Supplemental Information

Quantitative Expression of GATA3 Specifies Lineage Fates and Functions of Innate Lymphoid Cells

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Figure S1

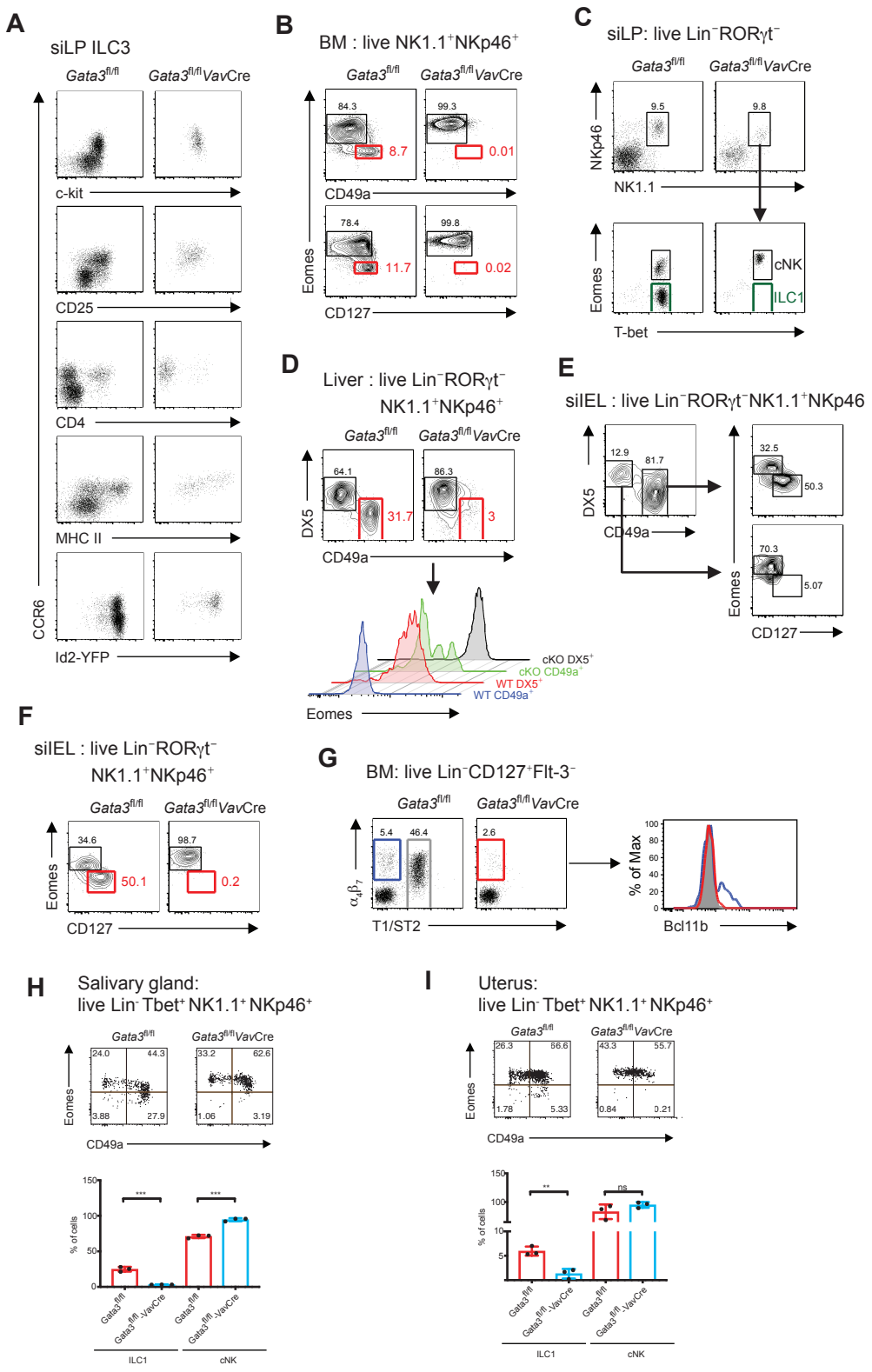
A
siLP ILC3
Gata3fl/fl
Gata3fl/flVavCre
c-kit
CD25
CD4
MHC II
CCR6
Id2-YFP
B
BM : live NK1.1+NKp46+
84.3
8.7
99.3
0.01
CD49a
78.4
11.7
99.8
0.02
Eomes
CD127
C
siLP: live Lin−RORγt−
9.5
9.8
NKp46
NK1.1
cNK
ILC1
Eomes
T-bet
D
Liver : live Lin−RORγt−
NK1.1+NKp46+
64.1
31.7
86.3
3
DX5
CD49a
cKO DX5+
cKO CD49a+
WT DX5+
WT CD49a+
Eomes
E
siIEL : live Lin−RORγt−NK1.1+NKp46
12.9
81.7
32.5
50.3
70.3
5.07
DX5
CD49a
Eomes
CD127
F
siIEL : live Lin−RORγt−
NK1.1+NKp46+
34.6
50.1
98.7
0.2
Eomes
CD127
G
BM: live Lin−CD127+Flt-3−
5.4
46.4
2.6
α4β7
T1/ST2
% of Max
Bcl11b
H
Salivary gland:
live Lin- Tbet+ NK1.1+ NKp46+
24.0
44.3
3.88
27.9
33.2
62.6
1.06
3.19
Eomes
CD49a
% of cells
***
ILC1
cNK
I
Uterus:
live Lin- Tbet+ NK1.1+ NKp46+
26.3
56.6
1.78
5.33
43.3
55.7
0.84
0.21
Eomes
CD49a
% of cells
**
ns
ILC1
cNK

