan Bioconductor package⁶⁶. To remove contaminating myeloid cells, we first identified lung myeloid- and NK cell-specific genes using the following sources: population comparison of splenic NK cells versus lung alveolar macrophages from the ImmGen RNA-seq database (accessed 26 October 2019)⁶⁷ (the analysis specified genes always expressed in one population and never in the other; the top 10 with the highest expression were selected); activated macrophage markers from Holtzman et al.⁶⁸; alveolar macrophage genes from Misharin et al.⁶⁹; and NK cell genes from Crinier et al.⁷⁰. Expression of myeloid-specific and NK cell-specific genes was largely mutually exclusive. Cells that expressed more myeloid-specific than NK cell-specific genes were classified as contaminating cells and were removed.

scRNA-seq analysis. Clustering was performed using the Louvain method on a shared nearest neighbor graph as implemented in the *scran* and *igraph* v.1.2.4.1 packages. Marker genes for each cluster were identified using the *findMarkers* function from *scran*, selecting genes differentially expressed compared to all other clusters. Cell-specific scores for cell cycle phases were calculated using the *CellCycleScoring* function from the *Seurat* v.3.1.1 package⁷¹ after converting supplied human phase-associated genes to their murine orthologs using *BioMart* (*biomaRt* v.2.40.5). Cluster abundances were modeled using a quasi-likelihood negative binomial GLM and differential abundances between PBS- and IL-33-treated samples tested with empirical Bayesian quasi-likelihood *F*-tests, as implemented in the *Bioconductor* package *edgeR* v.3.26.8 (ref.⁷²). Abundance analyses were performed by normalizing based on cell number and without estimating a trend on the dispersion due to the small number of clusters. After filtering for genes detected in at least 1% of cells, differential expression analysis controlling for cluster abundances and cluster-specific treatment effects was also performed through the *edgeR* negative binomial GLM framework⁷³, modeling gene expression as a function of sample, cluster and cluster-sample interactions. A likelihood ratio test was performed contrasting the sample coefficients of the IL-33-treated and PBS-treated samples. The analysis code for scRNA-seq is available at <https://github.com/MarioniLab/scRNAseqLungNKcells>.

Statistics. Analysis for two groups were calculated using an unpaired two-tailed Student's *t*-test; comparisons of more than two groups were calculated using a one-way analysis of variance (ANOVA) with Tukey post-analysis, or log-rank (Mantel–Cox) test where necessary. Data were analyzed using Prism 8 (GraphPad Software) with $P \leq 0.05$ considered significant.

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

Data availability

The transcriptomic data used in Fig. 6 and Extended Data Fig. 7 are available at the SRA under the BioProject no. [PRJNA637311](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA637311): lung NK cell transcriptomic. Bulk RNA-seq data are available under BioSample accession nos. [SAMN15099850](https://www.ncbi.nlm.nih.gov/biosample/SAMN15099850) (bulk PBS versus IL-33; the sample names are A1, D1, B2, C2, F2, E1 and H1 for the PBS-treated condition and D2, B1, F1, E2, A2 and G1 for the IL-33-treated condition) and [SAMN15099866](https://www.ncbi.nlm.nih.gov/biosample/SAMN15099866) (time course) with the following triplicate sample names: E4, A4, C3 (WT PBS day 2); H3, F4, D3 (WT IL-33 day 2); C4, G4, A3 (WT IL-33 day 7); B4, E3, G3 (WT IL-33 day 14); and D4, F3, B3 (ILC2 knockout IL-33 day 2).

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Author contributions

M.J.S. designed and conducted the experiments and wrote the manuscript. S. Png, A.C.R., A.T., G.J.H., S. Pinaud, C.G., A.N., J. Stockis, J. Su, A.R. and E.M.S. assisted with the experiments or analysis. J.D.S., M.D.E., A.N.J.M., H.-R.R., M.M., G.J.H., X.R.R., E.S.C., R.J.A.G., K.M.B. and J.C.M. provided reagents and/or advice. T.Y.F.H. supervised the study, designed and conducted experiments and wrote the manuscript.

Competing interests

G.H., X.R.R., R.J.A.G. and E.S.C. are employees of AstraZeneca and have stock/stock options in AstraZeneca.

Additional information

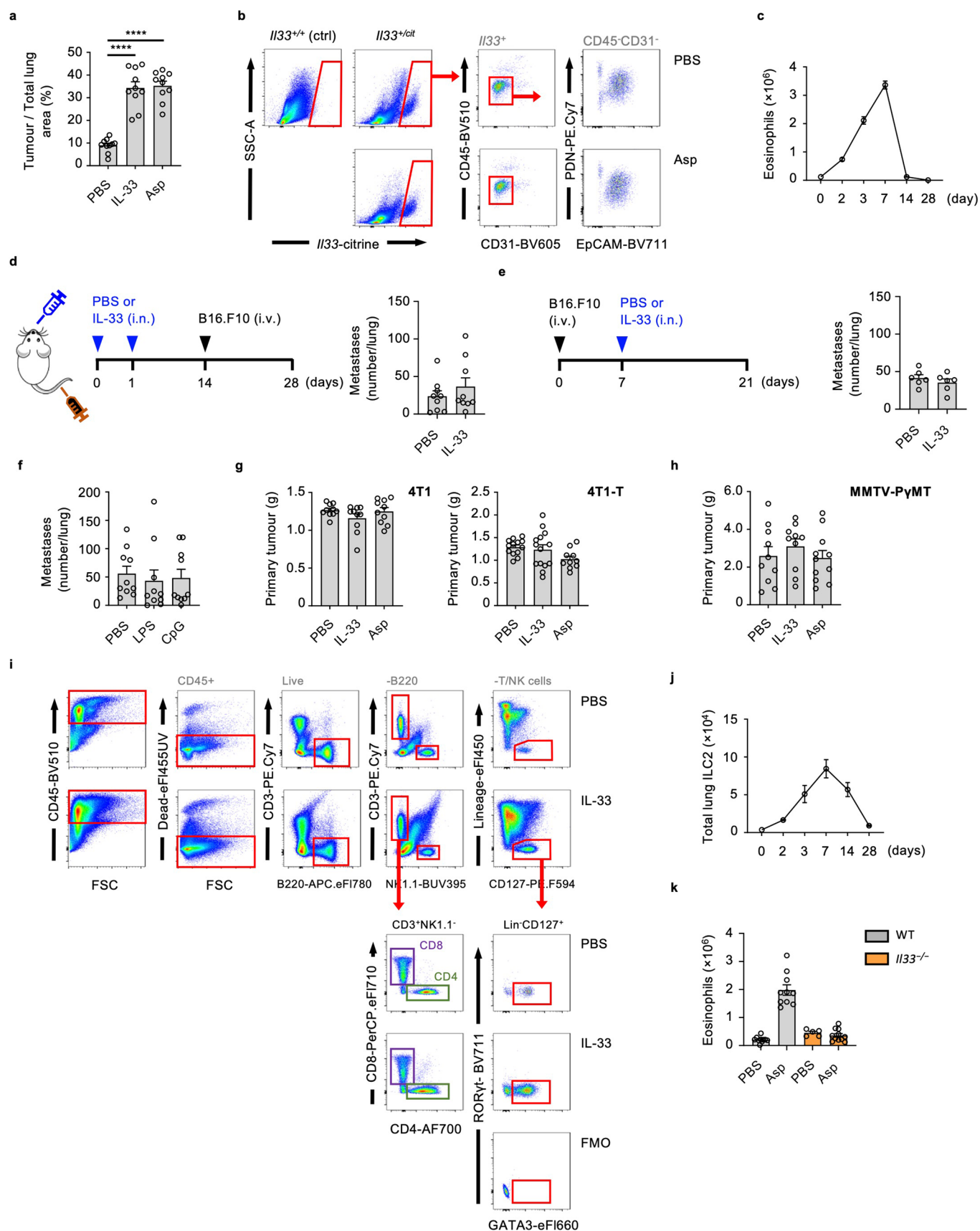
Extended data is available for this paper at <https://doi.org/10.1038/s41590-020-0745-y>.

Supplementary information is available for this paper at <https://doi.org/10.1038/s41590-020-0745-y>.

Correspondence and requests for materials should be addressed to T.Y.F.H.

