

Essential, dose-dependent role for the transcription factor *Gata3* in the development of IL-5⁺ and IL-13⁺ type 2 innate lymphoid cells

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Group 2 innate lymphoid cells (ILC2s; also called nuocytes, innate helper cells, or natural helper cells) provide protective immunity during helminth infection and play an important role in influenza-induced and allergic airway hyperreactivity. Whereas the transcription factor GATA binding protein 3 (*Gata3*) is important for the production of IL-5 and -13 by ILC2s in response to IL-33 or -25 stimulation, it is not known whether *Gata3* is required for ILC2 development from hematopoietic stem cells. Here, we show that chimeric mice generated with *Gata3*-deficient fetal liver hematopoietic stem cells fail to develop systemically dispersed ILC2s. In these chimeric mice, in vivo administration of IL-33 or -25 fails to expand ILC2 numbers or to induce characteristic ILC2-dependent IL-5 or -13 production. Moreover, cell-intrinsic *Gata3* expression is required for ILC2 development in vitro and in vivo. Using mutant and transgenic mice in which *Gata3* gene copy number is altered, we show that ILC2 generation from common lymphoid progenitors, as well as ILC2 homeostasis and cytokine production, is regulated by *Gata3* expression levels in a dose-dependent fashion. Collectively, these results identify *Gata3* as a critical early regulator of ILC2 development, thereby extending the paradigm of *Gata3*-dependent control of type 2 immunity to include both innate and adaptive lymphocytes.

asthma | innate immunity

Group 2 innate lymphoid cells (ILC2s; also known as natural helper cells, nuocytes, or innate helper cells) represent a population of non-T/non-B lymphocytes that promptly produce large amounts of the T helper 2 (Th2) cytokines IL-5 and -13. These IL-25- and IL-33-responsive innate cells have a broad distribution (1, 2) and were shown to be essential for the innate response to the helminth parasite *Nippostrongylus brasiliensis* (2–4). ILC2s can be distinguished by coexpression of CD25, CD127, IL-33R, and IL-25R. ILC2s in the respiratory tract play a crucial role in virus-induced airway hyperreactivity and in maintenance of epithelial barrier integrity during influenza infection (5, 6). Damaged epithelial cells or infected myeloid cells release inflammatory cytokines, including IL-25 and -33, that can potentially expand and activate ILC2s in mucosal tissues (7–10). Human ILC2s have been found in the lung and are associated with chronic rhinosinusitis (5, 11). In addition, we and others have shown that ILC2s are major IL-5 and -13 producers in various murine allergic asthma models (7–10, 12).

ILC2s belong to an emerging family of innate lymphoid cells (ILCs) that includes ILC1 [natural killer (NK) cells and other innate IFN- γ -producing cells] as well as a distinct group 3 ILC (ILC3) that includes subsets expressing the orphan transcription factor retinoid-related orphan receptor γ (ROR- γ) and producing IL-17 or -22 or having lymphoid tissue inducer function (reviewed by refs. 13, 14). The transcriptional regulator inhibitor

of differentiation 2 (Id2) has been identified as a common factor required for all ILC subsets (13, 14); however, the developmental stages from common lymphoid progenitors (CLPs) to fully mature ILC subsets have not been elucidated. Transient Notch signals appear critical for generation of ILC2s and ILC3s from CLPs (15–19). Nevertheless, the regulatory factors that orchestrate this process are poorly understood, although recently the transcription factor ROR- α was identified as an essential factor for efficient ILC2 development and in particular for IL-25-mediated expansion of the ILC2 compartment (10, 17).

Like Th2 cells, ILC2s promptly produce IL-5, -13, and, to a lesser extent, IL-4 (1–4). Regulation of *Il4*, *Il5*, and *Il13* expression is complex and in Th2 cells requires the zinc-finger transcription factor GATA binding protein 3 (*Gata3*), which is selectively up-regulated during Th2 differentiation (refs. 20 and 21 and reviewed in ref. 22). Conditional deletion of the *Gata3* gene in established Th2 cells demonstrated that *Gata3* is critical for IL-5 and -13, but not IL-4, production by Th2 cells (23). Likewise, deletion of the *Gata3* gene in ILC2s abolished their IL-5 and -13 production (24). In addition to its role in regulation of Th2 cytokine expression in Th2 cells and ILC2s, *Gata3* is required at multiple stages of T-cell development and has an essential role in the specification of the earliest T-cell progenitors in the thymus (25–27). In this work, hematopoietic chimeras, as well as mutant and transgenic mice in which *Gata3* gene copy number is altered, are used to address the essential, dose-dependent role for *Gata3* in ILC2 differentiation from lympho-hematopoietic precursors.