Figure S1. Analysis of the Residual ILCs in the *Gata3*$^{fl/fl}$*Vav*Cre Mice, Related to Figure 1
(A) ILC3 cells were isolated from the siLP of *Gata3*$^{fl/fl}$ and *Gata3*$^{fl/fl}$*Vav*Cre mice. The expression of c-Kit, CD25, CD4, MHC-II, and Id2 (Id2-YFP) in the CCR6$^+$ and CCR6$^-$ ILC3 subsets from *Gata3*$^{fl/fl}$ mice as well as in the residual CCR6$^+$ ILC3s from the *Gata3*$^{fl/fl}$*Vav*Cre mice was analyzed by flow cytometry. (B) The bone marrow immature ILC1 population (red gate, live NK1.1$^+$NKp46$^+$Eomes$^-$CD127$^+$) in the *Gata3*$^{fl/fl}$*Vav*Cre mice were analyzed by flow cytometry. (C) ILC1 (live Lin$^-$RORγt$^-$NK1.1$^+$NKp46$^+$T-bet$^+$Eomes$^-$) and conventional natural killer (cNK) cells (live Lin$^-$RORγt$^-$NK1.1$^+$NKp46$^+$T-bet$^+$Eomes$^+$) in the small intestine lamina propria (siLP) of *Gata3*$^{fl/fl}$ and *Gata3*$^{fl/fl}$*Vav*Cre mice were analyzed by flow cytometry. (D) Liver cells were isolated from *Gata3*$^{fl/fl}$ and *Gata3*$^{fl/fl}$*Vav*Cre mice, and the liver resident ILC1s (live Lin$^-$RORγt$^-$NK1.1$^+$NKp46$^+$CD49a$^+$DX5$^-$) and cNK cells (live Lin$^-$RORγt$^-$NK1.1$^+$NKp46$^+$CD49a$^-$DX5$^+$) as well as the Eomes expression (histogram) in these cells were analyzed. (E) The small intestine intraepithelial lymphocytes (siIEL) were isolated and analyzed by flow cytometry. After gating on the live Lin$^-$RORγt$^-$NK1.1$^+$NKp46$^+$ cells, the CD49a$^+$DX5$^-$ and CD49a$^-$DX5$^+$ subsets were analyzed for their Eomes and CD127 expression. (F) The absence of siIEL ILC1 cells in the *Gata3*$^{fl/fl}$*Vav*Cre mice was confirmed by staining Eomes and CD127 expression in the live Lin$^-$RORγt$^-$NK1.1$^+$NKp46$^+$cells. (G) The Bcl11b$^+$ ILC2 fate committed ILC progenitors in the αLP (live Lin$^-$CD127$^+$Flt-3$^+$T1/ST2$^-$$\alpha_4\beta_7^+$) population of *Gata3*$^{fl/fl}$ (blue gate) and *Gata3*$^{fl/fl}$*Vav*Cre (red gate) mice were analyzed. Bcl11b expression in the bone marrow immature ILC2s in the *Gata3*$^{fl/fl}$ mice (grey gate) was used as a negative control. (H) Cells from salivary gland were isolated and analyzed by flow cytometry. Live Lin$^-$T-bet$^+$NK1.1$^+$NKp46$^+$ cells were analyzed for their CD49a and Eomes expression. Percentages of ILC1s (Eomes$^-$CD49a$^+$) and cNK (Eomes$^+$) cells among the live Lin$^-$T-bet$^+$NK1.1$^+$NKp46$^+$ cell population were calculated (mean ± s.d.; n = 3; ***P < 0.001, Student's t-test). (I) Cells from uterus were isolated and analyzed by flow cytometry. Live Lin$^-$T-bet$^+$NK1.1$^+$NKp46$^+$ cells were analyzed for their CD49a and Eomes expression. Percentages of ILC1s (Eomes$^-$CD49a$^+$) and cNK (Eomes$^+$) cells among the live Lin$^-$T-bet$^+$NK1.1$^+$NKp46$^+$ cell population were calculated (mean ± s.d.; n = 3; **P < 0.01, ns, not significant, Student's t-test). Data are representative of three (A-G) and two (H-I) independent experiments.

