ILC1s control leukemia stem cell fate and limit development of AML

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Type I innate lymphoid cells (ILC1s) are critical regulators of inflammation and immunity in mammalian tissues. However, their function in cancer is mostly undefined. Here, we show that a high density of ILC1s induces leukemia stem cell (LSC) apoptosis. At a lower density, ILC1s prevent LSCs from differentiating into leukemia progenitors and promote their differentiation into non-leukemic cells, thus blocking the production of terminal myeloid blasts. All of these effects, which require ILC1s to produce interferon-γ after cell–cell contact with LSCs, converge to suppress leukemogenesis in vivo. Conversely, the antileukemia potential of ILC1s wanes when JAK–STAT or PI3K–AKT signaling is inhibited. The relevant antileukemic properties of ILC1s are also functional in healthy individuals and impaired in individuals with acute myeloid leukemia (AML). Collectively, these findings identify ILC1s as anticancer immune cells that might be suitable for AML immunotherapy and provide a potential strategy to treat AML and prevent relapse of the disease.

Allogeneic hematopoietic stem cell transplantation (HSCT) cures a small fraction of individuals with acute myeloid leukemia (AML), but the vast majority of other individuals relapse largely due to the persistence of leukemia stem cells (LSCs) that accumulate a multitude of mutations1,2. How immune cells interact with LSCs to prevent AML relapse is largely unknown. Identifying and exploiting the underlying mechanisms is an unmet medical need. Innate lymphoid cells (ILCs) are a heterogeneous population of non-B and non-T lymphocytes that originate from the common lymphoid progenitor. ILCs are classified into three groups: (1) group 1 ILCs composed of natural killer (NK) cells and type I ILCs (ILC1s), (2) group 2 ILCs (ILC2s), and (3) group 3 ILCs (ILC3s). Different from ILC2s and ILC3s, ILC1s usually reside in the liver, where they produce interferon-γ (IFNγ), granulocyte–macrophage colony-stimulating factor, tumor necrosis factor (TNF), TNF-related apoptosis-inducing ligand and so on. ILC2s and ILC3s play key roles in antiviral and antimicrobial immune responses, tumor surveillance and tumorigenesis, while ILC1s appear to have diverse functions, including promoting host defense against infection/microbiota, regulating tissue inflammation and protecting against acute tissue damage3,4. The roles of ILC1s in cancer remain unclear, and no available studies have examined the interaction of ILC1s and cancer stem-like cells.

To address this, we conducted a series of functional and mechanistic in vitro and in vivo studies. We demonstrated that ILC1s isolated from healthy mice or humans (healthy ILC1s) induced LSC apoptosis. Further, these ILC1s target LSCs to suppress leukemogenesis by preventing their differentiation into leukemia progenitors, thus blocking their differentiation into terminal myeloid blasts. These effects occurred via the production of IFNγ by ILC1s. Moreover, ILC1s produced more IFNγ than NK cells through the receptors DNAX accessory molecule 1 (DNAM-1) and interleukin-7Rα (IL-7Rα) interacting with LSCs. Because these functions are impaired in AML, ILC1s can no longer effectively target LSCs, which can then differentiate into terminal myeloid blasts. Collectively, we define an essential protective role for ILC1s in AML, which induce apoptosis and target differentiation of LSCs.

Results
Healthy ILC1s induce apoptosis of AML LSCs in vitro by secreting IFNγ. LSCs and normal HSCs (Lin−Sca-1+ c-Kit+ (LSK)) cannot be phenotypically distinguished in mice. Both are found mainly in bone marrow (BM) and spleen in AML11, while ILC1s reside in the liver. We isolated normal LSKs from the livers of healthy mice and LSCs from the livers of Kmt2aPTD/WT, Flt3ITD/ITD (MllPTD/WT; Flt3ITD/ITD) mice with AML12 for injection into immunodeficient Rag2−/−Il2γc−/− (Rag2−/−Il2γ−) mice. Mice injected with LSKs isolated from the livers of healthy mice lived, while all mice injected with LSKs from the livers of mice with AML died of AML, suggesting that LSCs are present in the livers of mice with AML (Extended Data Fig. 1a). This was validated by using a transplantable mouse LSC model in which CD45.1 mice were injected with LSCs (CD45.2+ cells) isolated from the spleens of MllPTD/WT, Flt3ITD/ITD mice with AML (Extended Data Fig. 1b). CD45.2+ LSCs trafficked to the livers of CD45.1 mice (~20% of total Lin− cells) 9 weeks after adoptive transfer (Extended Data Fig. 1b), consistent with a previous report13.

Using an AML mouse model (C1498 AML cells intravenously (i.v.) injected into C57BL/6 mice), we noted that the function of ILC1s (Lin−NK1.1+NKp46+CD49b−CD49a−; Extended Data Fig. 1c) from the liver and BM, but not the spleen, was impaired,

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as indicated by decreased production of IFNγ and TNF in AML mouse compared to in healthy mice (Extended Data Fig. 1d–f). Consistent with this, RNA sequencing (RNA-seq) of ILC1s indicated that NF-kB signaling, a pathway that controls ILC1 function, was inhibited in AML mice (Extended Data Fig. 1g). Because AML seemed to exert an immunosuppressive effect on ILC1s, we asked if ILC1s have an adverse effect on the genesis of AML. Sorted healthy liver ILC1s were cocultured for 3 d with splenic LSCs from the MIlT/WT, Flt3/STAMP AML mice. Purity of LSCs and ILC1s was over 95% (Extended Data Fig. 2a,b). LSCs were lysed by ILC1s at a ratio of 1:1 or 1:2, as evidenced by a decrease in the absolute number of live LSCs and an increase in the fraction of apoptotic LSCs compared to coculture without ILC1s (Fig. 1a–c and Extended Data Fig. 2c). Additionally, total caspase and caspase 3/caspase 7 activation of LSCs and the expression of the proapoptotic gene Bak1 significantly increased in coculture with ILC1s compared to in coculture without ILC1s (Fig. 1d–f and Extended Data Fig. 2d). We achieved similar results using ILC1s (Lin−CD56−CD127−c-Kit+Gr1−Th2−) isolated from healthy human peripheral blood (PB) that were cocultured with LSCs (CD45+Lin−CD34+CD38−) from individuals with AML (Fig. 1g–k and Extended Data Fig. 2e,f), further suggesting that healthy ILC1s induce apoptotic death of LSCs.

ILC1s lack a high level of cytolytic activity; they function primarily as immunoregulatory cells by secreting cytokines, including IFNγ and TNF. To determine how this affects leukemogenesis, we repeated our coculture experiment using healthy mouse ILC1s and LSCs in the presence of neutralizing antibodies against IFNγ or TNF. Neutralizing IFNγ, but not TNF, prevented ILC1s from mediating the death of LSCs (Fig. 1l,m). This requirement for IFNγ was validated using human cells (Fig. 1n). We cocultured the two cell types separately in a Transwell but did not observe LSC apoptosis (Extended Data Fig. 3a). Strikingly, IFNγ production by ILC1s significantly increased without the Transwell (Fig. 1o). Next, we observed LSC apoptosis when cultured with mouse IFNγ (Extended Data Fig. 3b) but not with TNF (Extended Data Fig. 3c). Collectively, our data demonstrate that cell–cell contact allows ILC1s to produce IFNγ, which induces LSC apoptosis.

ILC1s and secreted IFNγ inhibit differentiation of LSCs into leukemia progenitor cells. Initiation and differentiation of LSCs into leukemia progenitor cells drive the propagation of AML. To assess the effects of ILC1s on LSC differentiation, we cocultured LSCs isolated from the spleens of AML mice with or without ILC1s isolated from the livers of healthy mice for 4 d. The ratio of ILC1s to LSCs was 1:4, which was lower than in the apoptosis assay. Cultures with ILC1s contained a higher number of LSCs and a lower number Lin−Sca-1−c-Kit− leukemia progenitor cells (LS−K+ cells) compared to the group cocultured without ILC1s (Fig. 2a–c). Furthermore, the percentages and absolute cell numbers of Lin−Sca-1−c-Kit− non-leukemic cells (LS+K− cells) were significantly higher after coculture with ILC1s (Fig. 2d). No obvious difference was found in the Lin−Sca-1−c-Kit− cell population (Fig. 2e).

Previous studies demonstrated that non-leukemic LS−K+ cells contain early lymphoid-committed precursors that are highly apoptotic in mice with chronic myelogenous leukemia. Thus, ILC1s inhibit the differentiation of LSCs into LS−K+ leukemia progenitor cells while promoting their differentiation into non-leukemic LS+K− cells. To determine the mechanism, we included neutralizing antibodies against IFNγ and TNF in an ILC1–LSC coculture. IFNγ-neutralizing antibody, but not TNF-neutralizing antibody, inhibited both ILC1-mediated suppression of LSC differentiation into LS−K+ leukemia progenitor cells and induction of LSC differentiation into non-leukemic LS+K− cells (Fig. 2f–j). Results were validated by comparing LSCs cocultured with ILC1s from Ifng−/− (IFNγ−/−) or Tnf−/− (TNF−/−) mice with ILC1s from wild-type (WT) mice. ILC1s from IFNγ−/− mice could no longer inhibit the differentiation of LSCs into LS−K+ leukemia progenitor cells or promote their differentiation into non-leukemic LS+K− cells. However, ILC1s isolated from TNF−/− mice acted similar to ILC1s from healthy mice (Fig. 2f–j). Moreover, mouse IFNγ inhibited the differentiation of LSCs into LS−K+ leukemia progenitor cells and facilitated their differentiation into non-leukemic LS+K− cells (Fig. 2k,l and Extended Data Fig. 4a).