Results and Discussion

ILC2s Express High Levels of *Gata3* Protein and Require *Gata3* Expression for Development in Vivo. We first analyzed *Gata3* protein expression in ILC2s present in different tissue sites (Fig. 1A). ILC2s were identified as lineage-negative cells that coexpress CD25, CD127, and IL-33R as described (3, 4). We found that bone marrow (BM) and lung ILC2s expressed *Gata3* levels that exceeded by ~10-fold the levels expressed by mature CD4⁺ T cells (Fig. 1A). It remains unclear whether ILC2s in the BM represent immature ILC2 precursors or a “reservoir” of peripheral ILC2s (2), although BM ILC2s clearly produced cytokines

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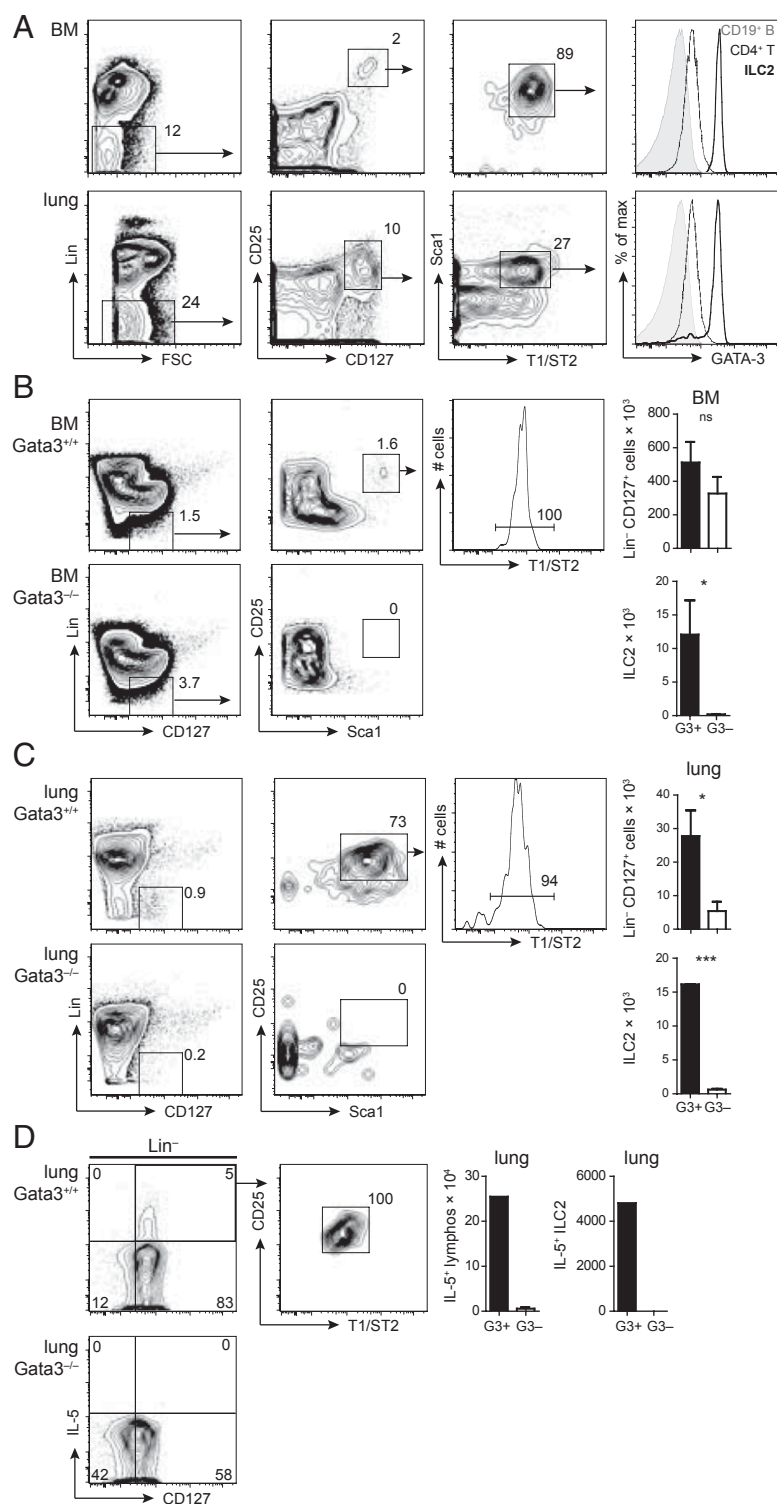