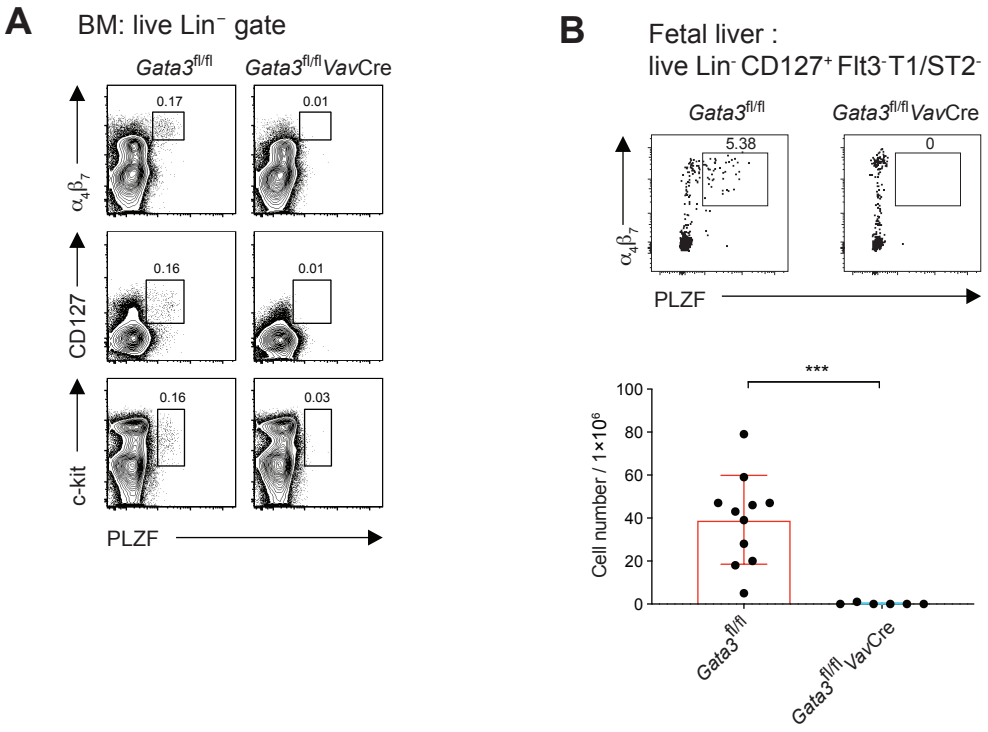

Figure S2. The Development of PLZF⁺ ILC Progenitors Depends on GATA3, Related to Figure 2
(A) BM cells were isolated from the wild-type (*Gata3*$^{fl/fl}$) and *Gata3*-deficient (*Gata3*$^{fl/fl}$*Vav*Cre) mice, and lineage$^-$PLZF$^+$ ILC progenitor population was analyzed by flow cytometry. Integrin $\alpha_4\beta_7$, CD127 and c-Kit expression by these lineage$^-$ PLZF$^+$ ILC progenitors was also assessed. (B) Cells were isolated from the fetal livers of wild-type (*Gata3*$^{fl/fl}$) and *Gata3*-deficient (*Gata3*$^{fl/fl}$*Vav*Cre) embryos (day 15.5), and integrin $\alpha_4\beta_7$ and PLZF expression by live Lin$^-$CD127$^+$Flt-3$^-$T1/ST2$^-$ were assessed. Total cell numbers of PLZF-expressing cells were calculated (mean ± s.d.; n = 6 and 11; ***P < 0.001, Student's *t*-test). Data are representative of three (A) and a combination of three (B) independent experiments.