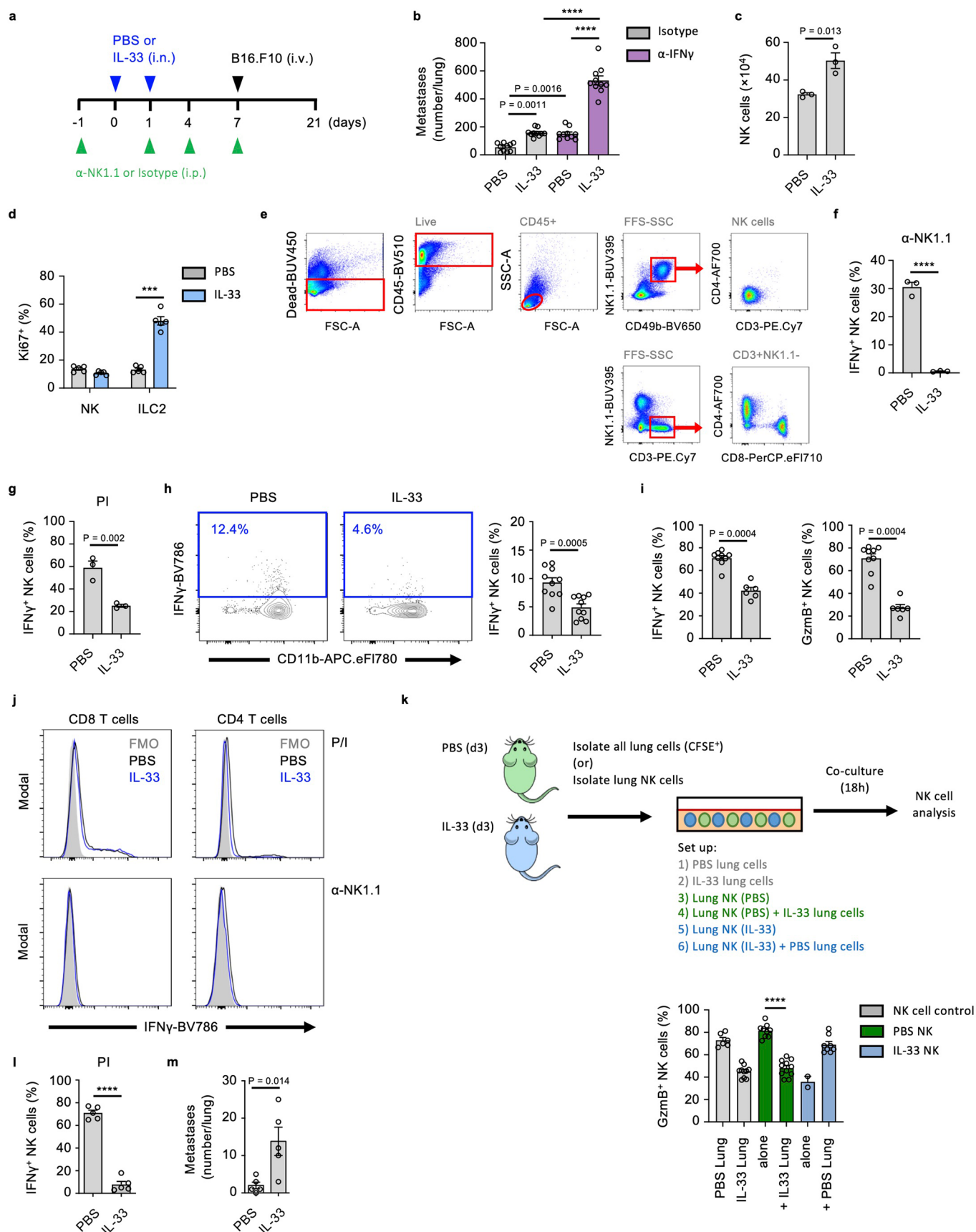
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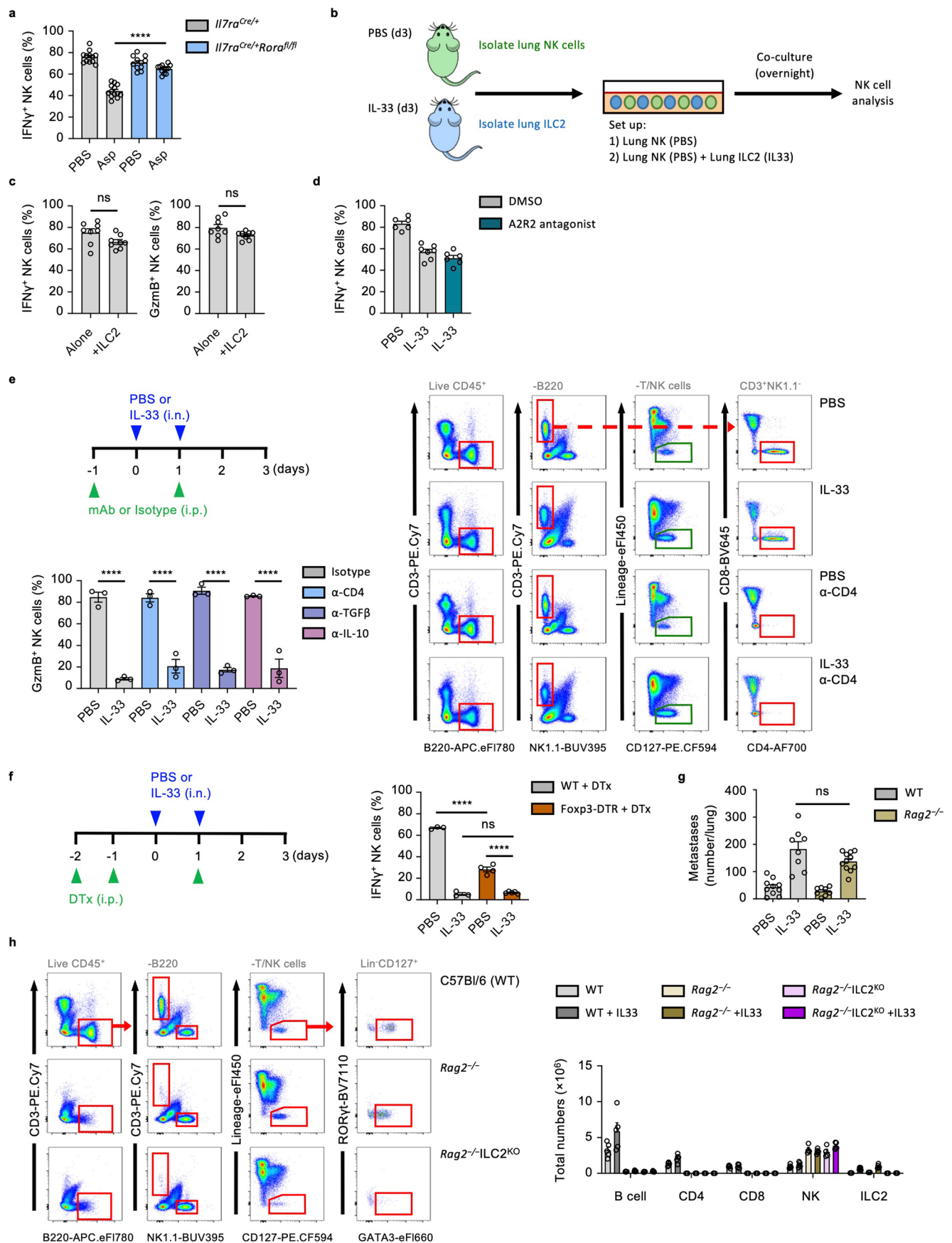
Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Pre-existing innate type-2 inflammation promotes lung metastasis formation. **a**, WT mice were treated as in (Fig. 1a) and tumor burden was assessed by histological staining for Ki67⁺ tumor foci and quantification of tumor area over total lung area by automated image analysis (n = 10). **b**, *Il33^{cre/+}* reporter mice were treated intranasally with PBS or IL-33 on days 0 and 1, and citrine⁺ lung cells were assessed on day 3. **c**, WT mice were treated intranasally with IL-33 on days 0 and 1 (or PBS injection for day 0), followed by quantification of total lung eosinophils by flow cytometry (see Extended Data Fig. 4a for gating strategy) at the indicated time-points (n = 3). **d**, WT mice were treated as indicated, followed by visual quantification of lung metastases on day 28 (n = 9). **e**, WT mice were treated as indicated, followed by visual quantification of lung metastases on day 21 (n = 6). **f**, WT mice were treated intranasally with LPS, CpG or PBS on days 0 and 1, followed by intravenous transfer of B16.F10 cells on day 7, and sacrifice on day 21, followed by visual quantification of lung metastases (n = 10). **g**, 4T1 (n = 10) or 4T1-T (n = 13,14,10) breast cancer cells were implanted in the mammary fat pad (see Methods), primary tumors were dissected and weighed. **h**, B6.MMTV-PyMT mice were randomized, treated intranasally with PBS, IL-33, or Asp (from week 12 to 20, see Methods), followed by measurement of total primary breast tumor(s) weight at 20 weeks of age (n = 10,10,11). **i**, Representative flow cytometry gating strategy of lung lymphocytes and innate lymphocyte populations from WT mice treated with PBS or IL-33 on day 0 and 1, followed by sacrifice on day 3 description of gated cells is listed above the dot plot, cell exclusion performed by Boolean-gating. **j**, WT mice were treated as in (c), followed by quantification of total lung ILC2 (Live CD45⁺CD3⁻B220⁻NK1.1⁻Lineage⁻CD127⁺RORγt⁺GATA3⁺) at the indicated time points (n = 3). **k**, WT and *Il33^{-/-}* mice were treated with PBS or Asp on day 0 and 1, followed by quantification of total lung eosinophils at day 3 (n = 10,10,5,10). Bar graphs indicate mean (±SEM) and show combined data of two (d-f, k, g) or three (a, h), or representative of three independent experiments (b, c, i, j, c). Statistical analyses were calculated using one-way ANOVA with **** = p ≤ 0.0001.