To determine if ILC1s regulate LSC differentiation through cell–cell contact (as proven to be critical for LSC apoptosis), we separated LSCs and ILC1s in a Transwell chamber. As expected, the percentages of LSCs, LS−K+ leukemia progenitor cells and LS+K− non-leukemic cells varied between LSCs cultured with and without ILC1s (Fig. 2m and Extended Data Fig. 4b). By contrast, the percentages were similar whether LSCs were separated from ILC1s by a Transwell or cultured without ILC1s (Fig. 2m and Extended Data Fig. 4b). These data suggest that cell–cell interaction is required for ILC1s to regulate LSC differentiation by inhibiting the differentiation of LSCs into LS−K+ leukemia progenitor cells while promoting their differentiation into non-leukemic LS+K− cells.

ILC1s and secreted IFNγ suppress differentiation of LSCs into terminal myeloid blasts. LSCs are hierarchical cells that differentiate into terminal myeloid blasts that sustain AML. When LSCs were cocultured with healthy ILC1s compared to coculture with no ILC1s, ILC1s significantly inhibited LSC differentiation into terminal myeloid blasts, as indicated by reduced populations of cells expressing macrophage-1 antigen (Mac-1) and myeloid differentiation antigen Gr-1 (Fig. 3a,b). When LSCs were cocultured with IFNγ−/− or TNF−/− ILC1s compared to WT ILC1s, we observed significantly increased populations of cells expressing Mac-1 and Gr-1 in the coculture with IFNγ−/− ILC1s but not in the coculture with TNF−/− ILC1s (Fig. 3c,d). Similar results were obtained in the
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ILC1s and IFNγ control LSC-derived AML in vivo. Next, we hypothesized that ILC1s could suppress leukemia development and growth in vivo. When we initiated the in vivo efficacy experiment, we did not know whether ILC1s could survive well in vivo after their adoptive transfer. Because IL-15 supports survival of ILC1s\(^7\), we first tested whether adoptively transferred WT ILC1s can suppress the development of LSCs co-injected with BM cells from IL-15 transgenic mice\(^8\) (Fig. 4a). Model mice treated with WT ILC1s had significantly fewer total WBCs than mice treated with IFNγ\(^−/−\) ILC1s and untreated groups, the latter two of which did not differ significantly from each other (Fig. 4b). A substantial reduction of the immature blast cell population in the blood and significantly prolonged survival were observed in mice treated with WT ILC1s compared to untreated mice or those treated with IFNγ\(^−/−\) ILC1s (Fig. 4c,d). Using mouse IFNγ to replace WT ILC1s in this experiment, an effect similar to that of WT ILC1s was observed; that is, the recombinant IFNγ had significantly fewer total WBCs than the untreated group (Extended Data Fig. 7a).

However, the above model could not distinguish LSC-derived and IL-15 transgenic BM-derived WBCs. Therefore, co-injected mouse CD45.2\(^+\) LSCs from AML mice along with WT CD45.1\(^+\) BM support cells were introduced into lethally irradiated CD45.1 recipient mice. The next day, we injected WT ILC1s or IFNγ\(^−/−\) ILC1s i.v. or IFNγ intraperitoneally (i.p.; Fig. 4e). Total WBCs (CD45.1\(^+\) and CD45.2\(^+\) WBCs), CD45.2\(^−\) WBCs, CD45.2\(^−\) LSCs and CD45.2\(^−\) immature blast cells (which have been reported to accumulate in AML\(^2\)) were counted 3 weeks after LSC implantation (Fig. 4e). Donor and host cells were distinguished by flow cytometry using anti-CD45.2 and anti-CD45.1 (Extended Data Fig. 7b). In this model, we also observed that compared to untreated or IFNγ\(^−/−\) ILC1-treated mice, mice treated with WT ILC1s or IFNγ had significantly reduced CD45.2\(^+\) WBCs (Fig. 4f) and total WBCs in PB (Extended Data Fig. 7c) and possessed significantly fewer CD45.2\(^−\) LSCs and CD45.2\(^−\) immature blast cells (Fig. 4g,h). The treated mice also survived significantly longer than the untreated or IFNγ\(^−/−\) ILC1-treated mice (Fig. 4i). Both mouse models indicate that ILC1s and IFNγ derived from them are sufficient to suppress leukemogenesis in vivo.

ILC1s, but not NK cells, require DNAM-1 and IL-7Rα for LSC-stimulated IFNγ production. Both ILC1s and NK cells express IFNγ, and, thus, we assessed each for their ability to produce IFNγ in the presence or absence of AML or LSCs. We sorted these two cell types from the livers of healthy mice and AML mice and

3 weeks later we killed the mice and analyzed donor hematopoietic and progenitor myeloid cell subsets and white blood cells (WBCs; Extended Data Fig. 6g). Consistent with our in vitro experiment, ILC1s did not significantly alter the absolute cell numbers of LSKs, myeloid progenitor cells (LSK\(^+\)), early lymphoid-committed precursors (LSK\(^+\)-Lin\(^−\)), short-term HSCs (ST-HSCs), long-term HSCs (LT-HSCs), multipotent progenitors 1 and 2 (MPP1 and MPP2), Mac-1\(^+\) and Lin\(^−\) cell subsets or WBCs (Extended Data Fig. 6h–j).

ILC1s do not substantially affect apoptosis and differentiation of normal HSCs. We next investigated whether ILC1s could promote apoptosis of normal HSCs (termed HSCs or WT LSKs or Lin\(^−\)Sca-1\(^+\)c-Kit\(^+\) cells). After sorting ILC1s from the livers of healthy mice, we cocultured them for 3 d with HSCs isolated from the BM of healthy mice at ILC1-to-HSC ratios of 1:1 or 1:2. The ILC1s did not display a significant difference in absolute cell numbers and percentages of cell subsets and white blood cells (WBCs). Consistent with our in vitro experiment, ILC1s did not significantly alter the absolute cell numbers of LSKs, myeloid progenitor cells (LSK\(^+\)), early lymphoid-committed precursors (LSK\(^+\)-Lin\(^−\)), short-term HSCs (ST-HSCs), long-term HSCs (LT-HSCs), multipotent progenitors 1 and 2 (MPP1 and MPP2), Mac-1\(^+\) and Lin\(^−\) cell subsets or WBCs (Extended Data Fig. 6h–j).
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cocultured each preparation separately with LSCs. The ILC1s isolated from AML mice produced significantly less IFNγ than those from healthy mice. This difference was not observed with NK cells (Fig. 5a). Additionally, healthy ILC1s cocultured with LSCs produced more IFNγ than cocultured NK cells (Fig. 5a). These results suggest that AML impairs IFNγ production by liver ILC1s but not by liver NK cells and that healthy liver ILC1s produce more IFNγ than healthy liver NK cells when they interact with LSCs. Finally, ILC1s are a critical supplier of IFNγ and appear to play a more important direct role than NK cells against LSCs.

Our previous data showed that ILC1s need cell–cell contact with LSCs to produce IFNγ (Fig. 1o). This led us to identify receptors and ligands required for the effector and target cells to interact. The activating receptor DNAM-1 is expressed more highly on ILC1s than on NK cells and is critical for IFNγ production24–26. We confirmed that the receptor was differentially expressed on these two types of innate immune cells and further showed that DNAM-1 expression on AML ILC1s was significantly downregulated compared to its expression on healthy ILC1s but not on NK cells (Fig. 5b). We also discovered that both ligands of DNAM-1 (CD155 and CD112)
were highly expressed on LSCs (Fig. 5c). Thus, we hypothesized that ILC1s recognize LSCs at least partially through DNAM-1. As expected, DNAM-1 neutralizing antibody significantly blocked the production of IFNγ in healthy ILC1s but not in healthy NK cells (compared to the control without DNAM-1-neutralizing antibody; Fig. 5d). That blockade was only partial, however, so we searched for
an additional mechanism underlying the interaction of ILC1s with LSCs. We focused on IL-7R, which is expressed during the development and maturation of all ILC subsets, including ILC1s, but is not expressed on liver NK cells. Likewise, IL-7 plays an important role in the development of ILC1s but not NK cells. Similarly, we observed high expression of IL-7R in liver ILC1s but not on liver NK cells (Fig. 5c). We also discovered that LSCs produced IL-7 (Fig. 5f). Therefore, we suspected that the IL-7–IL-7R signaling pathway upregulates IFNγ in ILC1s from healthy mice that were cocultured with LSCs, as recently described in a non-cancer liver injury model. Indeed, blockade of IL-7–IL-7R signaling with an IL-7R-neutralizing antibody downregulated the production of IFNγ in ILC1s from healthy mice.
we isolated LSCs from the spleens of CD45.2 MllPTD/WT: Flt3ITD/ITD CD45.1 mice (Fig. 5i and Extended Data Fig. 8g). Three days later, individual mice); MFI, mean fluorescence intensity. (\(=\), Representative flow histograms of CD155 and CD112 on mouse LSCs. Representative flow cytometry plots and statistics of IFN\(\gamma\) or NK cells after coculture with or without LSCs in the presence or absence of an anti-DNAM-1 or isotype IgG control (\(=\) individual mice). e, Expression of IL-7R\(\alpha\) on liver ILC1s or NK cells. RT–PCR analysis of mouse il7 mRNA expression in LSCs, ILC1s and NK cells. g, Healthy ILC1s or NK cells were cocultured with or without LSCs in the presence or absence of an anti-IL-7R\(\alpha\) or isotype IgG. Statistics of IFN\(\gamma\) LSCs (\(=\) individual mice). h, Percentages of IFN\(\gamma\) cells in ILC1s or NK cells after treatment with or without IL-7 in the presence of IL-12 plus IL-15 (\(=\) individual mice). i, Overall experimental design for j–l. j, Representative flow cytometry plots of the percentages of CD45.1 and CD45.2 cells. k, Statistical analysis of CD45.2+ WBC numbers (\(=\) individual mice). Box plots display the median and interquartile range (25th percentile to 75th percentile), with whiskers representing the upper and lower quartile (1.5x the 75th and 25th percentile values). Data are representative of two (c, e, f, j and k) or three (a, d, g and h) independent experiments. Data are presented as mean \(\pm\) s.d. and were assessed by unpaired (a) and paired (b, d, g and h) Student’s t-tests (two-tailed) or one-way ANOVA (k).