Figure S3

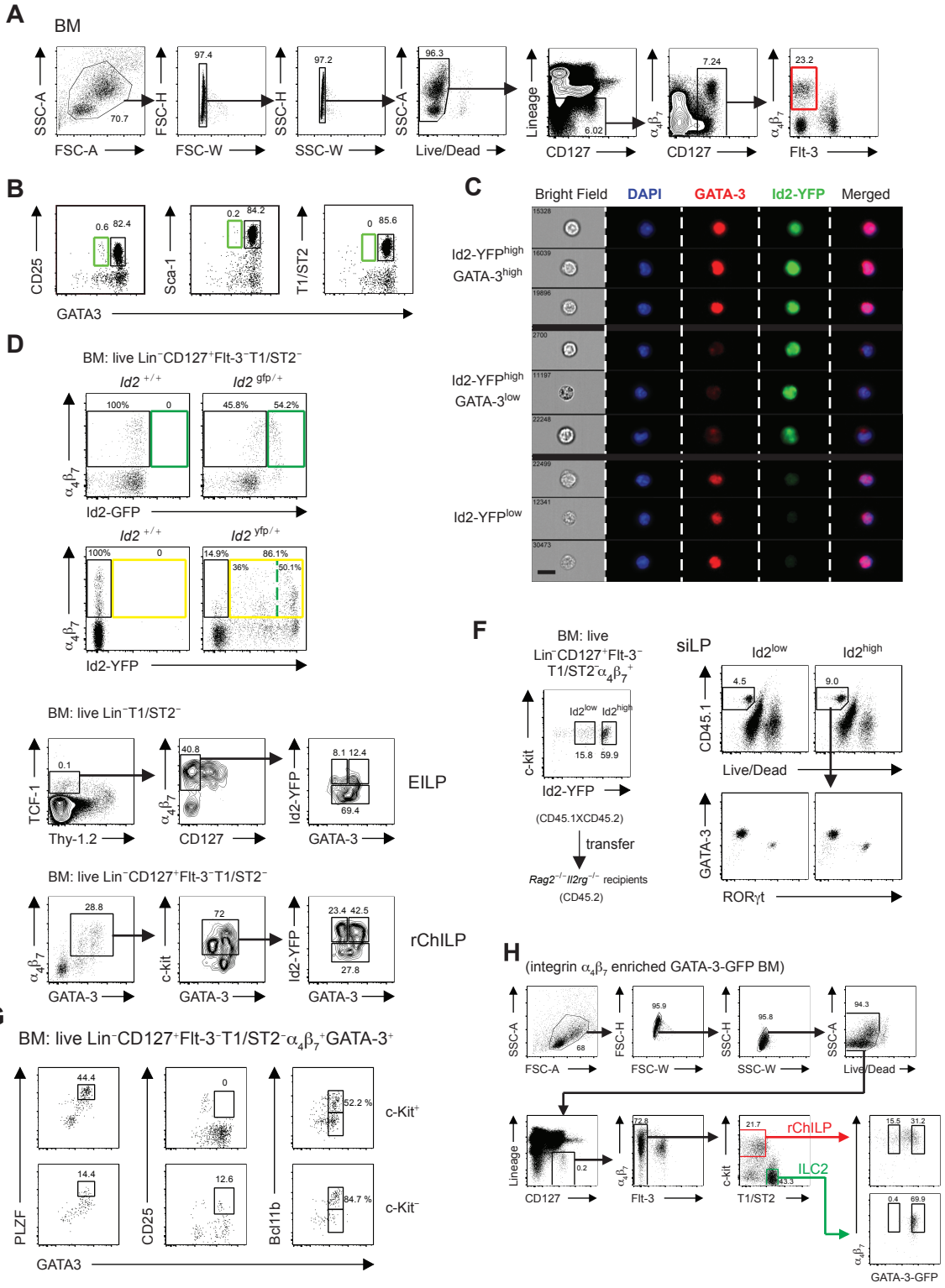
A
BM
SSC-A
FSC-A
70.7
FSC-H
FSC-W
97.4
SSC-H
SSC-W
97.2
SSC-A
Live/Dead
96.3
Lineage
CD127
6.02
α4β7
CD127
7.24
α4β7
Flt-3
23.2
B
CD25
GATA3
0.6
82.4
Sca-1
0.2
84.2
T1/ST2
0
85.6
C
Bright Field
DAPI
GATA-3
Id2-YFP
Merged
Id2-YFPhigh GATA-3high
Id2-YFPhigh GATA-3low
Id2-YFPlow
D
BM: live Lin⁻CD127⁺Flt-3⁻T1/ST2⁻
Id2 +/+
Id2 gfp/+
100%
0
45.8%
54.2%
α4β7
Id2-GFP
Id2 +/+
Id2 yfp/+
100%
0
14.9%
86.1%
36%
50.1%
α4β7
Id2-YFP
E
BM: live Lin⁻T1/ST2⁻
TCF-1
Thy-1.2
0.1
α4β7
CD127
40.8
Id2-YFP
GATA-3
8.1 12.4
69.4
EILP
BM: live Lin⁻CD127⁺Flt-3⁻T1/ST2⁻
α4β7
GATA-3
28.8
c-kit
GATA-3
72
Id2-YFP
GATA-3
23.4 42.5
27.8
rChILP
F
BM: live Lin⁻CD127⁺Flt-3⁻ T1/ST2⁻α4β7⁺
c-kit
Id2-YFP
Id2low Id2high
15.8 59.9
(CD45.1XCD45.2)
transfer
Rag2−/−Il2rg−/− recipients
(CD45.2)
siLP
Id2low
Id2high
CD45.1
4.5
9.0
Live/Dead
GATA-3
RORγt
G
BM: live Lin⁻CD127⁺Flt-3⁻T1/ST2⁻α4β7⁺GATA-3⁺
PLZF
GATA3
44.4
14.4
CD25
0
12.6
Bcl11b
52.2 %
84.7 %
c-Kit⁺
c-Kit⁻
H
(integrin α4β7 enriched GATA-3-GFP BM)
SSC-A
FSC-A
68
FSC-H
FSC-W
95.9
SSC-H
SSC-W
95.8
SSC-A
Live/Dead
94.3
Lineage
CD127
0.2
α4β7
Flt-3
72.8
c-kit
T1/ST2
21.7
43.3
rChILP
ILC2
15.5
31.2
0.4
69.9
α4β7
GATA-3-GFP

Figure S3. GATA3 Quantitatively Distinguishes Different ILC Lineages from the Refined Common Helper-liker Innate Lymphoid Progenitor (rChILP) Population, Related to Figure 3

(A) Gating strategy for flow cytometry analysis of the ILC progenitor cells in the bone marrow. (B) CD25, Sca-1 and T1/ST2 expression in the GATA3^{low} αLP cells (final gate in (A)) was analyzed by flow cytometry. (C) The three rChILP subsets, Id2-YFP^{high}GATA3^{high}, Id2-YFP^{high}GATA3^{low}, and Id2-YFP^{low} cells were analyzed on an ImageStream machine GATA3, Id2-YFP, as well as the nuclear staining (DAPI) were included. (D) The brightness of two Id2 reporters, *Id2*$^{gfp/+}$ and *Id2*$^{yfp/+}$, was assessed by comparing the Id2^{+} ILC progenitor populations (green and yellow gates). Their wild-type littermates (*Id2*$^{+/+}$) were used as background controls. The green dash line in the yellow gate indicated the separation of Id2^{high} and Id2^{low} cells in the *Id2*$^{yfp/+}$ mice. (E) The Id2 and GATA3 levels in TCF1^{+} early innate lymphoid progenitor (EILP, live Lin^{-}T1/ST2^{-}Thy1.2^{-}CD127^{-}$\alpha_4\beta_7^{+}$TCF1^{+}) were analyzed by flow cytometry and compared with those in rChILP cells (live Lin^{-}CD127^{+}Flt-3^{+}T1/ST2^{-} $\alpha_4\beta_7^{+}$c-Kit^{+}) within the same sample. Id2 level were surrogated by the Id2-YFP reporter, and GATA3 level were assessed by intracellular transcription factor staining. (F) Bone marrow cells were isolated from a congenically marked (CD45.1XCD45.2) Id2-YFP reporter mice, and the Id2^{high} and Id2^{low} rChILP (live Lin^{-}CD127^{+}Flt-3^{+}T1/ST2^{-}c-Kit^{+}$\alpha_4\beta_7^{+}$) subsets were sorted and transferred into sublethally irradiated *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (CD45.2) recipients. Repopulated progenies (CD45.1^{+}) in the small intestine were analyzed 4-6 weeks later for the expression of GATA3 and RORγt. (G) The c-Kit^{+} and c-Kit^{-} GATA3^{+}αLP cells were analyzed for the expression of PLZF, CD25, and Bcl11b in the GATA3^{high} and GATA3^{low} subsets. (H) Gating strategy for sorting the GATA3^{high} and GATA3^{low} rChILP cells from the GATA3-GFP reporter mice. The GATA3 level in the bone marrow immature ILC2 cells were used as a reference for the GATA3^{high} rChILP gate. Data are representative of at least three independent experiments (A-B), two independent experiments (C, F, H) or three independent experiments (D, E, G).