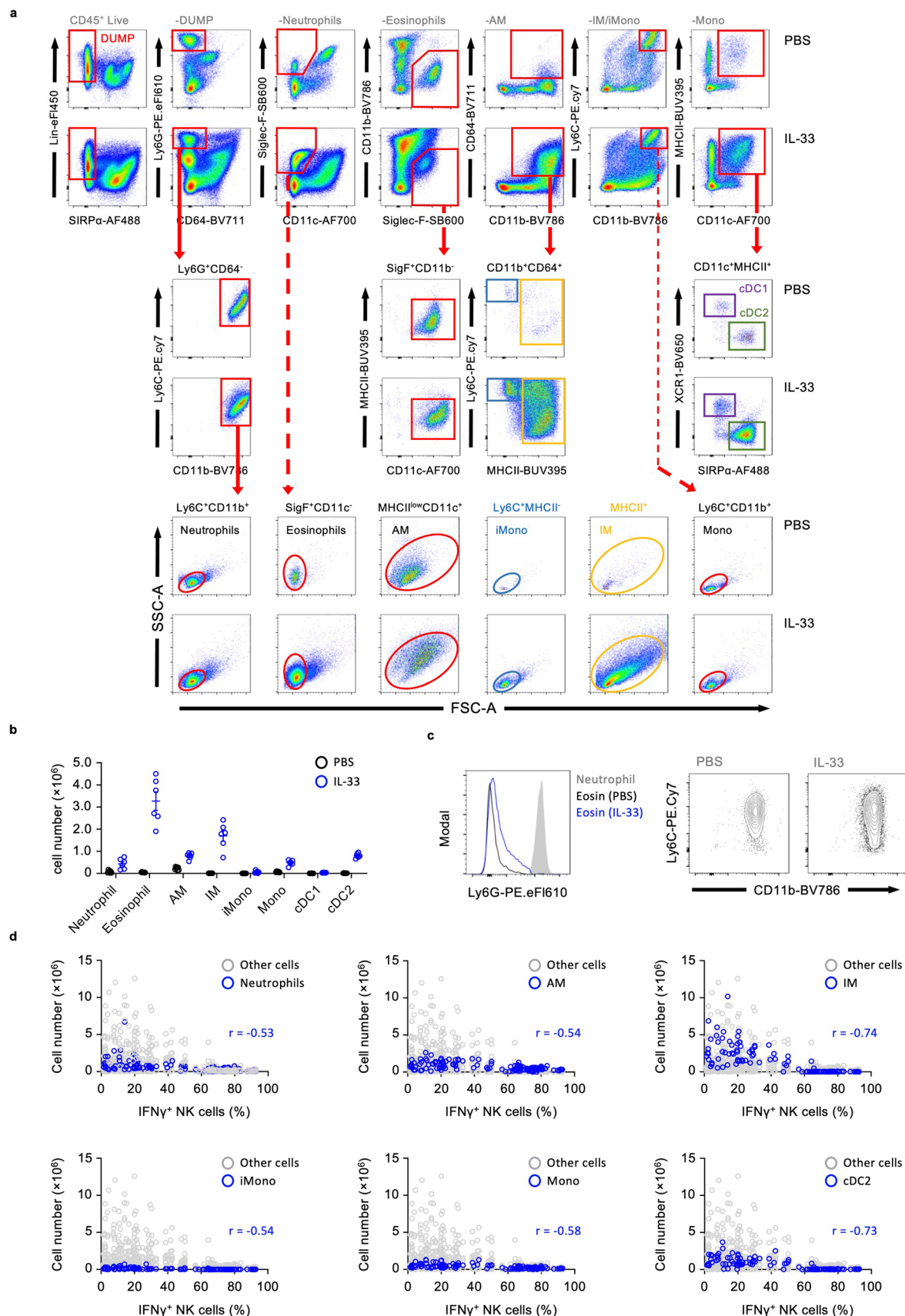
Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | IL-33 influences lung NK cells. **a**, WT mice were treated as indicated, followed by visual quantification of lung metastases on day 21. **b**, WT mice were treated with anti-IFN γ or control mAb similar to (**a**); Tumor burden was assessed on day 21 by visual quantification of lung metastases ($n=10$). **c**, Mice were treated intranasally with PBS or IL-33 on days 0 and 1, followed by quantification of total lung NK cells by flow cytometry on day 3 ($n=3$). **d**, WT mice were treated as in (**c**), followed by flow cytometric detection of Ki-67 $^{+}$ lung NK cells and ILC2 ($n=5$). **e**, Representative flow cytometry gating strategy of lung NK cells and T lymphocytes from WT mice treated with PBS or IL-33 on day 0 and 1, followed by sacrifice on day 3. **f,g**, WT mice were treated as in (**c**), followed by quantification of IFN γ^{+} NK cells (Live CD45 $^{+}$ NK1.1 $^{+}$ / low CD49b $^{+}$) after 3 hr stimulation of total lung cells with plate bound anti-NK1.1 (**f**) ($n=3$); or PI (**g**) ($n=3$). **h**, Total WT mouse lung cells were stimulated for 3 hrs *ex vivo* with a combination of IL-12 and IL-18, followed by quantification of IFN γ^{+} positive NK cells ($n=10$). **i**, WT mice were treated as in (**c**) and cardiac WAT NK cell were analyzed for intracellular IFN γ ($n=9,6$) and GzmB ($n=9,6$) after 3 hr stimulation with PI. **j**, Lung CD4 and CD8 T cells from PBS or IL-33 treated WT mice were analyzed for intracellular IFN γ after 3 hr stimulation with PI or anti-NK1.1 (representative gating shown in (**e**)). **k**, WT mice were treated as in (**c**), followed by lung NK cells purification and co-cultured with CFSE labelled whole lung homogenates from PBS or IL-33 treated WT mice (12 h), followed by 3 hr PI stimulation and detection of GzmB positive NK cells; grey bars indicate CFSE-labelled NK cells present in whole lung homogenates ($n=6,11,10,11,2,8$ biologically independent samples). **l-m**, WT mice housed at the MRC ARES facility were treated as in (**c**), followed by quantification of percent of IFN γ^{+} NK cells (after 3 hrs of PI) (**l**) ($n=5$), or treated as in Fig. 1a (50 K B16.F10) followed by visual quantification of lung metastases (**m**) ($n=5$). Bar graphs indicate mean ($\pm$ SEM) and show combined data of two (**b, i**) or three (**h, k**) independent experiments. (**e, j**) show representative flow cytometry plots of three independent experiments, whereas (**c, d, f, g, l** and **m**) show a representative bar graph of two independent experiments. Statistical analyses were calculated using one-way ANOVA or unpaired two-tailed Student's t-test (**c, f-i, l, m**) **** = $p \leq 0.0001$.