IFN\(\gamma\) in ILC1s from healthy mice, but not in NK cells, after interaction with LSCs (Fig. 5g and Extended Data Fig. 8a). However, treatment with IL-7 significantly increased IFN\(\gamma\) production in ILC1s but not in NK cells of healthy mice (Fig. 5h and Extended Data Fig. 8b). These results indicate that LSCs cognate ligands to DNAM-1 and IL-7R\(\alpha\) induce more potent IFN\(\gamma\) in healthy ILC1s than in NK cells, which in turn assists ILC1s in becoming more effective suppressors of leukemia.

**ILC1s collaborate with NK cells to control LSCs in vivo.** The above results do not exclude that liver NK cells are potent against LSCs, as their coculture enhanced LSC apoptosis to some extent (Extended Data Fig. 8c–f). However, IFN\(\gamma\)-neutralizing antibody did not affect their action, suggesting that, unlike ILC1s, the induction of LSC apoptosis by liver NK cells is not occurring primarily through IFN\(\gamma\) (Extended Data Fig. 8c–f).

To evaluate whether NK cells would slow the progression of AML in vivo, we i.p. injected anti-NK1.1 (resulting in depletion of both NK cells and ILC1s) or an optimized dose of anti-asialo GM1 (resulting in depletion of NK cells alone) into immunocompetent recipient CD45.1 mice (Fig. 5i and Extended Data Fig. 8g). Three days later, we isolated LSCs from the spleens of CD45.2 MB\(^{FLDTW}T\) F1\(^{FLDTDT}\) mice with AML and i.v. injected CD45.2 LSCs into the mouse depletion model. The preferential depletion of NK cells resulted in a small but significant increase in LSC-derived WBC counts compared to non-depletion, while depletion of both ILC1s and NK cells produced a ~20-fold increase in WBC cells compared to IgG control and >6-fold increase compared to the anti-asialo GM1 group with NK depletion alone (Fig. 5j,k).

**JAK–STAT and PI3K–AKT signaling in LSCs exploited by ILC1s or IFN\(\gamma\).** To uncover the mechanisms by which ILC1s regulate LSCs, we conducted RiboZero RNA-seq analysis of LSCs cocultured with or without ILC1s isolated from healthy mice or mouse IFN\(\gamma\). Of note, after ILC1–LSC coculture, the LSCs were separated from the LSCs by FACS (Extended Data Fig. 9a,b). Subsequent RNA-seq revealed that, compared to untreated LSCs, the LSCs cocultured with ILC1s had 445 significantly upregulated genes and 93 downregulated genes. In LSCs treated with IFN\(\gamma\), 320 genes were significantly upregulated, and 82 were downregulated (Extended Data Fig. 9c). Furthermore, LSCs cocultured with ILC1s or IFN\(\gamma\) had a large number of upregulated and downregulated genes in common (Fig. 6a and Extended Data Fig. 9d,e), supporting our conclusion that ILC1s regulate LSCs by producing IFN\(\gamma\). Among the upregulated LSC genes unique to the ILC1–LSC coculture, 3 of the top 10 were chemokines (Ccl3, Ccl4 and Xcl1; Extended Data Fig. 9f), suggesting that the interaction of ILC1s with LSCs may recruit additional immune cells into the tumor microenvironment (TME) to suppress the development of AML. Using gene set enrichment analysis (GSEA), we identified the top 10 pathways associated with those upregulated and downregulated genes (Fig. 6b and Extended Data Fig. 9g). LSCs cocultured with ILC1s or IFN\(\gamma\) activated apoptotic pathways in LSCs while significantly suppressing E2F targets, G2M checkpoints, MYC targets and mitotic spindle pathways (Extended Data Fig. 10a,b), consistent with our finding that ILC1-secreted IFN\(\gamma\) increased LSC apoptosis.

Additionally, after coculture with ILC1s or IFN\(\gamma\), LSCs showed activation of JAK–STAT and PI3K–AKT signaling (Fig. 6c) and increased expression of Akt3, Jak2, Stat1/Stat2, Ifi1l/Ifi2/Ifi7/Ifi8/Ifi9 and suppressor of cytokine signaling 1 (Socs1), all of which are downstream of IFN\(\gamma\) signaling (Fig. 6d–f and Extended Data Figs. 10c–d). This unbiased analysis strengthened our conclusion that ILC1s regulate LSCs via IFN\(\gamma\). It also suggests that ILC1s or secreted IFN\(\gamma\) use JAK–STAT or PI3K–AKT signaling to regulate LSCs. To confirm this, we pretreated LSCs for 30 min with inhibitors of signaling components involved in these two pathways and cocultured them with WT ILC1s or IFN\(\gamma\)−− ILC1s. Compared to coculture with ILC1s alone, the JAK2 inhibitor AZD1480 or the JAK1/JAK2/JAK3 inhibitor VX-509 combined with ILC1s significantly increased LSC differentiation into LS-K+ leukemic progenitor cells and decreased their differentiation into non-leukemic LS-K− cells. There was no significant alteration in the LS-K− cell population (Fig. 6g–k and Extended Data Fig. 10e–h), Predictably, the two JAK inhibitors did not affect LSCs cocultured with IFN\(\gamma\)−− ILC1s. We obtained similar results using aferesib, an AKT inhibitor (Fig. 6g,h and Extended Data Fig. 10e–h). These data suggest that ILC1-derived IFN\(\gamma\) regulates the differentiation of LSCs through JAK–STAT or PI3K–AKT signaling.

**ILC1-derived IFN\(\gamma\) inhibits LSC differentiation via JAK–STAT and PI3K–AKT signaling.** a, Mouse LSCs were cocultured with or without ILC1s or IFN\(\gamma\) for 3 d. LSCs were separated from cocultured ILC1s by FACS before RNA-seq. A heat map showing differential expression of the top 20 upregulated and downregulated genes is shown (\(n=3\) individual mice). b, Hallmark pathway analysis in LSC RNA pools (ILC1-treated versus untreated control). Left, signaling pathways downregulated in LSCs. Right, signaling pathways upregulated in LSCs (\(n=3\) individual mice); NES, normalized enrichment score; FDR, false discovery rate. c, GSEA plots showing enrichment of selected target genes in LSCs cocultured with ILC1s. The rank orders (ILC1 versus control) of all genes (\(n=3\) individual mice) are shown on the x-axis. d–f, Differential expression of Akt3 (d), Jak2 (e) and Stat1 and Stat2 (f) genes. Results are expressed as means compared with the control (\(n=3\) individual mice). g–k, Mouse LSCs were treated with or without the indicated JAK and AKT inhibitors for 30 min and then cocultured with or without WT or IFN\(\gamma\)−− ILC1s in the presence of IL-12 plus IL-15 for 3 d. Representative flow cytometry plots (g) and statistics of absolute cell numbers (h–k) of Lin–Sca-1−c-Kit+ Lin–Sca-1−c-Kit+, Lin–Sca-1−c-Kit+ and Lin–Sca-1−c-Kit+ cells are shown (\(n=3\) individual mice). Data in g, h, i, j and k are representative of three independent experiments, are presented as mean \(\pm\) s.d. and were assessed by one-way ANOVA models.
Fig. 7 | ILC1s are functionally impaired in AML. a, Mouse LSCs were cocultured with or without ILC1s isolated from healthy mice (healthy ILC1) or MllPTD/WT; Flt3ITD/ITD mice with AML (AML ILC1). Representative images of apoptotic LSCs (×5 magnification; n = 3 individual mice) are shown. b, Statistics of the percentages of viable human LSCs (n = 3 individual mice). c, Human LSCs from PB of individuals with AML were cocultured with or without ILC1s isolated from healthy donors (healthy ILC1) or individuals with AML (AML ILC1). Representative images of apoptotic LSCs (×5 magnification; n = 3 individual donors) are shown. d, Statistics of the percentages of viable human LSCs (n = 7 individual donors in the no ILC1 group and healthy ILC1 group; n = 6 individual donors in the AML ILC1 group). e, Supernatants from LSCs cocultured with or without healthy ILC1s or AML ILC1s were collected and subjected to enzyme-linked immunosorbent assay (ELISA) to determine levels of IFNγ in the AML ILC1 group. f–h, Mouse LSCs were cocultured with or without healthy ILC1s or AML ILC1s in the presence or absence of anti-IFNγ for 3 d. Representative flow cytometry plots of the percentages (f) and statistics of the percentages (g) and absolute cell numbers (h) of Lin−Sca-1−c-Kit+, Lin−Sca-1−c-Kit−, Lin−Sca-1+c-Kit− and Lin−Sca-1+c-Kit+ cells are shown (n = 7 individual mice). Data are representative of two independent experiments (a, b, f, g and h) or pooled from three independent experiments (d and e), are presented as mean ± s.d. and were assessed by one-way ANOVA models. The log transformation was used in g and h. Box plots (g and h) display the median and interquartile range (25th percentile to 75th percentile) with whiskers representing the upper and lower quartile (1.5x the 75th and 25th percentile values).
ILC1s become less able to target LSCs in AML. Our data showed that ILC1s isolated from the livers of AML mice produced less IFNγ and TNF than ILC1s isolated from healthy mice (Extended Data Fig. 1d). To determine whether ILC1s in AML are less able to target LSCs, we sorted ILC1s from the livers of healthy mice and from the livers of AML mice and cocultured each of them with splenic LSCs isolated from AML mice for 3 d. ILC1s isolated from AML mice were less able to lyse LSCs than ILC1s from healthy mice (Fig. 7a), resulting in increased LSC viability (Fig. 7b). We obtained similar results when we compared ILC1s from individuals with AML to those from healthy donors (Fig. 7c,d). Thus, healthy ILC1s cocultured with LSCs produced significantly more IFNγ than AML ILC1s (Fig. 7e). Compared to healthy ILC1s, AML ILC1s also induced less LSC differentiation into LS−K− non-leukemic cells and were less able to suppress LSC differentiation into LS+K+ leukemia progenitor cells (Fig. 7f–h). However, when IFNγ-neutralizing antibody was added to the culture, healthy mouse ILC1s and AML ILC1s were similarly ineffective against LSCs (Fig. 7f–h). These data suggest that the antileukemic function of ILC1s in humans with AML is as impaired as it is in mice.