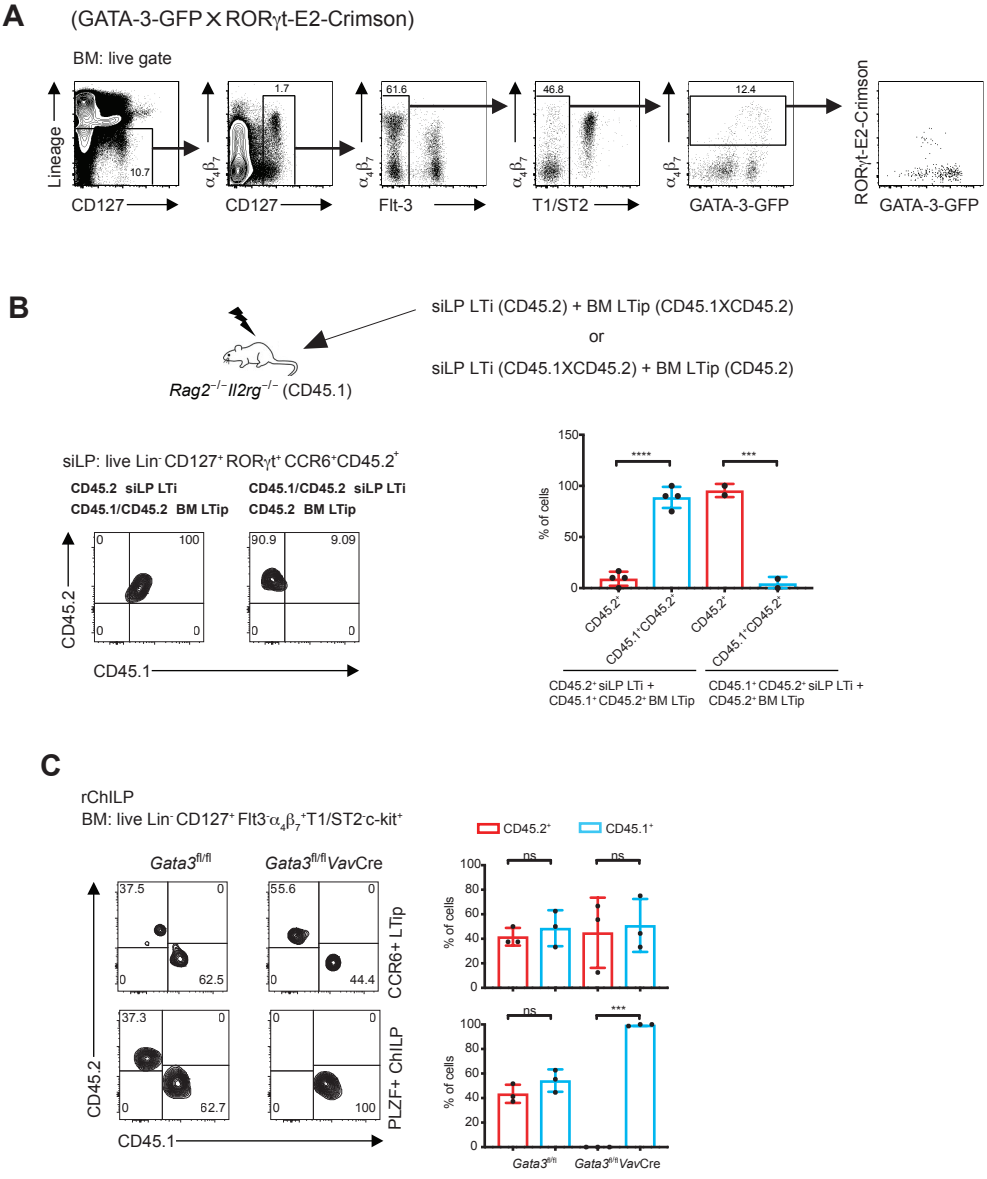

Figure S4. The CCR6^{+} GATA3^{low} rChILP Cells Express RORγt and Can Generate Mature LTi Cells; GATA3 Effects on the Development of ILC Progenitors are Cell-intrinsic, Related to Figure 4