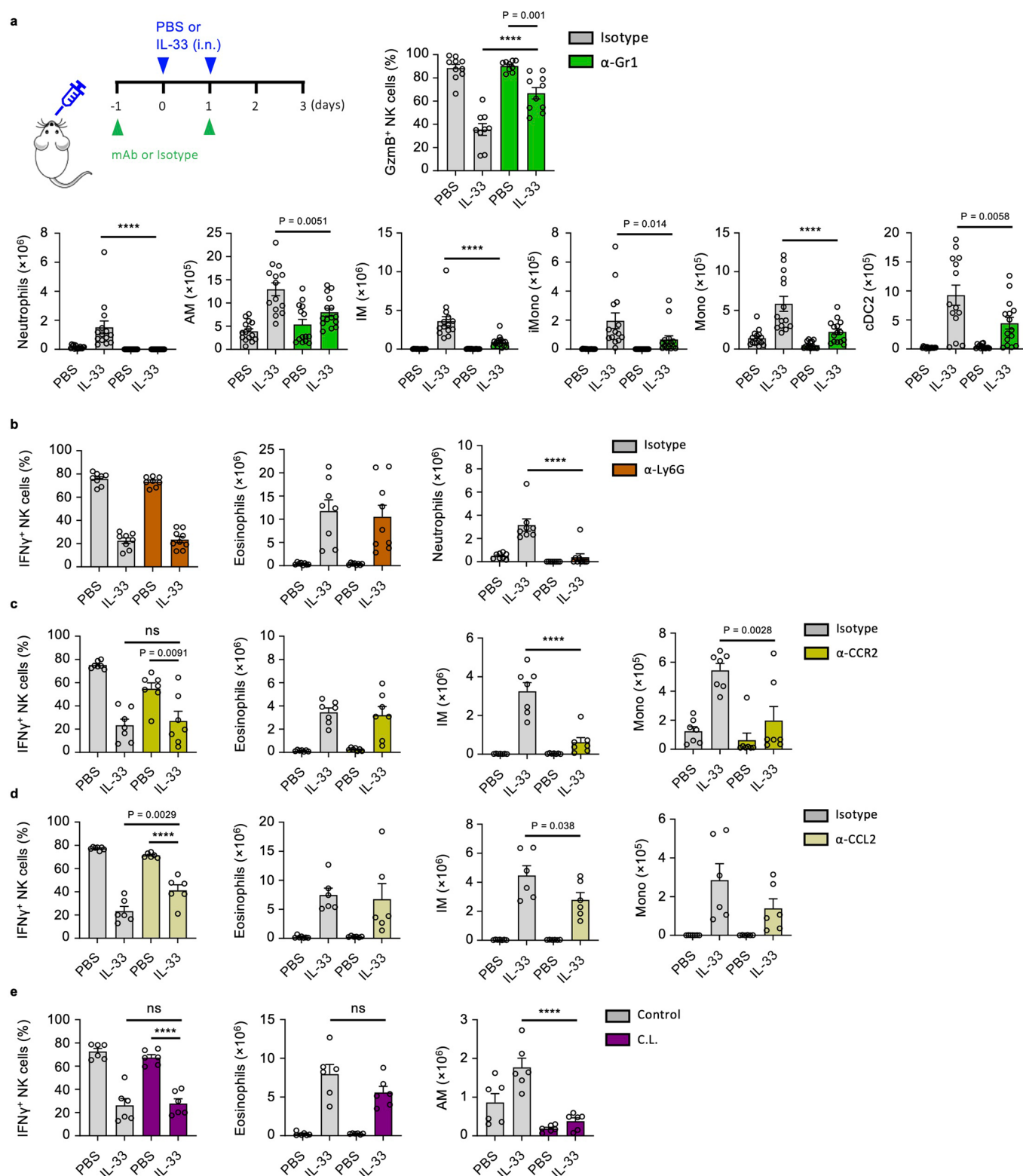
Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | ILC2 suppress NK cells via an indirect innate immune mechanism. **a**, *Il7ra*^{Cre/+} or *Il7ra*^{Cre/+}*Rora*^{fl/fl} mice were treated with PBS or Asp on day 0 and 1, followed by quantification of IFN γ ⁺ lung NK cells on day 3 ($n=12,13,11,12$). **b,c**, WT mice were treated as in **(a)**, followed by purification of lung ILC2 (flow cytometry) and lung NK cells (magnetic bead, see Methods) on day 3. Lung NK were cultured alone or with ILC2 and analyzed for intracellular IFN γ and GzmB (**c**) ($n=8$). **d**, WT mice were treated as in **(e)** with PBS or IL-33 and A2AR antagonist or DMSO followed by quantification of IFN γ ⁺ lung NK cells on day 3 ($n=6,7,6$). **e**, WT mice were treated as indicated, followed by quantification of GzmB⁺ lung NK cells ($n=3$, representative gating on right). **f**, WT and Foxp3^{DTR} mice were treated with PBS or IL-33 and DTx, followed by quantification of percent IFN γ ⁺ lung NK cells on day 3 ($n=3,3,4,5$). **g**, WT and *Rag2*^{-/-} mice were treated with PBS or IL-33 on days 0 and 1, and given adoptive transfer of LL/2 cells (i.v.) on day 7. Tumor burden was assessed on day 21 by visual quantification of lung metastases ($n=10,8,8,10$). **h**, WT, *Rag2*^{-/-} and *Rag2*^{-/-}*Il7ra*^{Cre/+}*Rora*^{fl/fl} mice were treated with PBS or IL-33 on day 0 and 1, followed by flow cytometry analysis for the indicated lung lymphoid cells on day 3 ($n=5$). Bar graphs indicate mean ($\pm$ SEM) and show combined data of two (**c**, **d**, and **g**) or three (**a**) independent experiments. (**e**, **f**, and **h**) shows a representative bar graph of two independent experiments. Statistical analyses were calculated using one-way ANOVA with ns = not significant, and **** = $p \leq 0.0001$.

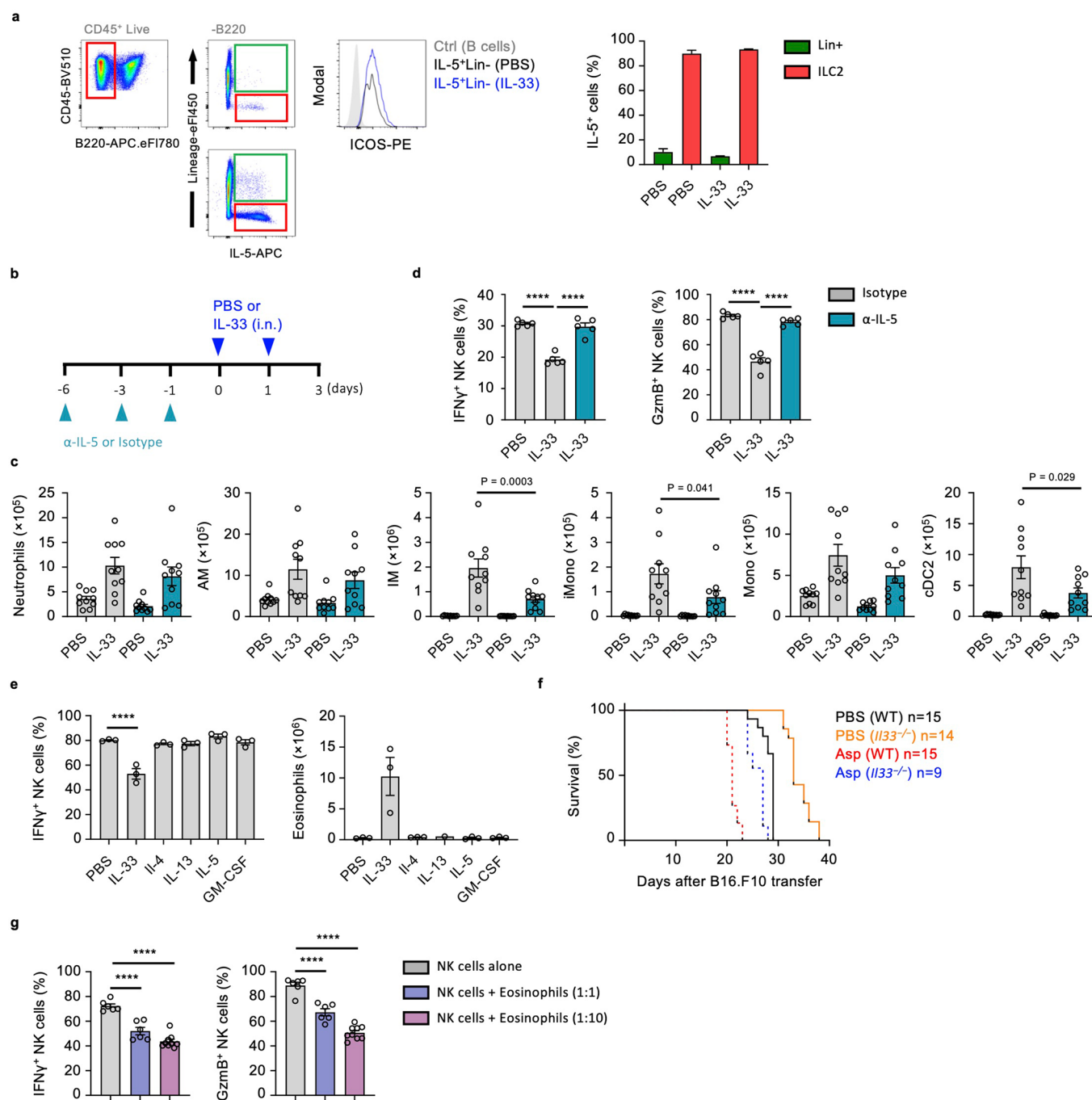


Extended Data Fig. 4 | See next page for caption.