Fig. 1. GATA-3 is expressed by ILC2s and is required for ILC2 development in vivo. (A) Gating strategy for analysis of BM and lung ILC2s. Histograms show intracellular expression of GATA-3 of ILC2s (bold line) compared with CD3⁺ CD4⁺ T cells (black line) and CD19⁺ B lymphocytes (gray area). Numbers indicate relative frequencies of gated populations. Shown are representative results from three independent experiments ($n \geq 6$ total mice analyzed). (B and C) Analysis of lymphocytes from BM (B) and lung (C) of *Rag2*^{-/-}*Il2rg*^{-/-} hosts transplanted with *Gata3*^{+/-} and *Gata3*^{-/-} precursors for expression of the indicated surface markers. Plots show gating strategy for ILC2s. Bar graphs show quantification of the indicated donor-derived populations in chimeras. (D) Analysis of pulmonary Lin⁻ lymphocytes for CD127 and intracellular IL-5 expression. Plots show gating strategy for IL-5⁺ lymphocytes and ILC2s. Bar graphs show quantification of the indicated populations in *Gata3*^{+/-} and *Gata3*^{-/-} chimeras. (B–D) Shown are representative results from two independent experiments with $n = 3$ (*Gata3*^{+/-}) or 5 (*Gata3*^{-/-}) total mice analyzed per genotype. ns, not significant. * $P < 0.05$; *** $P < 0.001$.

(see below) and had similar *Gata3* levels as lung ILC2s (Fig. 1A). *Gata3*⁺ ILC2s were also identified in spleen, lymph nodes (LNs), and intestine (see below).

To assess the requirement for *Gata3* in the development of ILC2s in vivo, we analyzed chimeric mice generated by using *Gata3*-deficient hematopoietic precursors cells as described (28, 29). To control for the resultant T-cell deficiency in these chimeras (30), we generated T-cell-deficient, *Gata3*-proficient chimeras using *Cd3e*^{-/-} hematopoietic precursors. Both *Gata3*^{+/+} and

Gata3^{-/-} hematopoietic precursors engrafted to a similar extent, leading to robust B, NK, and myeloid cell reconstitution (Fig. S14 and ref. 28).

Because *Rag2*^{-/-}*Il2rg*^{-/-} hosts lack ILC2s (3), all ILC2s in the chimeric mice were donor-derived, as expected (Fig. S1B). Both BM and lung ILC2s developed in *Gata3*^{+/+} chimeras (Fig. 1B and C), and these ILC2s normally expressed CD127, CD25, and IL-33R, as expected (5, 31). In *Gata3*^{-/-} chimeras, we found a similar number of BM Lin⁻CD127⁺ cells, although these cells

Gata3^{-/-}, chimeras (Fig. 2E and Fig. S34). Although we found that ILC2s were the major source of IL-5 produced by lung Lin⁻ lymphocytes, IL-5 was not detected in these cells in the absence of *Gata3* (Fig. S3B). Collectively, these results demonstrate that characteristic inflammatory responses following triggering of ILC2s by IL-25 and -33 are not elicited in chimeric mice made with *Gata3*^{-/-} hematopoietic precursors.

Failure of ILC2 Differentiation from *Gata3*^{-/-} Hematopoietic Precursors in Vitro. Notch-triggered differentiation of ILC2s from CLPs can be achieved in vitro by using stromal cells expressing delta-like ligand 4 in the presence of IL-7 and IL-33 (19). We found that culture of *Gata3*^{+/-} fetal liver (FL) hematopoietic precursors generated abundant ILC2s using this approach (Fig. S44), whereas cells expressing characteristic surface markers of ILC2s were markedly reduced in the absence of *Gata3* (Fig. S44). RT-PCR analysis of these FL cultures showed that in the absence of *Gata3*, *Id2* and *Rora* expression were not induced (Fig. S5); these transcription factors are highly expressed in ILC2s and are critical for their development (3, 17, 34). Moreover, we could not detect IL-5 or -13 production in these ILC2 *Gata3*^{-/-} cultures, whereas ILC2 cultures of *Gata3*^{+/-} fetal liver hematopoietic precursors gave rise to cells with characteristic IL-5 and -13 production (Fig. S4B).