(A) An GATA3 and RORγt double reporter strain (GATA3-GFPXRORγt-E2-Crimson) was used to assess RORγt expression in the GATA3^{low} rChILP population. (B) Sorted CCR6^{+} LTi cells from small intestine laminal propria (CD45.2 or CD45.1/CD45.2) and CCR6^{+} LTi progenitors from bone marrow (CD45.1/CD45.2 or CD45.2) were mixed at 1:1 ratio and transferred into sublethally irradiated *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (CD45.1) recipients. Lin^{-}CD127^{+}RORγt^{+}CCR6^{+}CD45.2^{+} cells in the small intestine were analyzed 4 weeks later for their origins based on the expression of CD45.1 and CD45.2. Percentages of CD45.2 or CD45.1/CD45.2 cells were calculated (mean ± s.d.; n = 2 or 4; ***P < 0.001, ****P < 0.0001, Student's t-test). (C) Bone marrow cells from *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$*Vav*Cre mice were mixed with bone marrow cells from CD45.1 C57BL/6 mice at 1:1 ratio and transferred into sublethally irradiated *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ recipients. Six weeks later, CCR6^{+} LTi progenitors and PLZF-expressing rChILPs were analyzed for their donor origins based on the expression of CD45.1 and CD45.2. Percentages of CD45.1 or CD45.2 cells were calculated (mean ± s.d.; n = 3; ***P < 0.001, ns, not significant, Student's t-test). Data are representative of three (A) and two (B-C) independent experiments.

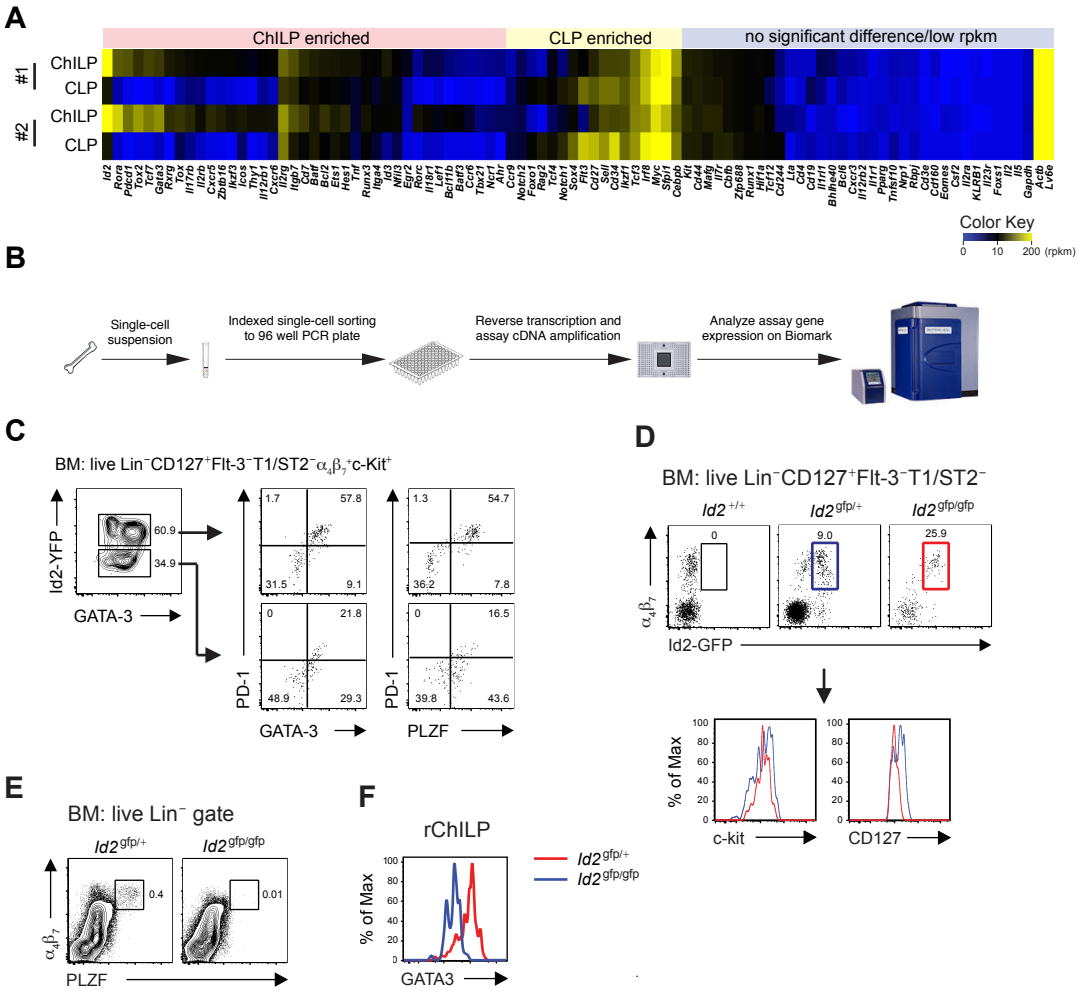

Figure S5. Gene Expression Analysis of rChILP Subsets and the Role of Id2 During ILC Development, Related to Figure 5