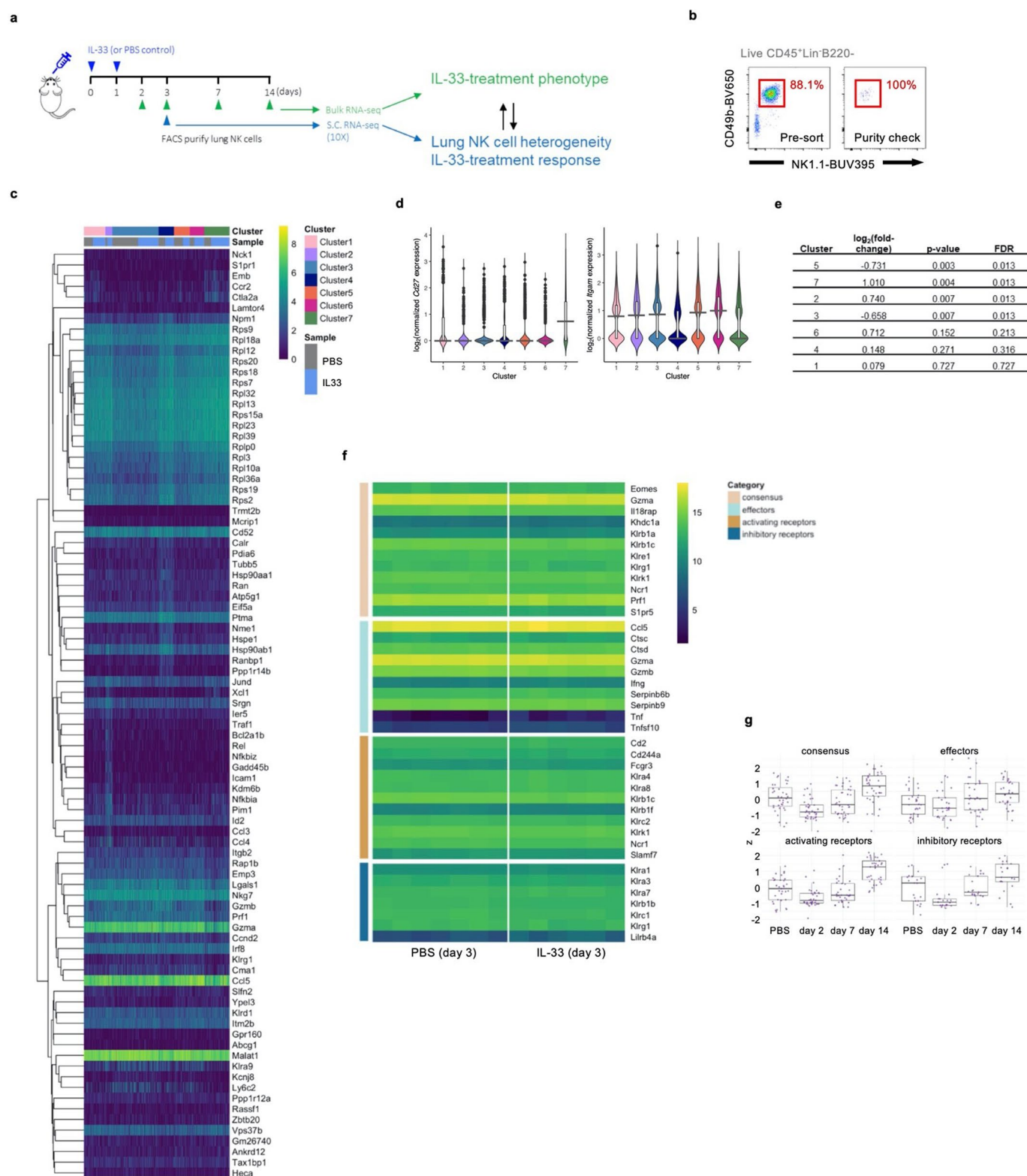
Extended Data Fig. 4 | Myeloid cell profiling after IL-33 administration and correlation with NK cell function. **a,b**, WT mice were treated with PBS or IL-33 on day 0 and 1, followed by **(a)** flow cytometry analysis and **(b)** quantification for the indicated lung myeloid cells on day 3 ($n=6$). **c**, Representative flow cytometry plot of eosinophils from mice injected and gated as in **(a)** assessed for expression of Ly-6G (left) Ly-6C (right). **d**, The correlation between percent IFN γ ⁺ NK cells and total numbers of the indicated myeloid cells in the lung of PBS or IL-33 injected WT mice (day 0 and 1, sacrificed on day 3) was analyzed on pooled results ($n=125$). Bar graphs indicate mean ($\pm$ SEM) and show representative data from three independent experiments (**a**, **b**, and **c**) and (**d**) representing Pearson r .



Extended Data Fig. 5 | IL-33-mediated suppression of NK cells is not dependent on neutrophils or alveolar macrophages. **a-e**, WT mice were treated with PBS or IL-33 on day 0 and 1, and the indicated mAb (or clodronate liposomes, C.L.) on day -1 and 1 followed by quantification of percent IFN γ ⁺ or GzmB⁺ lung NK cells (after PI stimulation) and quantification for the indicated lung myeloid cells on day 3 (**a**; GzmB $n=10,9,9,10$, and depicted myeloid cells $n=15,14,14,15$; **b**; $n=8,8,8,9$; **c**; $n=7$, **d,e**, $n=6$). Bar graphs indicate mean ($\pm$ SEM) and show combined data of two (**b-e**) or three (**a**) independent experiments. Statistical analyses were calculated using one-way ANOVA with ns = not significant, and **** = $p \leq 0.0001$.

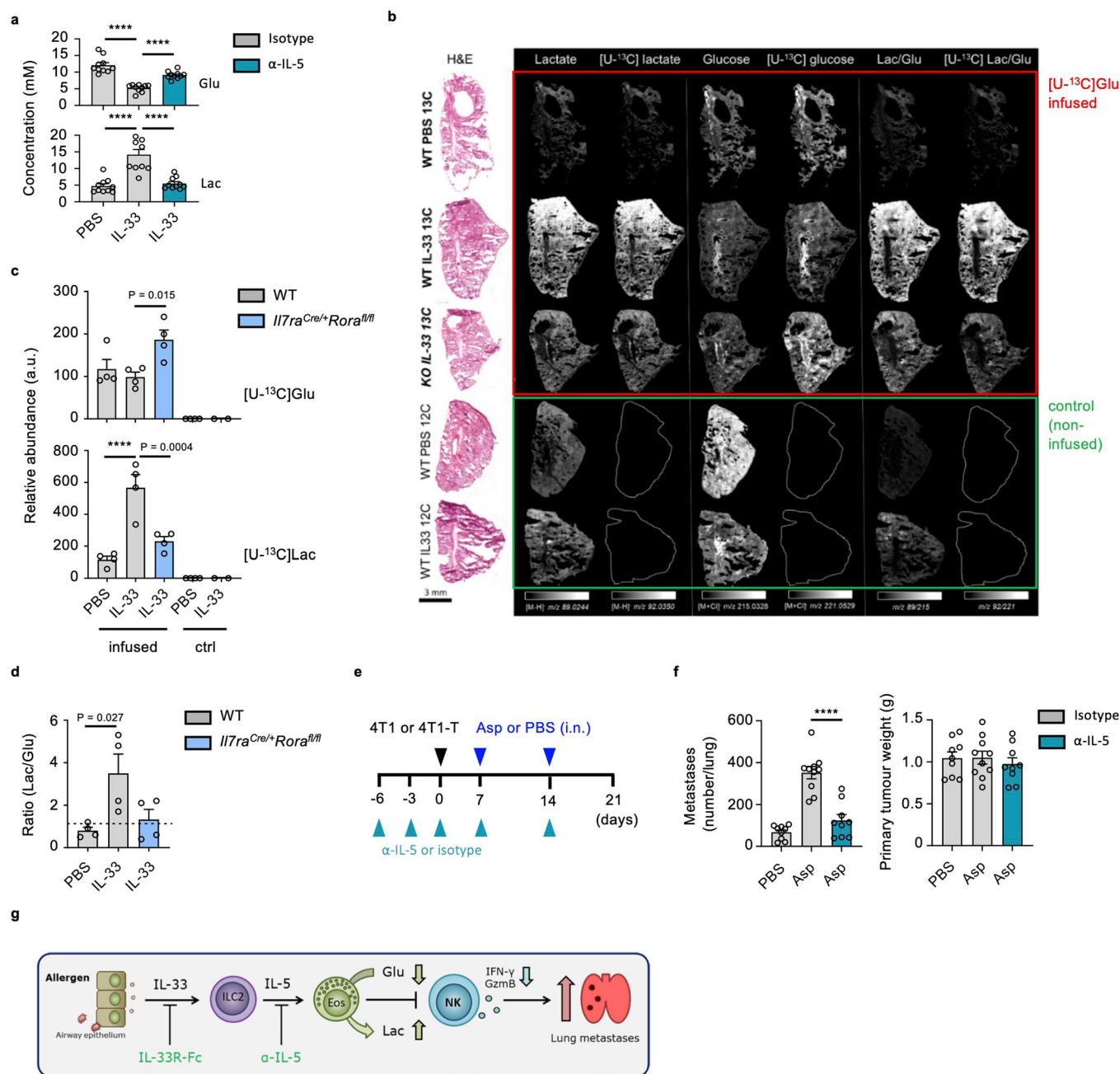


Extended Data Fig. 6 | IL-5 and eosinophils mediate IL-33-driven suppression of NK cells. **a**, WT mice were treated with PBS or IL-33 on day 0 and 1, followed by quantification of IL-5⁺ ILC2 (CD45⁺B220⁻Lineage⁻), or CD45⁺B220⁺lineage⁺ cells in the lungs on day 3 (n=5); the identity of IL-5⁺ ILC2 was further confirmed by ICOS expression. **b-d**, WT mice were treated with PBS or IL-33 on day 0 and 1, and anti-IL-5 or control on day -6, -3 and -1 followed by quantification of the total numbers of the indicated myeloid cells in the lung (**c**) (n=10), and the percent IFNγ⁺ and GzmB⁺ lung NK cells (after anti-NK1.1 stimulation) on day 3 (**d**) (n=5). **e**, WT mice were treated intranasally with PBS or the indicated cytokines on day 0 and 1, followed by quantification of percent IFNγ⁺ NK cells (after PI stimulation), or lung eosinophil numbers on day 3 (n=3). **f**, Mice of the indicated genotypes were treated intranasally Asp or PBS on days 0 and 1, followed by intravenous transfer of B16.F10 cells on day 7 and subsequent determination of lung metastases-related mortality by Kaplan-Meier survival curve (n=15,14,15,9). **g**, Purified WT mouse lung NK cells were cultured alone or with ex vivo bone marrow derived eosinophils at the indicated ratios for 18 hours, followed by a 3 hr re-stimulation with PI and quantification of IFNγ⁺ and GzmB⁺ NK cells by flow cytometry (n=6,6,9). Bar graphs indicate mean (±SEM) of combined data of two (**c**, **g**) or three (**f**) independent experiments. (**d** and **e**) show representative data of three independently performed experiments and (a) depicts representative flow cytometry plots. Statistical analyses were calculated using one-way ANOVA or Log-rank (Mantel-Cox) test (**f**) with **** = p ≤ 0.0001.