Discussion

ILC1s play critical roles in inflammation and the early antiviral response39,40. However, their role in preventing and/or promoting cancer, including AML, has not been explored. We showed that the progression of AML can be controlled by healthy ILC1s interacting with LSCs. ILC1s have dual roles in regulating LSCs in AML: (1) ILC1s induce apoptosis of LSCs at high effector-to-target ratios, and (2) at a lower dose of effector cells, ILC1s suppress the differentiation of LSCs into leukemia progenitor cells and then to myeloid blasts while facilitating the differentiation of LSCs into non-leukemic cells. However, ILC1s do not affect apoptosis and differentiation of HSCs. Mechanistically, although both IFNγ and TNF are secreted by ILC1s, IFNγ mediates ILC1s to act on LSCs via JAK–STAT or PI3K–AKT signaling. Additionally, ILC1s produce higher levels of IFNγ to control LSCs than do NK cells when DNAM-1 and IL-7Rα are expressed on LSCs interact with their cognate ligands expressed on ILC1s. Thus, ILC1s may normally perform critical surveillance by spotting and destroying LSCs; consequently, a dysfunction in this innate immune cell population can facilitate tumorigenesis.

In relapsed AML, a small population of LSCs is resistant to standard chemotherapy41. Elucidating the mechanism(s) of LSC resistance is a critical unmet challenge, and developing new approaches to targeting LSCs offers a potential strategy for prolonging relapse-free survival of individuals with AML. Chemotherapy and targeted therapy can kill leukemic blasts but may also enrich LSCs42. Healthy ILC1s act directly on LSCs to control AML progression. Therefore, given the special biologic function of ILC1s, expanding autologous ILC1s in vivo or administration of allogeneic ILC1s expanded ex vivo during times of remission or the combination of expanded ILC1s with an FDA-approved drug may have a positive impact on prolonging relapse-free survival of individuals with AML.

IFNγ plays important roles in antiviral and antitumor immunity and has been used clinically to treat several diseases43. However, IFNγ-based therapies have at least two limitations that preclude routine clinical use for individuals with cancer: (1) systemic delivery of IFNγ is difficult to reach local tumor sites to subsequently achieve effective concentrations in the TME without significant toxicity44, and (2) IFNγ is rapidly cleared from the blood after i.v. administration, further limiting the ability to achieve effective local concentrations. These clinical disadvantages necessitate the development of alternative methods to ensure the effectiveness of IFNγ in the local milieu of the marrow and/or other organs while limiting toxicity. Additionally, IFNγ can act on tumors, tumor stem cells and immune cells, resulting in PD-L1 induction, which can block T cell responses to tumor cells and cancer stem-like cells45, differentiation of cancer stem-like cells and activation of immune cells46. Although these roles are complex and should be considered before the clinical use of IFNγ, the ability of anti-PD-L1 to block the adverse effects of IFNγ-upregulated PD-L1 provides a good rationale for combining IFNγ or, if too toxic, combining cells that produce this cytokine, such as ILC1s, with anti-PD-L1 antibody to treat cancers, including AML.

Our study provides a promising approach to treating AML by using a cell-based source of IFNγ to target LSCs. Although ILC1s are a minute cell population, they express abundant IFNγ, especially when interacting with tumor cells in the TME. ILC1s also express high levels of chemokine receptors, including CXCR3 and CXCR6, the respective receptors for CXCL9–CXCL11 and CXCL16 expressed by AML cells27. These receptor–ligand interactions may help recruit ILC1s to the BM or tumor sites where most LSCs reside47. Leveraging chemotaxis to drive immune cells, including ILC1s, toward tumor cells may be important for controlling the disease. Furthermore, ILC1s rapidly and persistently produce IFNγ locally after contacting LSCs or more mature tumor cells, yielding sufficient cytokines to locally target AML blasts48. Our data suggest that ILC1s can also induce apoptosis and differentiation of LSCs within the TME. Moreover, ILC1s are associated with reducing severe progression of graft-versus-host disease after allogeneic HSCT treatment for AML. This suggests that ILC1s can control outcomes in AML through their multifaceted roles.

Like ILC1s, NK cells also belong to group 1 ILCs. Although more than a dozen studies have assessed the efficacy of infusing NK cells into individuals in remission following AML treatment, some of which showed promising results49, none have yet explored therapeutic ex vivo expansion and infusion of ILC1s during AML remission. Our data provide a strong rationale for developing methodologies to expand ILC1 populations rapidly and reproducibly for application as a cellular therapy to prolong relapse-free survival in individuals with AML who achieve complete remission but may carry quiescent LSCs. This would be especially valuable for individuals who are ineligible for HSCT, such as the elderly.

In summary, we identified previously unknown functions of ILC1s. ILC1s can closely regulate AML LSCs by inducing apoptosis, prevent LSCs from differentiating into leukemia progenitors and myeloid blasts and promote the differentiation of LSCs into a non-leukemic lineage. All these actions are mediated by IFNγ that ILC1s secrete when they form cell–cell contact with LSCs. We therefore believe that by uncovering the mechanisms underlying these processes, our study could unveil an innovative immunotherapeutic approach (administration of ILC1s that have been multiplied ex vivo) to prolong relapse-free survival of individuals diagnosed with AML.

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/s41590-022-01198-y.

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Methods

Human samples. Human specimens were collected from individuals with AML registered at City of Hope National Medical Center who consented to an Institutional Review Board (IRB)-approved protocol (IRB 18009); healthy donor samples were collected from donors who consented to IRB 870629. Mononuclear cells were isolated from PB samples of healthy donors or individuals with AML using Ficoll separation. LinCD54+CD34+CD38- cells were sorted using a BD FACSARia Fusion (BD Biosciences).

Mouse studies. C57BL/6J (B6, CD45.2), Rag2γc−/−, IFNγ−/−, TNF−/− and CD45.1 (B6.SL-Pepc−/−/BoyJ) mice were purchased from the Jackson Laboratory. MIP1α+/−, Flt3ITD−/− mice and IL-15 transgenic mice on the B6 background were generated by our group. All mice were maintained by the Animal Resource Center of City of Hope. Six- to 12-week-old CD45.2 and CD45.1 mice of both sexes were used as recipients for AML cell transplantation, and 18- to 28-week-old MIP1α+/−, Flt3ITD−/− mice with AML of both sexes were used as donor mice. For functional and survival studies, mice of the same age and sex were divided randomly into experimental groups. Experimenters were blinded to observe mouse survival. No mice were excluded from the analyses unless clearly indicated. Mice were fed with Picolab Rodent Diet 20 (5053) and housed in the City of Hope Animal Facility with a 12-h light/12-h dark cycle and temperatures of 18–23°C with 40–60% air humidity. Mouse care and experimental procedures were performed in accordance with federal guidelines and protocols approved by the Institutional Animal Care and Use Committee at City of Hope under protocols 18108 and 20003. Tumor-bearing mice were monitored twice per week and at more frequent intervals depending on the status of the mice. Any evidence of distress, discomfort, pain, lethargy, inability to properly groom or inability to obtain food and/or water were killed immediately via CO2 inhalation. Tumor-bearing mice with 20% weight loss from the age-matched controls without receiving tumor cell inoculation were killed.