Cell-Intrinsic *Gata3* Is Required for ILC2 Development. To exclude the possibility that cell-extrinsic effects caused by *Gata3* deficiency underlie the observed defects in ILC2 development, we generated and analyzed BM chimeras generated by using 9:1 mixtures of CD45.2⁺ *Gata3*^{-/-} and CD45.1⁺ wild-type (WT) C57BL/6 hematopoietic precursors, respectively. Mice injected with 9:1 mixtures of CD45.2⁺ *Gata3*^{-/-} and CD45.1⁺ *Gata3*^{+/-} precursors served as controls (Fig. 3A). Both sets of mixed chimeras reconstituted to a similar extent (Fig. S64). We confirmed that B and NK cell development occurred normally from both *Gata3*^{-/-} and *Gata3*^{+/-} precursors (Fig. 3B and Fig. S6B), and phenotypically mature ILC2s were generated in BM, lung, spleen, and small intestine (Fig. 3C and Fig. S6C). Further analysis revealed that all ILC2s in *Gata3*^{-/-} mixed BM chimeras expressed CD45.1⁺ (originating from *Gata3*^{+/-} precursors), whereas ILC2s in *Gata3*^{+/-} mixed chimeras originated from both CD45.2⁺ *Gata3*^{-/-} and CD45.1⁺ *Gata3*^{+/-} precursors (Fig. 3C and Fig. S6D). This result demonstrates that *Gata3* is required in a cell-intrinsic fashion for ILC2 development. Interestingly, although analysis of the origin of B and NK cells recapitulated the ratio in which precursors were injected (i.e., 86–87% of the B and NK cells were CD45.2⁺ independent of *Gata3* genotype), ILC2s derived from *Gata3*^{+/-} precursors were underrepresented relative to those derived from their *Gata3*^{+/-} counterparts in control chimeras (Fig. 3C). This result suggests that the *Gata3* gene dosage may influence overall ILC2 development.

GATA-3 Gene Copy Positively Correlates with ILC2 Development, Homeostasis, and Function. We next compared the effects of modifying GATA3 gene copy number on ILC2 development

and function. We characterized ILC2 homeostasis and function in three mouse strains: C57BL/6 mice with two functional *Gata3* alleles, *Gata3*^{lacZ/+} mice with one functional *Gata3* allele (the second allele is disrupted by insertion of a beta-galactosidase reporter (35)), and transgenic C57BL/6 mice that harbor a *Gata3* transgene under the control of the human CD2 promoter (36). We found that *Gata3* protein levels in BM and lung ILC2s were reduced in *Gata3*^{lacZ/+} mice compared with C57BL/6 mice, indicating that both *Gata3* alleles are apparently active in ILC2s (Fig. S7A). In contrast, ILC2s from CD2-*Gata3* Tg mice expressed the same high levels of *Gata3* protein as WT C57BL/6 mice (Fig. S7A). Because we detected increased *Gata3* RNA expression in BM ILC2s (Table S1), it is possible that the intracellular FACS assay is not sensitive enough to detect differences in *Gata3*^{hi} WT and CD2-*Gata3* Tg ILC2s.

We next assessed the impact of alterations in *Gata3* gene copy number on overall ILC2 homeostasis. Absolute numbers of mature ILC2s in the lung and BM were clearly reduced in *Gata3*^{lacZ/+} mice, whereas CD2-*Gata3* Tg mice had an approximately threefold increase in BM and lung ILC2s compared with *Gata3*^{+/-} C57BL/6 mice (Fig. 4A). ILC2 function also positively correlated with *Gata3* gene copy number: ILC2s from CD2-*Gata3* Tg mice produced somewhat higher levels of IL-5 and -13 following stimulation compared with ILC2s from B6 WT mice, whereas cytokine production was reduced in ILC2s from *Gata3*^{lacZ/+} mice having only one functional *Gata3* allele (Fig. 4A and Fig. S7B). Moreover, CD2-*Gata3* Tg ILC2s showed higher surface expression of IL-25R and IL-33R (Fig. S7C).