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

Extended Data Fig. 7 | Single-cell and bulk-RNA-seq of naive and IL-33-inflamed lung NK cells. **a**, Mice were treated with PBS or IL-33 on day 0 and 1, followed by FACS purification of lung NK cells at the indicated time-points for either scRNA-seq or bulk-RNA-seq analysis. **b**, Post FACS purity was assessed for all sorts. **c**, Clusters from (Fig. 6a) were annotated based on their gene expression patterns. Heatmap of genes significantly (FDR < 0.05) upregulated or downregulated in one cluster versus all others. Where more than 15 genes were significantly differentially expressed, only the 15 with the greatest average log-fold changes in each direction were included. Blue-to-yellow color gradient indicates $\log_2$ (normalized gene expression). **d**, scRNA-seq expression of *Cd27* and *Itgam* (encoding CD11b) separated by cluster. Specific clusters were annotated as follows (where individual genes were significantly upregulated in one cluster compared to all others, the 5 with greatest average log-fold-change are listed as marker genes): Cluster 2: signaling/inflammatory-chemokine-expressing NK cells (*Pim1*, *Nfkb1a*, *Gadd45b*, *Ccl4*, *Icam1*); Cluster 3: (*Kcnj8*, *Ly6c2*); Cluster 4: (*Hsp90ab1*, *Hspe1*, *Nme1*, *Ptma*, *Rps2*); Cluster 6: (*Ccl5*, *Cma1*, *Klrg1*, *Itm2b*); Cluster 7: immature NK cells (*Ctla2a*, *Emb*, *Ccr2*, *Rps15a*, *Rpl10a*). Cluster 2 was most similar to the previously identified splenic murine NK cell cluster 3, and Cluster 7 was most similar to the previously identified splenic and blood murine NK cell cluster 2 as identified by Crinier *et al.*³². **e**, Results of a differential abundance analysis comparing the abundance of cells in each cluster after IL-33 versus PBS treatment. P-values were calculated using empirical Bayes quasi-likelihood F-tests in a negative binomial GLM (as described in Methods). **f,g**, Expression of NK cell consensus³², effector, and both activating and inhibitory receptor transcripts from bulk-RNA-seq analysis of sorted lung NK cells. Data are represented as a heatmap of $\log_2$ -transformed normalized read counts of individual genes, grouped by category (**f**), or z-scaled expression values for genes within the 4 gene lists. Each point represents the expression value obtained by one replicate for a given gene at a given time point (**g**). Box plots represent mean (black line), first and third quartiles (box) and range within 1.5 times the interquartile range from the box (whiskers). Violin plots represent median (black line), interquartile ranges (box) and a kernel density plot.



Extended Data Fig. 8 | IL-33 increases glucose flux in the lung environment via ILC2 and IL-5. **a**, BALB/c mice were treated intranasally with IL-33 or PBS on days 0 and 1, and anti-IL-5 or control antibody (i.p.) on day -6, -3, and 0 and sacrificed on day 3. Lung homogenates were cultured for 18 hours and glucose (Glu) and lactate (Lac) concentrations were measured in the supernatant by NMR analysis ($n = 10, 9, 9$). **b**, Spatial resolving glycolytic activity in lung by MSI. WT or $Il7ra^{Cre/+}Rora^{fl/fl}$ (KO) mice were dosed with PBS or IL-33 on day 0 and 1, and sacrificed on day 3 and infused or not with [U- 13 C] glucose (as described in Methods). (Right to left) H&E stained lungs and post-DESI-MSI molecular images of lactate, [U- 13 C] lactate, glucose, [U- 13 C] glucose, normal and [U- 13 C] lactate to glucose ratio (pixel per pixel). Intensity scale is fixed for each molecular species independently, and monochromatic lighter colors correspond to higher relative abundance. **c**, **d**, Bar graphs indicate mean relative abundances of [U- 13 C] glucose or [U- 13 C] lactate (**c**) ($n = 4$), or the ratio of [U- 13 C] lactate over glucose (**d**) ($n = 4$). **e**, BALB/c were treated intranasally with Asp or PBS on days 0 and 1, and anti-IL-5 or control antibody (i.p.) on day -6, -3, 0 and 3, followed by injected with 4T1-T breast cancer cells in the mammary fat pad on day 7, and sacrifice on day 21. **f**, Tumor burden of mice treated as in (**e**) with 4T1-T cells was quantified by visual examination and primary tumor weight was recorded ($n = 9, 10, 9$). **g**, Graphical abstract. Bar graphs indicate mean ($\pm$ SEM) of combined data of two (**a**, **c**, **d**, and **f**) independent experiments. (**b**) depicts representative MSI images of two independent experiments. Statistical analyses were calculated using one-way ANOVA with **** = $p \leq 0.0001$.

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Give P values as exact values whenever suitable.
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Software and code

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Data collection

No software was used for data collection.

Data analysis

Bulk RNA-seq data were analyzed as described in the Methods using the following packages:
 STAR (v2.7.3) for alignment to GRCm38 reference genome.
 subread (v1.5.2) featureCounts to assign reads to transcripts from Ensembl release 98.
 DESeq2 (v1.26.0) was used for normalization and differential expression
 10x Genomics Chromium scRNAseq data was aligned and counted using Cell Ranger (v3.1.0).
 scRNAseq data was further processed and analyzed as described in the Methods, using the following packages in R (v3.6.1):
 DropletUtils (v1.4.3)
 scran (v1.12.1)
 scater (v1.12.2)
 SingleCellExperiment (v1.6.0)
 igraph (v1.2.4.1)
 Seurat (v3.1.1)
 biomaRt (v2.40.5)
 edgeR (v3.26.8)
 Gene set enrichment analysis (GSEA Desktop) (v4.0.3)
 No custom codes have been developed in this study.

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Transcriptomic data used in Fig. 6 and Extended Data Fig. 7 are available at the Sequence Read Archive (SRA) under the Bioproject PRJNA637311 : Lung NK cell transcriptomic. Bulk RNAseq data are available with the following BioSample accession numbers: SAMN15099850 (bulk PBS vs IL33) and the following sample names are A1, D1, B2, C2, F2, E1 and H1 for the PBS-treated condition and D2, B1, F1, E2, A2 and G1 for IL33-treated condition; SAMN15099866 (time-course) with the following triplicate sample names E4, A4, C3 (WT PBS day 3), H3, F4, D3 (WT IL33 day 3), C4, G4, A3 (WT IL33 day 7), B4, E3, G3 (WT IL33 day 14) and D4, F3, B3 (IL2KO IL33 day 3). Analysis code for scRNA-seq is available at (<https://github.com/MarioniLab/scRNAseqLungNKcells>).

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Life sciences study design

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

Sample size	Power calculations to determine exact sample sizes were not performed. Sample sizes were chosen based on pilot study results.
Data exclusions	We have included all data, with the exception in cases of explicit technical failures (i.e. entire experiment excluded due to omission of key reagents, degraded reagents - critically, these cases were assessed using the positive and negative controls), or suboptimal quality of individual data points (Extended Data Fig. - 1 bulk RNA seq dataset did not meet QC, see materials and methods).
Replication	As detailed in the manuscript, all experiments were conducted at least in duplicate. We observed no experiments where results did not repeat. All pooled experiments were individually significant, although in some cases we present individual experiments (where two experiments repeat the findings, but cannot be pooled due to global shifts in values - likely due to technical variability).
Randomization	Mice were randomly assigned to study groups. For RNA sequencing experiments, samples were randomized by the genomics core facility. Other experiments were not randomized.
Blinding	We did not explicitly blind investigators, although practically many studies were performed blinded due to assignment of blind labels to treatment groups (Fig. 1b, 1e-l, 3a, 4b, 4f, 7e, 8c-f, and Ext. Fig. 1d-h, 2b, 8a, 8f).

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

target (clone, provider) (fluorophore)(Catalogue number, Lot number)
 B220 (RA3-6B2, Life Technologies) (APC.eFl780) (47-0452-82, 4317799)
 CCL2 (MCP-1, BioXcell) (unconjugated) (BE0185)
 CCR2 (2H5, gift from Prof. Matthias Mack) (unconjugated)
 CD3e (145-2C11, eBioscience) (PE.Cy7) (25-0031-83, 4304567)