(A) RNA-Seq analysis was performed to assess gene expression in CLP and rChILP cells. Based on the result and other references from the literature, 92 genes were then selected for the follow-up single cell gene expression analysis on a 96.96 Fludigm chip, together with 18sRNA and three spike RNAs. The 92 gene expression in bulk CLP and rChILP cells were presented by heatmap. (B) Work flow indicates the procedure for single cell multiplex gene expression analysis through RT-qPCR. (C) The correlation between PD-1 expression and GATA3 or PLZF expression in rChILPs. (D) Id2-GFP-expressing "ChILP" cells were analyzed in the *Id2*-deficient (*Id2*$^{gfp/gfp}$) reporter mice. *Id2*$^{+/+}$ mice were used as a background control, and *Id2*$^{gfp/+}$ mice were used as an *Id2*-sufficient control. The c-Kit and CD127 expression in *Id2*-sufficient (*Id2*$^{gfp/+}$) and -deficient (*Id2*$^{gfp/gfp}$) GFP^{+} cells was also compared by flow cytometry. (E) The Lin^{-}PLZF^{+} non-LTi ILC progenitors were analyzed in the *Id2*-deficient mice. (F) The expression of GATA3 by the rChILP cells from the *Id2*-sufficient (*Id2*$^{gfp/+}$) and -deficient (*Id2*$^{gfp/gfp}$) mice was assessed by flow cytometry. Data are from two biological duplicates (A) or representative of three (C-F) independent experiments.

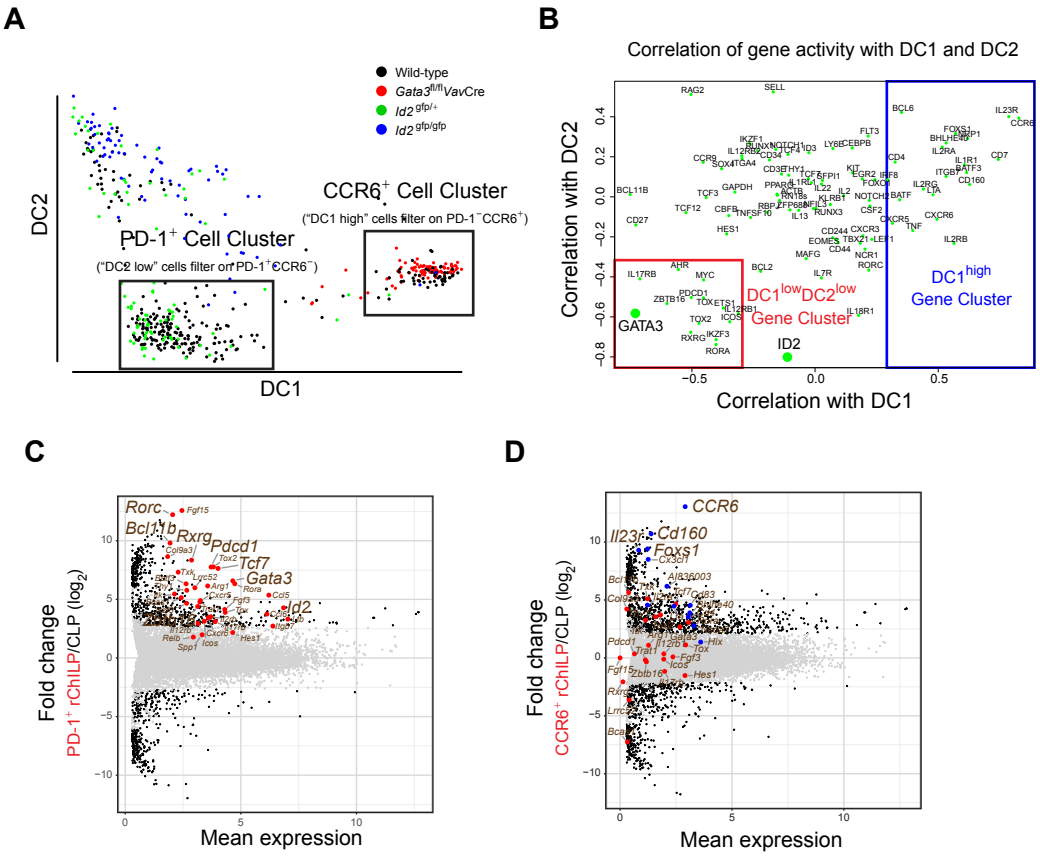

Figure S6. Gene Expression Profile in the Non-LTi and LTi Progenitors, Related to Figure 6

(A) The single cell gene expression data from Figure 5 C-E were pooled together as indicated, and analyzed with the diffusion map algorithm. DC2 is a subversion of that in the Figure 5 to simplify the calculation. The "PD-1$^+$ Cell Cluster" for the non-LTi progenitor was defined by the DC1^{low}DC2^{low} cells with further filter with the PD-1$^+$CCR6$^-$ population, and the "CCR6$^+$ Cell Cluster" for the LTi progenitor was defined by DC1^{high} cells with further filtered with the PD-1$^-$CCR6$^+$ population. (B) The 92 genes were scored based on their correlation with DC1 and DC2 values in (A). Based on the DC1 and DC2 value of the two ILC lineage progenitors, the non-LTi ILC progenitor related genes were defined as the "DC1^{low}DC2^{low} Gene Cluster" in the red gate, and the LTi progenitor related genes were defined as the "DC1^{high} Gene Cluster" in the blue gate. (C) The gene expression of non-LTi ILC progenitors (PD-1$^+$ rChILP) was analyzed by RNA-Seq. An MA-plot was performed to identify the non-LTi ILC progenitor specifically expressed genes compared with the CLP cells. (D) Similar as in (C), the gene expression of LTi progenitors (CCR6$^+$ rChILP) were analyzed by RNA-Seq. An MA-plot was performed to identify the LTi progenitor specifically expressed genes compared with the CLP cells. Data are pooled from two (A-B) independent experiments or from two biological duplicates (C-D).