Cells and culture. Human LSCs were cultured in StemSpan serum-free expansion medium II (STEMCELL) with penicillin (100 U ml−1), streptomycin (100 µg ml−1), Stemline (20 ng ml−1), IL-3 (10 ng ml−1) and IL-6 (10 ng ml−1). Mouse LSCs were cultured in Iscove's modified Dulbecco's medium (Thermo Fisher Scientific) with 10% fetal bovine serum (FBS), penicillin (100 U ml−1), streptomycin (100 µg ml−1), Stemline (20 ng ml−1), thrombopoietin (20 ng ml−1), Flt3-L (20 ng ml−1), IL-3 (10 ng ml−1) and IL-6 (10 ng ml−1). Human and mouse ILC1s or NK cells were cultured in RPMI 1640 (Thermo Fisher Scientific) with 10% FBS, penicillin (100 U ml−1), streptomycin (100 µg ml−1), IL-12 (10 ng ml−1) and IL-15 (10 ng ml−1). The mouse AML cell line C1498 (ATCC) was cultured in RPMI 1640 with 10% FBS, penicillin (100 U ml−1) and streptomycin (100 µg ml−1). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2. All cytokines were from PeproTech. Penicillin and streptomycin were from Thermo Fisher Scientific.

Flow cytometry. ILC1s from human PB were identified using surface staining with a live/dead cell viability cell staining kit (Invitrogen) and the following monoclonal antibodies: lineage (FITC-conjugated anti-CD3, anti-CD4, anti-CD8, anti-CD14, anti-CD19, anti-CD20, anti-CD11c, anti-CD33, anti-CD34, anti-CD203c and anti-Flt3), CD38 (PE-Cy7-conjugated anti-CD38), Gr-1 (PE-conjugated anti-Gr-1), CD117, CD127, CD150, CD199, CD49a, CD49b, CD48 and NK1.1 (BV510-conjugated anti-NK1.1), CD49d (AF647-conjugated anti-CD49d), CD10 (AF700-conjugated anti-CD10), CD11c (AF700-conjugated anti-CD11c), CD69 (AF700-conjugated anti-CD69), NKp46 (AF647-conjugated anti-NKp46), CD49b (AF700-conjugated anti-CD49b) and CD49a. Thirty minutes later, the cells were washed three times and then sorted using a BD FACSARia Fusion.

ILC1s and ILC1s in vitro coculture assays. For mouse LSC coculture assays, LSCs from MIP1α+/−, Flt3ITD−/− mice with AML were labeled with 5 mM CellTrace Violet (CTV; Thermo Fisher Scientific) and cocultured in the presence of mouse IL-12 (10 ng ml−1) and IL-15 (10 ng ml−1) with various numbers of ILC1 or NK cells isolated from the livers of healthy mice or AML mice. For human LSC coculture assays, LSCs from individuals with AML were labeled with 5 µM CTV and cocultured in the presence of human IL-12 (10 ng ml−1) plus IL-15 (10 ng ml−1) with various numbers of ILC1s isolated from the PB of healthy donors or individuals with AML. For coculture of LSCs and ILC1s in the Transwell coculture system, LSCs were seeded in the lower chamber of a 96-well Transwell plate, while varying numbers of mouse ILC1s were seeded in the upper chamber. For coculture assays with cytokines and antibodies, mouse or human LSCs were cocultured with various doses of mouse TNF (0.25 µg ml−1, 0.5 µg ml−1, 0.75 µg ml−1 and 1 µg ml−1), mouse IFNγ (0.1 ng ml−1, 1 ng ml−1, 10 ng ml−1, 0.25 µg ml−1, 0.5 µg ml−1, 0.75 µg ml−1 and 1 µg ml−1), anti-TNF (10 µg ml−1) or anti-IFNγ (10 µg ml−1). For all coculture assays, cells were collected after 3 d and analyzed by flow cytometry.

In vivo HSC transplantation assay. In all transplantation experiments, recipient mice were killed 3 d after reconstitution, and luminescence was determined with Peace (Zeiss AxioCam M2). To compare the LSC differentiation assay, LSCs were isolated from MIP1α+/−, Flt3ITD−/− mice with AML and cocultured with or without ILC1s isolated from the livers of healthy mice or MIP1α+/−, Flt3ITD−/− mice with AML for 1 to 4 d in the presence or absence of anti-TNF (10 µg ml−1) or anti-IFNγ (10 µg ml−1). Cells were collected and analyzed by flow cytometry.

Caspase 3/caspase 7 activity assay. ILC1s were cocultured with LSCs at a ratio of 1:1 or 1:2 for 6 h. Next, 100 µl of Caspase-Glo 3/7 reagent was added to each well. Plates were then shaken at 300 r.p.m. for 1 min, incubated for 60 min at room temperature and read on a luminometer (Promega, Glomax). Background luminescence was determined with 100 µl of culture medium without cells and subtracted before fold changes were calculated.

In vitro colony-forming unit assay. LSCs were obtained from MIP1α+/−, Flt3ITD−/− mouse spleens and cocultured with or without WT, IFNγ−/− or TNF−/− ILC1s for 3 d. Cells were then plated into mouse methylcellulose complete medium (R&D, HSCI07) supplemented with human transferrin (200 µg ml−1), recombinant human insulin (0.5 µg ml−1), recombinant SCF (100 ng ml−1), recombinant GM-CSF (100 ng ml−1), recombinant IL-3, IL-6 (10 ng ml−1) and recombinant mouse erythropoietin (31 µg ml−1). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 for 10–14 d. Colony numbers were counted using a microscope.

In vivo HSC transplantation assay. In all transplantation experiments, recipient mice were fed with sulfatrim-based food (5SW8, TestDiet) after transplantation to avoid any infection/toxicity associated with irradiation. HSAs (3×104) were isolated from BM cells of 6- to 12-week-old healthy CD45.2 male mice and i.v. co-injected with 5×106 to 12-week-old female CD45.1 BM cells (as support...
cells) into lethally irradiated (900 cGy) 6- to 12-week-old female C57BL/6J (CD45.1) recipients. One day later, 3 × 10^5 ILC1s (CD45.2) isolated from the livers of healthy mice were i.v. injected into these recipient mice. The LSKs, Lin− Sca-1+ c-Kit+ cells, Lin− Sca-1− c-Kit− Kit+ cells, STHSCs, LTILHSCs, MPP1s, MPP2s, Mac-1+Gr-1+ cells and WBCs derived from donor mice were analyzed 3 weeks after HSC transplantation using an Element HT5 hematology analyzer (Heska) and flow cytometry (BD Biosciences).

In vivo LSC transplantation assay. In all transplantation experiments, recipient mice were fed with sulfathiazole-based food (SW/SE; Test Diet) after transplantation to avoid any infection/toxicity associated with irradiation. BM cells (0.5 × 10^6) from 6- to 12-week-old female CD45.1 WT mice or BM cells isolated from male IL-15 transgenic mice (CD45.2) depleted of NK1.1+ NKp46+ cells were transplanted by i.v. injection with 3 × 10^5 LSCs obtained from 18- to 28-week-old MllPTD/WT, Flt3ITD/WT mice with LSCs isolated from the livers of healthy mice or AML mice using a BD FACSAria Fusion. For stimulation with recombinant mouse IL-7, mouse ILC1s or NK cells were sorted from the livers of healthy mice and were treated with or without control, mouse ILC1s or NK cells were sorted from the livers of healthy mice and were treated with or without IFNγ−/− mice labeled with CTV at a ratio of 4:1 or treated with IFNγ (10 ng ml−1). Thirty days later, cells were collected and analyzed by flow cytometry.

ELISA. Cell supernatants were collected and analyzed for cytokine content by ELISA according to the manufacturer's protocols. LSCs isolated from the PB of individuals with AML were cocultured with the ILC1s isolated from healthy donors or individuals with AML in the presence of IL-12 (10 ng ml−1) and IL-15 (100 ng ml−1) for 3 days. Levels of IFNγ in culture supernatants were measured using the human IFNγ Quantikine ELISA kit (DIF50C, R&D). Samples for each condition were assayed in three duplicates.