CD3e (145-2C11, eBioscience) (eFl450) (48-0031-82, 4311331)
 CD4 (RM4-5, eBioscience) (AF700) (56-0042-82, 4280294)
 CD5 (53-7.3, eBioscience) (eFl450) (48-0051-82, 4312832)
 CD8 (53-6.7, eBioscience) (PerCP.eFl710) (46-0081-82, NIL)
 CD8 (53-6.7, eBioscience) (SB645) (64-0081-82)
 CD11b (M1/70, eBioscience) (eFl450) (48-0112-82, 4289827)
 CD11b (M1/70, eBioscience) (APC.eFl780) (47-0112-82, NIL)
 CD11b (M1/70, Biolegend) (BV785) (101243, NIL)
 CD11c (N418, eBioscience) (eFl450) (48-01140-82, 4300055)
 CD11c (N418, eBioscience) (AF700) (56-0114-82, 4300335)
 CD16/32 (93, Biolegend) (101320, B234518)
 CD19 (1D3, eBioscience) (eFl450) (48-0199-42, NIL)
 CD31 (390, Biolegend) (BV605) (102427)
 CD45 (30-F11, Biolegend) (BV510) (103138, B235434)
 CD49b (HMA2, BD) (BV650) (740496, NIL)
 CD64 (X54-5/7.1 Biolegend) (BV711) (139311, NIL)
 CD127 (SB/199, BD) (PE.CF594) (562419, 7059620)
 CD172a (P84, Biolegend) (AF488) (144001, NIL)
 EpCam (G8.8, Biolegend) (BV711) (118233)
 FceR1a (MAR-1, eBioscience) (eFl450) (48-5898-82, NIL)
 FceR1a (MAR-1, eBioscience) (PerCP.eFl710) (46-5898-82, 4329352)
 F4/80 (BM8, eBioscience) (eFl450) (48-4801-82, 4278803)
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 Foxp3 (FJK-16s, eBioscience) (AF488) (53-5773-82, 4306753)
 GATA3 (TWAJ, eBiosciences) (eFl660) (50-9966-42, 4292231)
 Granzyme B (NGZB, eBioscience) (PerCP.eFl710) (46-8898-82, NIL)
 I-A/I-E (CI2G9, BD) (BUV395) (743876, NIL)
 ICOS (7E.17G9, eBioscience) (PE) (12-9942-82)
 IFNg (XMG1.2, Biolegend) (BV786) (505838, NIL)
 IgG2a isotype control (2A3, BioXcell) (unconjugated) (BP0089)
 IgG1 isotype control (HRPN, BioXcell) (unconjugated) (BE0088)
 IL-5 (TRFK5, BD) (APC) (554396, NIL)
 IL-5 (TRFK5, BioXcell) (unconjugated) (BE0198)
 IL-10 (JES5-2A5, BioXcell) (BE0049)
 Ki67 (CIB56, BD) (BV786) (563756, 7032783)
 Ly-6C/G (Rb6-8C5, eBioscience) (eFl450) (48-5931-82, 4274410)
 Ly-6G (1A8-Ly6g, eBioscience) (PE.eFl610) (61-9668-82, 4308004)
 Ly-6G (1A8, Bio X cell) (unconjugated) (BE0075-1, NIL)
 Ly-6C/G (RB6-8C5, Bio X cell) (unconjugated) (BE0075, NIL)
 Ly-6C (HK1.4, eBioscience) (PE.Cy7) (25-5932-82, 4306741)
 NK1.1 (PK136, BD) (BUV395) (564144, 6326906)
 NK1.1 (PK136, eBioscience) (eFl450) (48-5941-82, 4306758)
 NK1.1 (PK136, Miltenyi Biotec) (PE) (130-102-400, NIL)
 NK1.1 (PK136, Bio X cell) (unconjugated) (BE0036, NIL)
 RELM α (DS8RELM, Invitrogen) (APC) (17-5441-82, NIL)
 Phospho-S6 (cupk43k, eBioscience) (PE) (12-9007-42)
 Podoplanin (8.1.1, Biolegend) (PE.Cy7) (127412)
 Roryt (Q31.378, eBioscience) (PerCP.eFl710) (46-6981-82, 4300066)
 SiglecF (1RNM44N, eBioscience) (SB600) (63-1702-82, 4343350)
 ST2 (RMST2-2, eBioscience) (PE) (12-9335-82, NIL)
 Ter119 (TER-119, eBioscience) (eFl450) (48-5921-82, 4295840)
 TGF- β (1D11, BioXcell) (unconjugated) (BE0057)

Validation

validation of primary antibodies incorporated published literature, manufacturers data, and experimental data included in the manuscript.

B220 (RA3-6B2, Life Technologies) (APC.eFl780) (47-0452-82, 4317799)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD45R-B220-Antibody-clone-RA3-6B2-Monoclonal/47-0452-82>

CCL2 (MCP-1, BioXcell) (unconjugated) (BE0185)
 - reported by manufacturer: <https://bxccl.com/product/m-r-h-ccl2-mcp-1/>

CCR2 (2H5, gift from Prof. Matthias Mack) (unconjugated)
 - Mack M. et al. (2001) J Immunol, 166 (7) 4697-4704; DOI: <https://doi.org/10.4049/jimmunol.166.7.4697>

CD3e (145-2C11, eBioscience) (PE.Cy7) (25-0031-83, 4304567)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD3e-Antibody-clone-145-2C11-Monoclonal/25-0031-82>

CD3e (145-2C11, eBioscience) (eFl450) (48-0031-82, 4311331)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD3e-Antibody-clone-145-2C11-Monoclonal/48-0031-82>

CD4 (RM4-5, eBioscience) (AF700) (56-0042-82, 4280294)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD4-Antibody-clone-RM4-5-Monoclonal/56-0042-82>

CD5 (53-7.3, eBioscience) (eF1450) (48-0051-82, 4312832)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD5-Antibody-clone-53-7-3-Monoclonal/48-0051-82>

CD8 (53-6.7, eBioscience) (PerCP.eF1710) (46-0081-82, NIL)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-53-6-7-Monoclonal/46-0081-82>

CD8 (53-6.7, eBioscience) (SB645) (64-0081-82)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-53-6-7-Monoclonal/64-0081-82>

CD11b (M1/70, eBioscience) (eF1450) (48-0112-82, 4289827)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/48-0112-82>

CD11b (M1/70, eBioscience) (APC.eF1780) (47-0112-82, NIL)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/48-0112-82>

CD11b (M1/70, Biolegend) (BV785) (101243, NIL)

- reported by manufacturer: <https://www.biolegend.com/en-gb/products/brilliant-violet-785-anti-mouse-human-cd11b-antibody-7958>

CD11c (N418, eBioscience) (eF1450) (48-01140-82, 4300055)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD11c-Antibody-clone-N418-Monoclonal/48-0114-82>

CD11c (N418, eBioscience) (AF700) (56-0114-82, 4300335)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD11c-Antibody-clone-N418-Monoclonal/56-0114-82>

CD16/32 (93, Biolegend) (101320, B234518)

- reported by manufacturer: <https://www.biolegend.com/en-gb/products/trustain-fcx-anti-mouse-cd16-32-antibody-5683>

CD19 (H1B19, eBioscience) (eF1450) (48-0199-42, NIL)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD19-Antibody-clone-H1B19-Monoclonal/48-0199-42>

CD31 (390, Biolegend) (BV605) (102427)

- reported by manufacturer: <https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd31-antibody-9963>

CD45 (30-F11, Biolegend) (BV510) (103138, B235434)

- reported by manufacturer: <https://www.biolegend.com/en-gb/products/brilliant-violet-510-anti-mouse-cd45-antibody-7995>

CD49b (HMa2, BD) (BV650) (740496, NIL)

- reported by manufacturer: <https://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-mouse-antibodies/cell-surface-antigens/bv650-hamster-anti-mouse-cd49b-hm2/p/740496>

CD64 (X54-5/7.1 Biolegend) (BV711) (139311, NIL)

- reported by manufacturer: <https://www.biolegend.com/en-gb/products/brilliant-violet-711-anti-mouse-cd64-fcgammari-antibody-9920>

CD127 (SB/199, BD) (PE.CF594) (562419, 7059620)

- reported by manufacturer: <https://www.bdbiosciences.com/eu/applications/research/b-cell-research/surface-markers/mouse/pe-cf594-rat-anti-mouse-cd127-sb199/p/562419>

CD172a (P84, Biolegend) (AF488) (144001, NIL)

- reported by manufacturer: <https://www.biolegend.com/en-gb/products/alexa-fluor-488-anti-mouse-cd172a-sirpalpa-antibody-14089>

EpCam (G8.8, Biolegend) (BV711) (118233)

- reported by manufacturer: <https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-cd326-ep-cam-antibody-13763>

FceR1a (MAR-1, eBioscience) (eF1450) (48-5898-82, NIL)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/FceR1-alpha-Antibody-clone-MAR-1-Monoclonal/48-5898-82>

FceR1a (MAR-1, eBioscience) (PerCP.eF1710) (46-5898-82, 4329352)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/FceR1-alpha-Antibody-clone-MAR-1->

Monoclonal/46-5898-82

F4/80 (BM8, eBioscience) (eFl450) (48-4801-82, 4278803)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/F4-80-Antibody-clone-BM8-Monoclonal/48-4801-82>

F4/80 (BM8, eBioscience) (APC.eFl780) (47-4801-82, NIL)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/F4-80-Antibody-clone-BM8-Monoclonal/47-4801-82>

Foxp3 (FJK-16s, eBioscience) (AF488) (53-5773-82, 4306753)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/53-5773-82>

GATA3 (TWAJ, eBiosciences) (eFl660) (50-9966-42, 4292231)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/Gata-3-Antibody-clone-TWAJ-Monoclonal/50-9966-42>

Granzyme B (NGZB, eBioscience) (PerCP.eFl710) (46-8898-82, NIL)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/Granzyme-B-Antibody-clone-NGZB-Monoclonal/46-8898-82>

I-A/I-E (CI2G9, BD) (BUV395) (743876, NIL)

- reported by manufacturer: <https://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-mouse-antibodies/cell-surface-antigens/buv395-rat-anti-mouse-i-ai-e-2g9/p/743876>

ICOS (7E.17G9, eBioscience) (PE) (12-9942-82)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD278-ICOS-Antibody-clone-7E-17G9-Monoclonal/12-9942-82>

IFN γ (XMG1.2, Biolegend) (BV786) (505838, NIL)