These results suggest that *Gata3* expression levels can modify ILC2 generation from hematopoietic precursors and thereby influence ILC2 homeostasis. Whereas absolute numbers of CLPs were normal in CD2-*Gata3* Tg mice, we found that *Gata3* levels in CLPs were modestly but significantly increased in CLPs in CD2-*Gata3* Tg mice compared with nontransgenic controls (Fig. 4B). This finding would be consistent with hCD2 Tg control elements being active in CLPs, a hypothesis that we confirmed using CD2-GFP mice (37) (Fig. 4C). To assess the influence of increased *Gata3* levels in determining ILC2 potential from CLPs, we analyzed expression of ILC2-related genes (*Rora*, *Id2*, *Il17rb*, and *Il1rl1*) in CLPs from WT and CD2-*Gata3* Tg mice. Whereas *Gata3* expression was threefold increased, the levels of other ILC2-related genes were unaffected (Table S1). We next cultured single CLPs from *Gata3*^{lacZ/+}, *Gata3*^{+/-}, or CD2-*Gata3* Tg mice on stromal cells in the presence of IL-7 and -33. ILC2s developed from *Gata3*^{+/-} CLPs at the expected frequency (*f* = 1/5) (19), whereas CD2-*Gata3* Tg CLPs were almost twice as efficient in generating ILC2s (*f* = 1/2.6). In contrast, *Gata3*^{lacZ/+} CLPs showed a 2.6-fold reduction in ILC2 potential (*f* = 1/13) compared with their WT counterparts (Table 1, Fig. 4D, and Fig. S8). Collectively, these results suggest a model in which *Gata3* levels in CLPs control initial commitment to the ILC2 lineage and confirm the important role for sustained *Gata3* expression in maintenance of type 2 cytokine secretion from mature ILC2s (24).

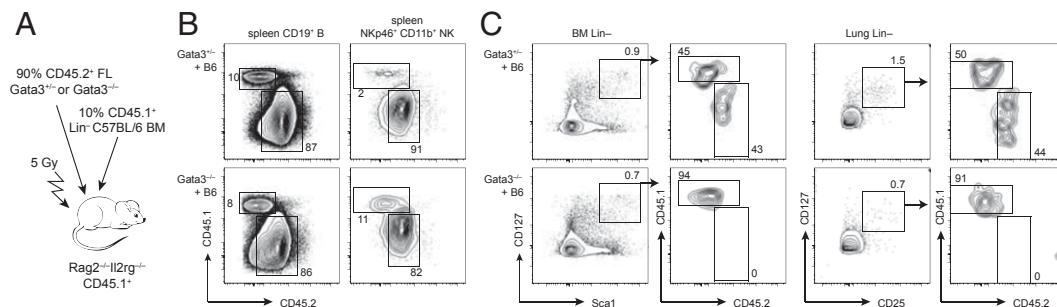
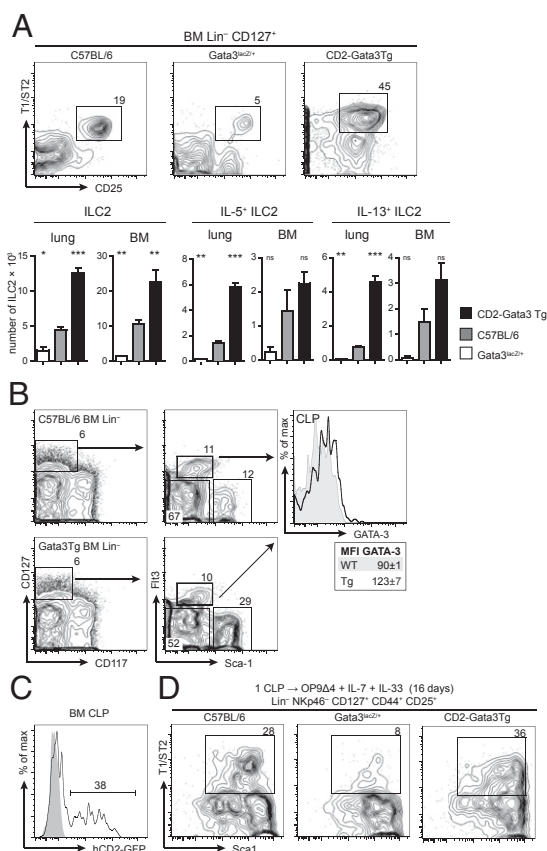


Fig. 3. Cell-intrinsic GATA-3 is required for ILC2 development. (A) Experimental protocol. Analysis was performed 5 wk after transplantation. (B) Analysis of B and NK cell development in chimeric mice. (C) ILC2 analysis in mixed chimeras. Numbers indicate relative frequencies of gated populations.