Statistical analyses. For continuous endpoints, a Student's t-test or paired t-test was used to compare two independent or matched conditions, and one-way ANOVA models were used to compare three or more independent conditions. Linear mixed models were used to account for the variance–covariance structure due to repeated measures. Mouse survival functions were estimated by the Kaplan–Meier method and compared by log-rank tests. All tests were two-sided. P values were adjusted for multiple comparisons by Holm's procedure. No statistical methods were used to predetermine sample sizes, but sample sizes are similar to those reported in previous publications. Data distribution was assumed to be normal, but this was not formally tested. For RNA-seq analysis, sequencing reads were trimmed from sequencing adapters using Trimmomatic and poly(A) tails using FASTP and mapped back to the mouse genome using STAR (v.0.20.0). The gene-level count table was created by high-throughput sequence (HTSeq v0.6.0) and normalized by the trimmed mean of mean values method. General linear models based on negative binomial distributions (R package EdgeR) were used to compare gene expression levels between two specific cell types. Genes with a false discovery rate-adjusted P value less than 0.05 and a fold change greater than 1.5 (upregulated) or less than 0.7 (downregulated) were considered as differentially expressed genes. Pathway analysis and enrichment analysis were performed using the GSEA48,49 program, which runs the GSEA Permutation algorithm on a ranked list of genes. Data are presented as mean ± s.d. Prism software v.8 (GraphPad) and SAS v.9.4 (SAS Institute) were used to perform statistical analyses.
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Author contributions
J.Y. and M.A.C. conceived and designed the study. Z.L., R.M., S.M., L.T. and T.L. developed the methodology. Z.L., R.M., S.M., L.T., T.L., B.L.M.-B., B.Z. and G.M. acquired the data (for example, provided animals, acquired and managed donors, provided facilities and so on). Z.L., J.Y. and J.Z. analyzed and interpreted data (for example, statistical analysis, biostatistics and computational analysis). Z.L., R.M., S.M., J.Y. and M.A.C. wrote, reviewed and/or revised the manuscript. J.Z. provided administrative, technical or material support (for example, reporting or organizing data and constructing databases). J.Y. and M.A.C. supervised the study and acquired funding. All authors discussed the results and commented on the manuscript.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | ILC1s are functionally impaired in mice with AML. a, 0.2×10^6 LSKs isolated from the liver of healthy mice or MllPTD/WT: Flt3ITD/ITD mice with AML were i.v. injected into immunodeficient Rag2^-/-γc^-^- mice. The survival of those mice was analyzed by the Kaplan-Meier method and log-rank test (n=3 individual mice). b, LSCs were isolated from the spleen of MllPTD/WT: Flt3ITD/ITD mice with AML (CD45.2) and then transplanted into lethally irradiated mice (CD45.1). The percentages of LSCs in the liver of the CD45.1 mice were analyzed 9 weeks after LSC transplantation using flow cytometry. c, Gating strategy for flow cytometry analysis of the mouse ILC1s isolated from livers. The mouse ILC1s were defined as CD3^-CD19^-NK1.1^-NKp46^-CD49b^CD49a^- CD49b^-CD49a^+. d-f, 2×10^6 C1498 cells were i.v. injected into C57BL/6J mice. Twenty-one days later, the production of IFN-γ and TNF by ILC1s from the liver (d), bone marrow (e), and spleen (f) of those healthy mice or mice with AML are shown (n=5 individual mice). g, GSEA plot shows the relative abundance of genes involved in the TNF–NF-κB signaling pathways in liver ILC1s isolated from mice with AML or healthy mice (n=3 individual mice). Data are representative of two (a, b, d, e, and f) independent experiments. Data (d-f) are shown as mean ± s.d. and are assessed by unpaired two-tailed Student’s t test. NS, not significant.
Extended Data Fig. 2 | Cell purity and gating strategy for flow cytometry analysis. 
a, b, Purity of LSCs (a) and ILC1s (b) after cell sorting. 
c, Gating strategy for flow cytometric analysis of apoptosis of LSCs co-cultured with or without ILC1s, using 7-AAD. CTV: CellTrace™ Violet. 
d, Gating strategy for flow cytometry analysis of apoptosis of LSCs cocultured with ILC1s using the Violet Live Cell Caspase Probe. 
e, Gating strategy for flow cytometry analysis of human ILC1s isolated from peripheral blood. Lineage markers: CD3, CD4, CD8, CD14, CD15, CD16, CD19, CD20, CD33, CD34, CD203c, and FceRI. Human ILC1s were defined as Lin−CD56−CD127+c-Kit−CRTH2−. 
f, Gating strategy for flow cytometry analysis of human LSCs. Lineage markers: CD2, CD3, CD4, CD8, CD14, CD16, CD19, Mac-1, CD56, and CD235a. Human LSCs were defined as Lin−CD45dimCD34+CD38−.
Extended Data Fig. 3 | IFN-γ—but not TNF—induces apoptosis of LSCs. a, 5,000–10,000 mouse liver ILC1s were sorted and transferred into the top wells of a 96-well Transwell plate. The bottom chambers of the plate were loaded with 10,000–20,000 LSCs from the spleens of MllPTD/WT:Flt3ITD/ITD mice with AML. The cells were then cocultured for 3 days. The percentages of LSCs that were apoptotic were measured by flow cytometry (n = 3 individual mice). b, c, LSCs from the spleen of MllPTD/WT:Flt3ITD/ITD mice with AML were treated with or without the indicated doses of IFN-γ or TNF for 3 days. Representative images (top, 5× magnification, scale bar 200 µm) and flow cytometry plots (bottom) of the percentages of apoptotic cells in LSCs are shown. Data in a are shown as mean ± s.d. and are assessed by one-way ANOVA models. Data in a, b, and c are representative of two independent experiments. NS, not significant.
Extended Data Fig. 4 | ILC1s and IFN-γ transform the differentiation of LSCs. **a**, LSCs from the spleen of MllPTD/WT: Flt3ITD/ITD mice with AML were cocultured with or without 0.1 ng/ml, 1 ng/ml, or 10 ng/ml recombinant mouse IFN-γ. The percentages of Lin^−^Sca-1^+^c-Kit^+^, Lin^−^Sca-1^−^c-Kit^+^, and Lin^−^Sca-1^c-Kit^−^ cells were measured by flow cytometry (n = 4 individual mice). **b**, ILC1s from healthy mouse liver were sorted and transferred into the top well of a 96-well Transwell plate. The bottom chamber of the plate was loaded with LSCs from the spleen of MllPTD/WT: Flt3ITD/ITD mice with AML, and coincubated for 3 days (n = 3 individual mice). Then the percentages of Lin^−^Sca-1^c-Kit^+^, Lin^−^Sca-1^c-Kit^−^, and Lin^−^Sca-1^c-Kit^−^ cells were measured by flow cytometry. All data are representative of three independent experiments, shown as mean ± s.d., and assessed by one-way ANOVA.
Extended Data Fig. 5 | ILC1s and IFN-γ do not affect leukemia progenitor cell differentiation into myeloid blasts. a, b, Mouse LSCs labeled with CTV were cocultured with or without mouse ILC1s in the presence or absence of anti-IFN-γ or anti-TNF antibody. Statistics of absolute cell numbers of Mac-1⁺ (a) and Gr-1⁺ (b) cells are shown (n = 3 individual mice). c, Leukemia progenitor cells were sorted from the spleen of MllPTD/WT, Flt3ITD/ITD mice with AML and cocultured with or without WT ILC1s, IFN-γ⁻/⁻ ILC1s, or IFN-γ. Representative flow cytometry plots (top) and statistics of the percentages (bottom) of Mac-1⁺ and Gr-1⁺ cells are shown (n = 4 individual mice). d, A working model of how ILC1s and their secreted IFN-γ regulate differentiation of LSCs. Data in a, b, and c are representative of three independent experiments, shown as mean ± s.d., and assessed by one-way ANOVA models. NS, not significant.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | ILC1s do not induce HSC apoptosis nor impair their differentiation. a, Wild-type mouse HSCs from bone marrow of mice were cocultured with or without ILC1s. Representative images and statistics of the percentages of apoptotic cells (5× magnification, scale bar 200 μm, n=5 individual mice). b, HSCs from blood of healthy donors were cocultured with or without ILC1s. Representative images and statistics of the percentages of apoptotic cells (n=4 individual donors). c-e, Mouse HSCs were cocultured with or without ILC1s, and representative flow cytometry plots (c), statistics of cell numbers (d), and percentages (e) of Lin−Sca-1+c-Kit+ and Lin−Sca-1−c-Kit+ cells (n=4 individual mice). f, Representative flow cytometry plots and statistics of cell numbers of Mac-1+Gr-1+ cells (n=4 individual mice). g, Experimental scheme for (h-j). Mouse HSCs from CD45.2 mice were injected into lethally irradiated CD45.1 mice. One day later, ILC1s were injected into those CD45.1 mice. Three weeks later, donor hematopoietic and progenitor cells, myeloid cell subsets, and WBCs were analyzed. h, The cell numbers of donor LSKs, myeloid progenitor cells (L−S−K+, Lin−Sca-1−c-Kit+ cells), early lymphoid-committed precursors (L−S−K−, Lin−Sca-1−c-Kit+ cells), short-term hematopoietic stem cells (STHSC, Lin−Sca-1−c-Kit+Flt3−CD150−CD48− cells), long-term hematopoietic stem cells (LTHSC, Lin−Sca-1−c-Kit+Flt3+CD150+CD48− cells), multipotent progenitors 1 and 2 (MPP1, Lin−Sca-1−c-Kit+Flt3−CD150−CD48− cells; MPP2, Lin−Sca-1−c-Kit+Flt3+CD150−CD48− cells), Mac-1+Gr-1+ cell subsets, and WBCs derived from CD45.2 mice were analyzed (n=4 individual mice in no ILC1 group; n=3 individual mice in ILC1 group). i, Representative flow cytometry plots and statistics of cell numbers of Mac-1+Gr-1+ cells derived from CD45.2 mice (n=4 individual mice in no ILC1 group; n=3 individual mice in ILC1 group). j, Statistics of cell numbers of WBCs (n=4 individual mice in no ILC1 group; n=3 individual mice in ILC1 group). Data in a, b, d, e, f, h, i, and j are representative of two independent experiments and shown as mean ± s.d. Statistics are assessed by one-way ANOVA (a and b) or unpaired two-tailed Student’s t test (d, e, h, i, and j). NS, not significant.
Extended Data Fig. 7 | ILC1s control the leukemia burden in mice transplanted with LSCs. a, LSCs were i.v. co-injected into lethally irradiated (900 cGy) CD45.2 recipient mice on day 0 along with bone marrow cells isolated from IL-15 transgenic mice (CD45.2) as support cells. On day 1, the mice were i.v. injected with WT ILC1s from the liver of C57BL/6 J (CD45.2) mice or i.p. injected daily with recombinant mouse IFN-γ (0.5 μg/mouse/day). Statistics of the numbers of total WBCs at week 5 (n = 5 individual mice in no ILC1 group; n = 4 individual mice in WT ILC1 group; n = 6 individual mice in recombinant IFN-γ group). All absolute cell numbers of WBCs were determined by cell counting with the Element HT5 Hematology Analyzer. b, Representative flow cytometry plots of the percentages of CD45.1+ and CD45.2+ cells. c, LSCs were co-injected into lethally irradiated (900 cGy) CD45.1 recipient mice on day 0 along with bone marrow cells isolated from CD45.1 mice (as support cells). Mice were injected with WT ILC1s or IFN-γ−/− ILC1s from the liver of C57BL/6 J (CD45.2) mice on day 1 or injected daily with recombinant mouse IFN-γ (0.5 μg/mouse/day). Statistics of the number of total WBCs at week 3 (n = 7 individual mice). All absolute cell numbers of WBCs were determined by cell counting with the Element HT5 Hematology Analyzer followed by flow cytometry. For box plots, boxplots (a and c) display the median and interquartile range (25th percentile-75th percentile) with whiskers representing the upper- and lower-quartile (1.5x the 75th and 25th percentile values). Data in a and c are representative of two independent experiments and shown as mean ± s.d. and assessed by one-way ANOVA.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | The role of ILC1s and ILC1-derived IFN-γ in controlling LSCs. a, Healthy mouse liver ILC1s or NK cells were cocultured with or without LSCs in the presence or absence of anti-IL-7Rα neutralizing antibody or isotype IgG control for 12 h along with IL-12 plus IL-15. Representative flow cytometry plots of IFN-γ production by ILC1s (n = 6 individual mice). b, Representative flow cytometry plots of IFN-γ production in healthy liver ILC1s or healthy liver NK cells after treatment with or without IL-7 (100 ng/ml) in the presence of IL-12 plus IL-15 (n = 5 individual mice). c,d, Mouse LSCs were cocultured with or without mouse ILC1s or NK cells for 3 days in the presence or absence of mouse anti-IFN-γ antibody. Representative images (c; 5x magnification, scale bar 200 µm) and statistics of absolute cell numbers (d) are shown (n = 3 individual mice). e,f, Representative flow cytometry plots (e) and statistics of the percentages of apoptotic LSCs (f; n = 3 individual mice). g, To deplete ILC1s or NK cells, WT mice were i.p. injected with IgG control (CTRL), anti-NK1.1, or anti-asialo-GM1 antibody. Three days later, the percentages of NK cells (Lin−NK1.1+NKp46+CD49b+) and ILC1s (Lin−NK1.1+NKp46−CD49a+) in the liver of WT mice were measured by flow cytometry. Data (d and f) are representative of two independent experiments and shown as mean ± s.d. and are assessed by one-way ANOVA models. NS, not significant.
Extended Data Fig. 9 | RNA-seq identifies gene transcriptional changes and signaling pathways in LSCs treated with ILC1s or IFN-γ. a, Experimental design for RNA sequencing (RNA-Seq). Mouse LSCs were sorted and treated with or without sorted ILC1s or IFN-γ for 3 days. LSCs were resorted from cocultured ILC1s or IFN-γ using FACS before RNA-Seq. b, Purity of LSCs (left) and ILC1s (right) after cell sorting. c, A heat map showing differential expression of RNA of 627 genes (n = 3 individual mice) is shown. d-f, Volcano plots showing significantly differentially expressed genes in RNA pools from AML LSCs treated with ILC1s vs. Ctrl (untreated) (d), IFN-γ vs. Ctrl (e), and IFN-γ vs. ILC1s (f) (n = 3 individual mice). g, Hallmark pathway analysis in LSC RNA pools (IFN-γ vs. Ctrl). The left panel shows signaling pathways downregulated in LSCs. The right panel shows signaling pathways upregulated in LSCs (n = 3 individual mice). Genes with an FDR-adjusted P-value < 0.05 and a fold change (FC) > 1.5 or < 0.7 were considered as significantly upregulated and downregulated genes, respectively.
Extended Data Fig. 10 | ILC1s or IFN-γ inhibit LSC differentiation via the JAK-STAT and AKT signaling pathways. a, GSEA plots show enrichment of the indicated target genes in LSCs co-cultured with ILC1s. The x axis shows the rank orders (ILC1s vs. Ctrl) of all the genes. b, GSEA plots show enrichment of the indicated target genes in LSCs treated with IFN-γ. The x axis shows the rank orders (IFN-γ vs. Ctrl) of all the genes. c-d, Heat maps showing differential expression of RNAs of genes downstream of IFN-γ. e-h, Mouse LSCs labeled with CTV were treated with or without the indicated JAK and AKT inhibitors for 30 min and then cocultured with or without WT or IFN-γ−/− ILC1s in the presence of IL-12 and IL-15 for 3 days. Statistics of the percentages of Lin−Sca-1+c-Kit+, Lin−Sca-1−c-Kit+, Lin−Sca-1−c-Kit−, and Lin−Sca-1−c-Kit− cells (n = 3 individual mice). Genes with an FDR-adjusted P-value < 0.05 and a fold change (FC) > 1.5 or < 0.7 were considered to be significantly upregulated or downregulated. Data in e-h are representative of three independent experiments, shown as mean ± s.d., and assessed by one-way ANOVA models. NS, not significant.
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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated.