- reported by manufacturer: <https://www.biolegend.com/en-gb/products/brilliant-violet-785-anti-mouse-ifn-gamma-antibody-7987>

IgG2a isotype control (2A3, BioXcell) (unconjugated) (BP0089)

- reported by manufacturer: <https://bxccl.com/product/invivoplus-rat-igg2a-isotype-control-anti-trinitrophenol/>

IgG1 isotype control (HRPN, BioXcell) (unconjugated) (BE0088)

- reported by manufacturer: <https://bxccl.com/product/rat-igg1-isotype-control>

IL-5 (TRFK5, BD) (APC) (554396, NIL)

- reported by manufacturer: <https://www.bdbiosciences.com/eu/applications/research/t-cell-immunology/th-2-cells/intracellular-markers/cytokines-and-chemokines/human/apc-rat-anti-mouseanti-human-il-5-trfk5/p/554396>

IL-5 (TRFK5, BioXcell) (unconjugated) (BE0198)

- reported by manufacturer: <https://bxccl.com/product/m-h-il-5/>

IL-10 (JES5-2A5, BioXcell) (BE0049)

- reported by manufacturer: <https://bxccl.com/product/m-il-10/>

Ki67 (CIB56, BD) (BV786) (563756, 7032783)

- reported by manufacturer: <https://www.bdbiosciences.com/eu/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-human-antibodies/bv786-mouse-anti-ki-67-b56/p/563756>

Ly-6C/G (Rb6-8C5, eBioscience) (eFl450) (48-5931-82, 4274410)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/Ly-6G-Ly-6C-Antibody-clone-RB6-8C5-Monoclonal/48-5931-82>

Ly-6G (1A8-Ly6g, eBioscience) (PE.eFl610) (61-9668-82, 4308004)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/Ly-6G-Antibody-clone-1A8-Ly6g-Monoclonal/61-9668-82>

Ly-6G (1A8, Bio X cell) (unconjugated) (BE0075-1, NIL)

- reported by manufacturer: <https://bxccl.com/product/invivomab-anti-m-ly-6g/>

Ly-6C/G (RB6-8C5, Bio X cell) (unconjugated) (BE0075, NIL)

- reported by manufacturer: <https://bxccl.com/product/m-ly-6g-2/>

Ly-6C (HK1.4, eBioscience) (PE.Cy7) (25-5932-82, 4306741)

- reported by manufacturer: <https://www.thermofisher.com/antibody/product/Ly-6C-Antibody-clone-HK1-4-Monoclonal/25-5932-82>

NK1.1 (PK136, BD) (BUV395) (564144, 6326906)

- reported by manufacturer: <https://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-mouse-antibodies/cell-surface-antigens/buv395-mouse-anti-mouse-nk-11-pk136/p/564144>

NK1.1 (PK136, eBioscience) (eFl450) (48-5941-82, 4306758)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/NK1-1-Antibody-clone-PK136-Monoclonal/48-5941-82>

NK1.1 (PK136, Miltenyi Biotec) (PE) (130-102-400, NIL)
 - reported by manufacturer: <https://www.miltenyibiotec.com/GB-en/products/nk1-1-antibody-anti-mouse-pk136.html#pe-vio-770:30-ug-in-200-ul>

NK1.1 (PK136, Bio X cell) (unconjugated) (BE0036, NIL)
 - reported by manufacturer: <https://bxccl.com/product/nk-1-1/>

RELMα (DS8RELM, Invitrogen) (APC) (17-5441-82, NIL)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/RELM-alpha-Antibody-clone-DS8RELM-Monoclonal/17-5441-82>

Phospho-S6 (cupk43k, eBioscience) (PE) (12-9007-42)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/Phospho-S6-Ser235-Ser236-Antibody-clone-cupk43k-Monoclonal/12-9007-42>

Podoplanin (8.1.1, Biolegend) (PE.Cy7) (127412)
 - reported by manufacturer: <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-podoplanin-antibody-6674>

Roryt (Q31.378, eBioscience) (PerCP.eFl710) (46-6981-82, 4300066)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/ROR-gamma-t-Antibody-clone-B2D-Monoclonal/46-6981-82>

SiglecF (1RNM44N, eBioscience) (SB600) (63-1702-82, 4343350)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/CD170-Siglec-F-Antibody-clone-1RNM44N-Monoclonal/63-1702-82>

ST2 (RMST2-2, eBioscience) (PE) (12-9335-82, NIL)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/IL-33R-ST2-Antibody-clone-RMST2-2-Monoclonal/12-9335-82>

Ter119 (TER-119, eBioscience) (eFl450) (48-5921-82, 4295840)
 - reported by manufacturer: <https://www.thermofisher.com/antibody/product/TER-119-Antibody-clone-TER-119-Monoclonal/48-5921-82>

TGF-β (1D11, BioXcell) (unconjugated) (BE0057)
 - reported by manufacturer: <https://bxccl.com/product/m-h-tgf-beta/>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	YAC-1 (Dr. Maïke de la Roche) (TIB-160, ATCC) B16.F10 (Dr. Jacqueline Shields) (CRL-6475, ATCC) 4T1 (prof. Greg Hannon) (CRL-2539, ATCC) 4T1-T (prof. Greg Hannon) (doi: 10.1038/nature14403) LL/2 (Dr. Maïke de la Roche) (CRL-1642, ATCC)
Authentication	All lines were authenticated by STR profiling (CRUK-CI, research instrumentation and cell services CORE facility).
Mycoplasma contamination	All lines tested negative for Mycoplasma testing (CRUK-CI, research instrumentation and cell services CORE facility).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	All mouse studies were carried out with sex matched groups (male and female), and age matched to our best abilities. All animals were used between the ages of 8 - 20 weeks (B6.MMTV-PyMT mice were used at 20 weeks). Mice housed at the CRUK-CI were kept in individually ventilated cages, between 19-23 °C with 45-65% humidity and a 12hour dark/light cycle. C57BL/6J (B6) (Charles River) BALB/c (Charles River) NOD/SCID.IL2rg ^{-/-} (Charles River) B6(Cg)-Tyrc-2J/J (albino B6, Charles River) B6.FVB-Tg(B6.MMTV-PyVT)634Mul/LelJ (JAX) B6.IL33cit/cit (IL33 reporter/KO, Andrew McKenzie)
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B6.117raCre (Hans-Reimer Rodewald)
 B6.Roraflox (Andrew McKenzie)
 B6.129(Cg)-Foxp3tm3(DTR/GFP)Ayr/J (JAX)
 B6(Cg)-Rag2tm1.1Cgn/J (JAX)
 B6.129S2-Ighmtm1Cgn/J (B6.µMT) (JAX)

B6.117raCre/+;Roraflox/fl and B6.117raCre/+;Roraflox/fl;Rag2-/- mice were generated by crossing the animals mentioned above. Heterozygous animals were generated by crossing the animals mentioned above with C57BL/6J mice (same genetic background).

Wild animals

This study did not use wild animals.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All animal work was conducted under project license PD7484FB9 at the CRUK-CI (with approval from the Cancer Research UK - Cambridge Institute, Animal Welfare Ethical Review Body) or at the Medical Research Councils ARES facility (with approval from Babraham Institute, Animal Welfare Ethical Review Body) all in accordance with Home Office regulation.

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

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

Cell suspensions were prepared from lung by mechanical dissociation, followed by digest in 5 ml of RPMI-1640 containing collagenase I (500 U/ml) and DNase I (0.2 mg/ml) for 45 minutes at 37 °C on a shaker (220 rpm), followed by filtration through a 70 µm strainer and 25% Percoll gradient enrichment of leukocytes, and red blood cell (RBC) lysis. Tumour cell were recovered without Percoll enrichment. Blood cells were lysed in 5 ml RBC lysis buffer 3 times for 5 minutes and spleens were strained through a 70 µm filter in RPMI-1640 before lysing erythrocytes with RBC lysis buffer for 5 minutes

Instrument

BD FACS Fortessa, BD FACS Symphony, BD FACS Aria II

Software

BD DIVA
FlowJo X

Cell population abundance

All cell purification strategies (magnetic bead enrichment, negative selection, FACS) were followed by purity checks. For FACS purified cells, an aliquot was analyzed post-sort, and only samples >95% purity, as defined by inclusion in original gating parameters, were used for downstream experimentation.

Gating strategy

General gating strategies used: Dead cell exclusion by fixable (eFl780, UV450) or non-fixable stains (i.e. DAPI) was used in all experiments, followed by gating on CD45+ leukocytes. Doublet exclusion and FSC/SSC gating (i.e. lymphocyte) strategies were used in some experiments where exclusion of myeloid cells improved signal/noise. Gating strategies were consistent across repeat experiments (copied and pasted, with minor corrections applied). Gating strategies for general myeloid, lymphoid and intracellular cytokine panels were consistent across the majority of experiments, and presented in the Extended Data Figures. Single stain controls were run before the majority of experiments, although only minor deviations were observed in compensation values (using FlowJo autocompensation). For all critical measurements we performed both positive and negative biological controls (i.e. stimulated & non-stimulated WT), as well as staining controls (i.e. fluorescence minus one, or similar). We used a combination of these controls to fine-tune gating thresholds, which were consistent for each experiment. We provide examples in the Extended Data Figures.

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