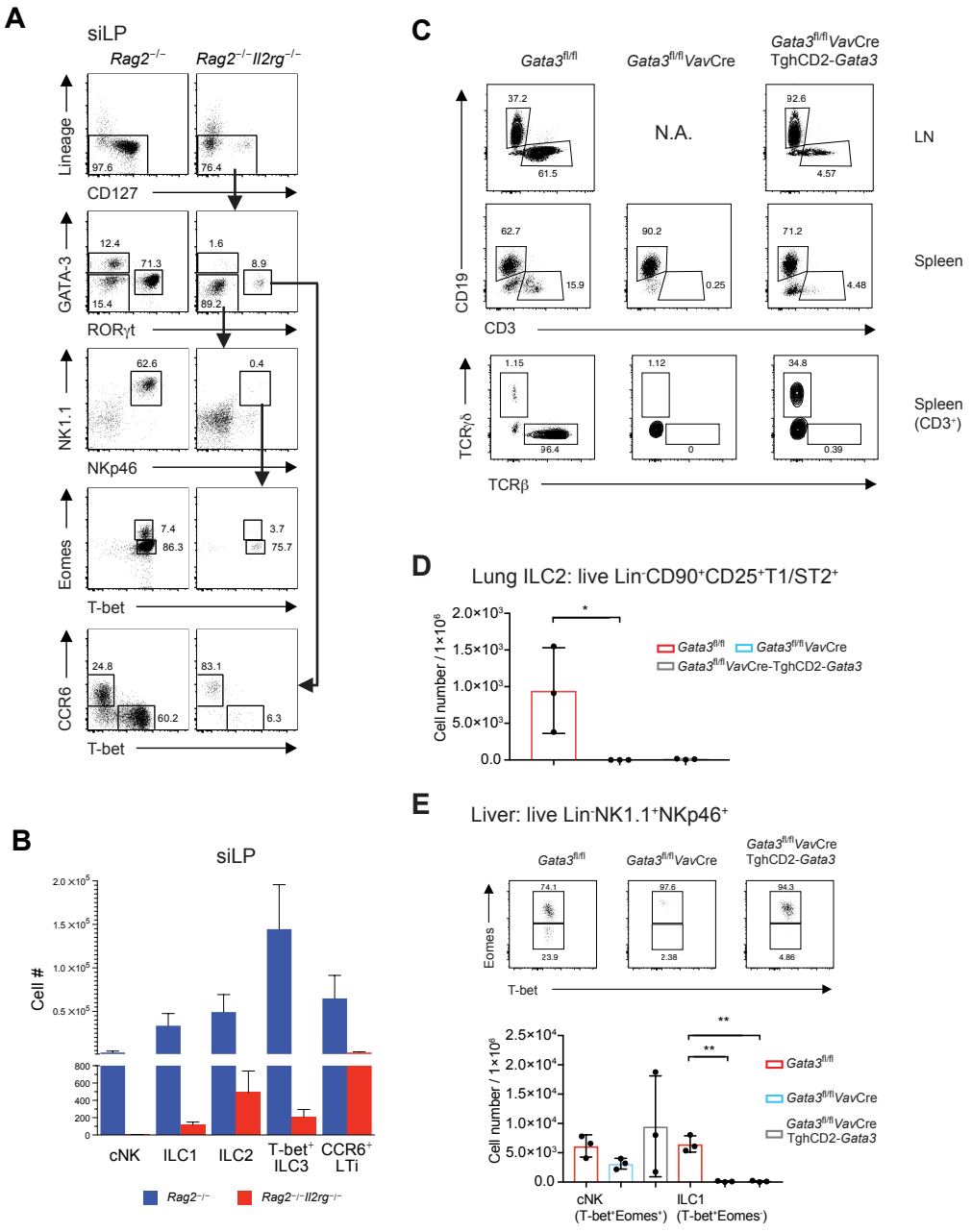

Figure S7. Dose-Dependent GATA3-Mediated Transcriptional Regulation Dictates the Development of ILCs Prior to Cytokine Signals, Related to Figure 7

(A) The siLP ILC subsets from *Rag2*$^{-/-}$ (γc cytokine signal sufficient) and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (γc cytokine signal deficient) mice were analyzed by flow cytometry. (B) The total cell numbers of distinct ILC subsets in the siLP of *Rag2*$^{-/-}$ and *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice were counted and plotted. (C) Cells harvested from the lymph nodes and spleen of the *Gata3*$^{fl/fl}$*Vav*Cre-TghCD2-*Gata3* mice were analyzed by flow cytometry. (D) ILC2s (live Lin^{-}CD90^{+}CD25^{+}T1/ST2^{+}) harvested from the lungs of the *Gata3*$^{fl/fl}$*Vav*Cre-TghCD2-*Gata3* and the control mice were analyzed by flow cytometry and their cell numbers were calculated and plotted (mean ± s.d.; n = 3; **P* < 0.05, Student's *t*-test). (E) Liver cells harvested from the *Gata3*$^{fl/fl}$*Vav*Cre-TghCD2-*Gata3* mice were analyzed by flow cytometry. The plots were gated on live Lin^{-} NK1.1^{+}NKp46^{+} cells. Liver cNK cells and ILC1s were further separated as T-bet^{+}Eomes^{+} and T-bet^{+}Eomes^{-}, respectively. Their cell numbers were calculated and plotted (mean ± s.d.; n = 3; ***P* < 0.01, Student's *t*-test). Data are representative of three independent experiments (A-C, E) and the combination of three independent experiments (D-E).