ILC2 containing cultures 1 CLP → OP9Δ4 + IL-7 + IL-33 (14-18 d). Single CLPs were cultured as described in Fig. 4 and analyzed for ILC2 development. Frequencies and 95% confidence interval are shown. Data from three pooled, independent experiments are shown. Frequencies were calculated by using an Extreme Limiting Dilution Assay. Statistical significance of differences between groups was determined by using a χ^2 test. χ^2 test for differences between any of the groups: $\chi^2 = 42.4$ ($P = 6.09 \times 10^{-10}$). n/a, not applicable.

factor in early T-cell development in the thymus (25, 26). These observations provide another example of the remarkable similarities between the molecular mechanisms that regulate the differentiation of innate and adaptive lymphocytes (13).

Materials and Methods

Mice and Generation of Chimeric Mice. *Rag2-Il2rg* double-deficient on the C57BL/6 background, *Cd3e*-deficient, *Gata3-lacZ*, *CD2-Gata3*, and *CD2-GFP* Tg mice were maintained at the animal facilities of the Institut Pasteur, Erasmus University Medical Center, and the Faculdade de Medicina de Lisboa. C57BL/6 mice and BALB/c *Rag2-Il2rg* mice were obtained from Harlan and Charles River, respectively. Mice were analyzed at 8–16 wk of age. Five-gray-irradiated *Rag2-Il2rg* double-deficient animals as hosts for hematopoietic stem cell grafts and were analyzed 5–10 wk after transplantation. All animals were kept under specific pathogen-free conditions and provided with food and water ad libitum. All experiments were approved and performed in accordance with local regulations (Institutional Animal Care and Use Committee, Institut Pasteur, Paris).

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- IL-25 and -33 Administration. For IL-25-induced inflammation, mice received 2.5 μg of recombinant mouse IL-25 (BioLegend) in PBS for four subsequent days intraperitoneally (33). Three doses of 1 μg of recombinant mouse IL-33 (BioLegend) in PBS were administered i.n. to anesthetized mice to induce eosinophilic airway inflammation (9).
- Phenotypic and Functional Analysis.** Lamina propria lymphocytes from small intestine were isolated as described (47). Single-cell suspensions from lungs, BAL, spleen, LNs, and BM were prepared and analyzed by FACS as described (9). CLPs (Lin[−]CD117^{low}Sca1^{low}CD135⁺) were sorted from WT and CD2-Gata3 Tg mice for quantitative PCR analysis. Antibodies used for analysis of cell surface phenotype, intracellular cytokines, and transcription factors are described in *SI Materials and Methods*.
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Supporting Information

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SI Materials and Methods

Generation of Chimeric Mice. Sublethally irradiated (5 Gy), 6- to 12-wk-old recombinase activating gene 2 (*Rag2*)–*Il2rg* double-deficient mice were used as hosts to generate GATA binding protein 3 (*Gata3*^{−/−}) and *Cd3e*-deficient chimeras. *Gata3*-deficient chimeras were generated by injection of *Gata3*-deficient fetal liver cells (embryonic day 15.5) obtained from noradrenalin-treated pregnant *Gata3*^{+/−} dams mated to *Gata3*^{+/−} males as described (1). The genotype of the embryos was confirmed by PCR. For *Cd3e*-deficient chimeras, bone marrow (BM) cells from 4- to 6-wk-old mice were depleted of lineage-positive cells [CD4, CD8 α , CD11b, CD11c, CD19, CD45R (B220) Ter-119, Gr-1, CD161 (NK1.1), Fc fragment of IgE, high affinity I, receptor for alpha polypeptide (Fc ϵ RI α), T-cell receptor β (TCR- β), and TCR- $\gamma\delta$] by using magnetic beads (Miltenyi), and $1\text{--}2 \times 10^6$ cells were injected into the immunodeficient hosts. For some experiments, Ly5.1⁺ *Rag2*–*Il2rg* mice were used as hosts, and CD45.1/CD45.2 were used as congenic markers. In other experiments, *Rag2*–*Il2rg* deficient hosts were on the BALB/c background (H2D^{d+}) and transplanted with cells on the C57BL/6 background (H2D^{b+}). Mixed chimeras were generated by injection of a mix of *Gata3*^{+/−} or *Gata3*^{−/−} fetal liver precursors supplemented with C57BL/6 lineage-depleted BM precursors. Levels of chimerism were verified by screening B lymphocytes, NK cells, and myeloid cells in spleen. Chimeric mice were analyzed 5–10 wk after transplantation.