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
- BD LSRFortessaTM X20 and BD FACSAriaTM Fusion were used for flow cytometry data collection. Zeiss AxioCam 702 was used for acquisition of microscopy data. Element HT5 hematoloy analyzer was used for data collection of WBCs. Quantstudio 12K Flex was used to collect real-time quantitative reverse transcription PCR data.

Data analysis
- Flow Cytometry data were analyzed by FlowJo V10 (Treestar) and Novoexpress 1.3.0 (Agilent); Statistical analysis was conducted using GraphPad Prism V8 and SAS V.9.4. Trimmomatic (v.0.38), FASTP (v.0.19.4), RseQC (v.2.5), edgeR (v.3.28.1), GSEA (v.3.0), and Cluster (v.3.0) were used for RNA-sequencing analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. Github). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The sequencing datasets are accessible from GEO with accession number GSE 198783.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [ ] Life sciences
- [ ] Behavioural & social sciences
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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-list.pdf

Life sciences study design

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

Sample size
A reasonable sample sizes were determined based on experimental group size, mouse availability, leukemia burden variability, the experience of ours and other investigators, and similar research reported in the literature [e.g., Zhang et al., Nature Medicine. 2018. PMID: 29505034] to ensure adequate reproducibility of results. The sample size and associated statistics are indicated in the figures and corresponding legends. For in vitro studies, the experiments were repeated at least 2 times with samples size at least 3 to control technical variations.

Data exclusions
No data were excluded from the analyses.

Replication
All experiments were reliably reproduced and results are represented as mean +/- SD as appropriate, which is indicated in figure legends. One-way ANOVA model was utilized to compare three or more conditions. Linear mixed models were used to account for the variance-covariance structure due to repeated measures. Experiments were repeated with at least two to three biologically independent times for all results presented in the manuscript. P values were adjusted for multiple comparisons using Holm’s procedure. A P value of 0.05 or less was considered statistically significant, which is described in the methods section of the main text.

Randomization
Peripheral blood cones used to isolate healthy HLCs or peripheral blood from patients with AML to isolate LSCs were de-identified and randomly picked up. For animal studies, 6- to 12-week old mice were matched by age and sex and randomly assigned to specific treatment groups.

Blinding
Experimenter were blinded to observe survival of mice. Otherwise, blinding was not performed, such as during in vitro experiments, where experimenter were required to know the conditions of each well.