Antibodies for Extracellular Flow Cytometric Analysis. Antibodies used for flow cytometry were against CD4 (GK1.5), CD8 α (53-6.7), CD11b (M1/70), CD11c (N418), CD19 (1D3), CD25 (PC61.5), CD44 (IM7), CD45 (I3/2.3), CD45.1 (A20), CD45.2 (104), CD117 (2B8), CD127 (A7R34), B220 (RA3-6B2), NK1.1 (PK136), Fc ϵ RI α (MAR-1), Gr-1 (RB6-8C5), Ter-119 (TER-119), stem cell antigen-1 (Sca-1) (D7), IL-33R (DJ8; MD Bioproducts), H2Db (28-14-8), H2Dd (34-1-2S), Siglec-F (E50-2440), and immune costimulator ICOS (C398.4A). Different conjugated antibodies were purchased from eBioscience, BD Biosciences, or BioLegend, unless indicated otherwise. Biotin-conjugated antibodies were detected by using Pacific Blue (Invitrogen), eFluor 780, or PerCPy5.5 (both eBioscience) conjugated streptavidin.

Intracellular Flow Cytometric Analysis. For measurement of intracellular cytokines, cells were stimulated for 4 h at 37 °C by using phorbol 12-myristate 13-acetate and ionomycin (Sigma-Aldrich) in the presence of GolgiStop (BD Biosciences). After extracellular staining, cells were fixed by using 2% (wt/vol) paraformaldehyde in PBS (Electron Microscopy Sciences) and stained for intracellular cytokines in PBS containing 0.5% saponin (Sigma-Aldrich). Antibodies against IL-4 (11B11), IL-5 (TRFK5), and IL-13 (eBio13A) were purchased from BD Biosciences and eBioscience. For *Gata3* protein detection, cells were fixed and permeabilized by using the Foxp3 staining kit (eBioscience) according to the manufacturer's

protocol. PE-conjugated, Alexa Fluor 647-conjugated, or eFluor 660-conjugated antibodies against *Gata3* were purchased from BD Biosciences or eBioscience.

Flow Cytometric Analysis and Data Analysis. After antibody staining, cells were analyzed by using an LSRII (three lasers) or LSRFortessa (four or five lasers) flow cytometer (both BD Biosciences) equipped with FACSDiva software. Sorting was performed by using FACSARIA or FACSARIAII (both three lasers; BD Biosciences) equipped with FACSDiva software. Data analysis was performed by using FlowJo (Version 8.8.7; Treestar).

In Vitro Development of Group 2 Innate Lymphoid Cells from Hematopoietic Precursors. Stroma cells expressing Notch ligand delta-like 4 (OP9 Δ 4) were grown in OptiMEM medium (Invitrogen) supplemented with 10% FCS (Thermo Scientific), 2-mercaptoethanol (Sigma or Invitrogen), and penicillin/streptomycin (Invitrogen) and seeded onto multiwell plates (BD Biosciences or TPP).

Hematopoietic cell precursors were cultured on OP9 Δ 4 cells in IL-7 (5 ng/mL; in-house hybridoma production) alone or in combination with IL-33 (20 ng/mL; Biolegend). For common lymphoid progenitor (CLP) cultures, single Lin[−] CD127⁺ CD117^{low} Sca1^{low} CD135⁺ cells were sorted directly into 96-well plates containing OP9 Δ 4 cells and IL-7/IL-33. Medium and cytokines were refreshed every 3 or 4 d, and cultures were split when necessary. Cultures were screened for growth by using an inverted phase microscope, and positive cultures were analyzed by flow cytometry on the indicated days.

Gene Expression Analysis. RNA was purified (RNeasy Micro Kit; Qiagen) from OP9 Δ 4 stromal cell cultures at various time points, and cDNA was synthesized by using RevertAid H Minus Reverse Transcriptase and random hexamer primers in the presence of Ribolock RNase inhibitor (all from Thermo Scientific). Quantitative RT-PCR (qRT-PCR) was performed by using an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Life Technologies) using gene-specific primers (Invitrogen) combined with a Universal Probe Library amplicon-specific probe (Roche Diagnostics).

Statistical Analysis. Unpaired *t* tests were used for comparisons between two normally distributed groups. For groups that were not normally distributed, significance of difference between groups was determined by using Mann–Whitney *u* tests. For comparisons between multiple groups, one-way ANOVA was used, followed by a Dunnett posttest. Differences were considered significant at *P* < 0.05. All statistical analyses were performed by using Prism 4 and 5 (GraphPad). Significance levels of statistical tests are not significant (ns); **P* < 0.05; ***P* < 0.01; or ****P* < 0.001.

1. Kaufman CK, et al. (2003) GATA-3: An unexpected regulator of cell lineage determination in skin. *Genes Dev* 17(17):2108–2122.

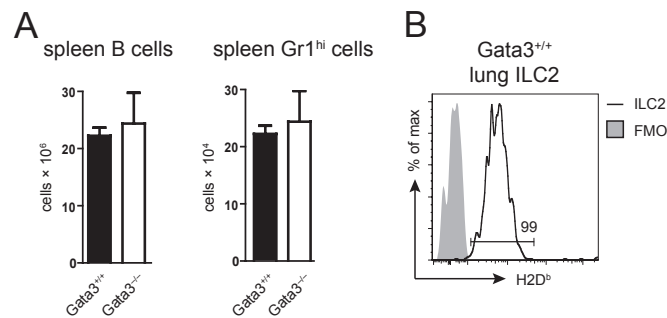


Fig. S1. Engraftment of *Gata3*^{+/+} and *Gata3*^{-/-} hematopoietic precursors. (A) Analysis of donor-derived (H2D^b) CD19⁺ B cells and Gr-1⁺ myeloid cells in spleen from hosts transplanted with *Gata3*^{+/+} or *Gata3*^{-/-} hematopoietic precursors. (B) Analysis of H2D^b expression by Lin⁻ CD127⁺ CD25⁺ Sca-1⁺ T1/ST2⁺ lung cells (*Gata3*^{+/+} chimeric mouse). Shown are results from two independent experiments, each with *n* = 3 mice (*Gata3*^{-/-}) or 2 (*Gata3*^{+/+}) mice per group.

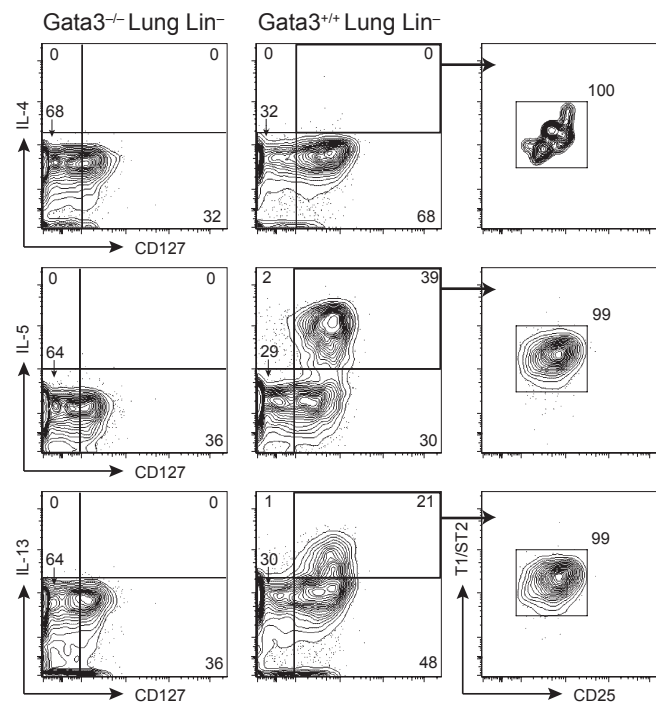


Fig. S2. Pulmonary IL-5 and -13 production by Lin⁻ lymphocytes upon IL-33 challenge is group 2 innate lymphoid cell (ILC2)-derived. Lin-negative lymphocytes from lungs of IL-33-challenged chimeras were analyzed for the expression of CD127 and the indicated intracellular cytokines. Expression of the indicated ILC2 markers is shown for the boxed *Gata3*^{+/+} population, and relative frequencies are indicated. Shown are representative results from two independent experiments with *n* ≥ 6 total mice analyzed per genotype.

A

BM
Gata3^{+/+}

6.5

2.4

cells

65

T1/ST2

BM
Gata3^{-/-}

Lin

19

CD127

CD25

Sca1

Lung
Gata3^{+/+}

1.1

23

cells

42

T1/ST2

Lung
Gata3^{-/-}

Lin

0.4

0

CD127

CD25

Sca1

B

Lung Lin⁻

Gata3^{+/+} Lung

0 6 9 85

T1/ST2

83

CD25

Gata3^{-/-} Lung

IL-5

0 0 48 52

CD127