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 | Methods |
|---------------------------------|---------|
| n/a Involved in the study       | n/a Involved in the study |
| □ X Antibodies                 | □ X ChiP-seq |
| □ X Eukaryotic cell lines      | □ X Flow cytometry |
| □ X Palaeontology and archaeology | □ X MRI-based neuroimaging |
| □ X Animals and other organisms | □ X Clinical data |
| □ X Human research participants | □ X Dual use research of concern |

Antibodies

- Anti-Mouse CD3e-PE-Cy7 BD Biosciences Cat# 552774; Clone: 14S-2C11; RRID:AB_394460
- Anti-Mouse CD19-PE/Cy7 BD Biosciences Cat# 552854, Clone: 1D3; RRID:AB_394495
- Anti-Mouse CD45R/B220-PE-Cy7 BD Biosciences Cat# 552772, Clone: RA3-6B2; RRID:AB_394458
- Anti-Mouse CD11b/Mac-1-PE-Cy7 BD Biosciences Cat# 552850, Clone: M1/70; RRID:AB_394491
- Anti-Mouse Ter119-PE-Cy7 BD Biosciences Cat# 557853, Clone: TER-119; RRID:AB_396988
- Anti-Mouse Ly6G and Ly6C-PE-Cy7 BD Biosciences Cat# 565033, Clone: RB6-8C5; RRID:AB_27390409
- Anti-Mouse Ly6A/E [Sca-1]-BV510 BD Biosciences Cat# 565507, Clone: D7; RRID:AB_2739272
- Anti-Mouse Ly6A/E [Sca-1]-PE-CF394 BD Biosciences Cat# 562730, Clone: D7; RRID:AB_2737751
- Anti-Mouse CD117 [c-Kit]-BV711 BD Biosciences Cat# 563160, Clone: 2B8; RRID:AB_2722510
- Anti-Mouse CD335 [MKp46]-AF647 BD Biosciences Cat# 560755, Clone: 29A1.4; RRID:AB_1271464
- Anti-Mouse NK1.1-BV510 BD Biosciences Cat# 563096, Clone: PK136; RRID:AB_2738002
- Anti-Mouse NK1.1-FITC BD Biosciences Cat# 561082, Clone: PK136; RRID:AB_394676
- Anti-Mouse CD49b-PE BD Biosciences Cat# 552585, Clone: DX5; RRID:AB_395094
- Anti-Mouse CD49b-BV421 BD Biosciences Cat# 740250, Clone: HMa2; RRID:AB_2739996
- Anti-Rat/Mouse CD49a-PE BV711 BD Biosciences Cat# 564863, Clone: Ha31/8; RRID:AB_2738987
Validation

All mouse antibodies were used at 1:200. All human antibodies were used at 1:50, except for CD34, CD38, CRTH2, and CD117, which were used at 1:100.

Anti-Mouse CD3e-PE-Cy7 BD Biosciences Cat# 552774; Clone: 145-2C11; RRID:AB_394460

https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruu/pe-cy7-7-hamster-anti-mouse-cd3e.552774

Anti-Mouse CD19-PE-Cy7 BD Biosciences Cat# 552854; Clone: 1D3; RRID:AB_384495

https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruu/pe-cy7-7-rat-anti-mouse-cd19.552854

Anti-Mouse CD45R/3220-PE-Cy7 BD Biosciences Cat# 552772; Clone: RA3-6B2; RRID:AB_394458

https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruu/pe-cy7-7-rat-anti-mouse-cd45r.3220.552772

Anti-Mouse CD11b/Mac-1-PE-Cy7 BD Biosciences Cat# 552850; Clone: M1/70; RRID:AB_394491

https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruu/pe-cy7-7-rat-anti-mouse-cd11b.552850

Anti-Mouse Ter119-PE-Cy7 BD Biosciences Cat# 557853; Clone: TER-119; RRID:AB_396898

https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruu/pe-cy7-7-rat-anti-mouse-ter-119-erythroid-cells.557853

Anti-Mouse Ly6G and Ly6C-PE-Cy7 BD Biosciences Cat# 565033; Clone: RB6-8C5; RRID:AB_2739049

https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruu/pe-cy7-7-rat-anti-mouse-ly6g-and-ly6c.565033
Anti-Mouse Ly-6A/E [Sca-1]-BV510 BD Biosciences Cat# 565507, Clone: D7; RRID:AB_2739272
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv510-ant-mouse-ly-6a-e-565507

Anti-Mouse Ly-6A/E [Sca-1]-PE-CF594 BD Biosciences Cat# 562730, Clone: D7; RRID:AB_2737751
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cf594-ant-mouse-ly-6a-e-562730

Anti-Mouse CD117 (c-Kit)-BV711 BD Biosciences Cat# 563160, Clone: 2B8; RRID:AB_2722510
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv711-ant-mouse-cd117.563160

Anti-Mouse CD335 [NKp46]-AF647 BD Biosciences Cat# 560755, Clone: 29A1.4; RRID:AB_1727464
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/af647-ant-mouse-cd335-nkp46.560755

Anti-Mouse NK1.1-BV510 BD Biosciences Cat# 563096, Clone: PK136; RRID:AB_2738002
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv510-ant-mouse-nk1-1.563096

Anti-Mouse NK1.1-FITC BD Biosciences Cat# 561082, Clone: PK136; RRID:AB_394676
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-ant-mouse-nk1-1.561082

Anti-Mouse CD49b-PE BD Biosciences Cat# 553858, Clone: DX5; RRID:AB_385094
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-ant-mouse-cd49b.553858

Anti-Mouse CD49b-BV429 BD Biosciences Cat# 740250, Clone: HM3a2; RRID:AB_2739996
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv429-ant-mouse-cd49b.740250

Anti-Rat/Mouse CD49a-BV711 BD Biosciences Cat# 564863, Clone: Ha31/8; RRID:AB_2738987
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv711-ant-mouse-rat-mouse-cd49a.564863

Anti-Mouse CD135 [Flt3]-APC BD Biosciences Cat# 560718, Clone: A2F10.1; RRID:AB_1727425
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-ant-mouse-cd135.560718

Anti-Mouse IFN-γ-BV786 BD Biosciences Cat# 563773, Clone: XMG1.2; RRID:AB_2738419
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv786-ant-mouse-ifi-563773

Anti-Mouse Ly-6G and Ly-6C-FITC BD Biosciences Cat# 553126, Clone: RB6-8C5; RRID:AB_394643
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-ant-mouse-ly-6g-and-ly-6c.553126

Anti-Mouse CD11b/Mac-1-PE BD Biosciences Cat# 553311, Clone: M1/70; RRID:AB_396680
https://www.bdbiosciences.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-ant-mouse-cd11b.553311

Anti-Mouse 1H-1-α-AF-700 BioLegend Cat# 506338, Clone: MP6-X122; RRID:AB_2562918
https://www.biologend.com/en-us/products/alpha-fluor-700-anti-mouse-tnf-alpha-antibody.9146

Anti-Mouse CD48-FITC BD Biosciences Cat# 557484, Clone: HMA4-1; RRID:AB_396724
https://www.biologend.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-ant-mouse-cd48.557484

Anti-Mouse CD150-PE BD Biosciences Cat# 562651, Clone: Q38-480; RRID:AB_2737705
https://www.biologend.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-ant-mouse-cd150.562651

Anti-Mouse CD112 [Nectin-2]-BV786 BD Biosciences Cat# 748050, Clone: 829038; RRID:AB_2872511
https://www.biologend.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv786-ant-mouse-cd112.748050

Anti-House CD155-APC BD Biosciences Cat# 566630, Clone: 3F1; RRID:AB_2869798
https://www.biologend.com/en-us/products/reagents/cytometry-reagents/research-reagents/single-color-antibodies-ruo/alpha-fluor-647-ant-mouse-cd155.566630

Anti-Mouse CD226 [DNAM-1]-BV421 BioLegend Cat# 133615, Clone: TX42.1; RRID:AB_2715977
https://www.biologend.com/en-us/products/brilliant-violet-421-anti-mouse-cd226-dnam-1-antibody.14753

Anti-Mouse CD127 (IL-7Ra)-PerCP/Cyanine5.5 BioLegend Cat# 121114, Clone: 5B/199; RRID:AB_1134205
https://www.biologend.com/en-us/products/percp-cyanine5.5-ant-mouse-cd127-il-7ralpha-antibody.4517
Anti-Mouse CD45.1-BV605 Biologend Cat# 110737, Clone: A20; RRID:AB_11204076
https://www.biologend.com/en-us/products/brilliant-violet-605-anti-mouse-cd45-1-antibody-7850

Anti-Mouse CD45.2-APC/Fire™750 Biologend Cat# 109852, Clone: 104; RRID:AB_2629723
https://www.biologend.com/en-us/products/apc-fire-750-anti-mouse-cd45-2-antibody-13589

Anti-Mouse CD45.2-FITC BD Biosciences Cat# 553772, Clone: 104; RRID:AB_395041
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-mouse-cd45-2-553772

Anti-Human CD3-FITC BD Biosciences Cat# 561802, Clone: HIT3a; RRID:AB_395745
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd3-561802

Anti-Human CD4-FITC BD Biosciences Cat# 555346, Clone: RPA-T4; RRID:AB_395751
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd4-555346

Anti-Human CD8-FITC BD Biosciences Cat# 555634, Clone: HIT8a; RRID:AB_395996
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd8-555634

Anti-Human CD14-FITC BD Biosciences Cat# 555397, Clone: MSE2; RRID:AB_395798
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd14-555397

Anti-Human CD15-FITC BD Biosciences Cat# 555401, Clone: H98; RRID:AB_395801
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd15-555401

Anti-Human CD16-FITC BD Biosciences Cat# 555406, Clone: 3G8; RRID:AB_395806
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd16-555406

Anti-Human CD19-FITC BD Biosciences Cat# 555412, Clone: HIB19; RRID:AB_395812
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd19-555412

Anti-Human CD20-FITC BD Biosciences Cat# 555622, Clone: 2H7; RRID:AB_395998
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd20-555622

Anti-Human CD33-FITC BD Biosciences Cat# 555626, Clone: HIM3-4; RRID:AB_395992
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd33-555626

Anti-Human CD34-FITC BD Biosciences Cat# 555821, Clone: 581; RRID:AB_396150
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd34-555821

Anti-Human CD203C (NP4D6)-FITC Thermo Fisher Scientific Cat# MA5-28586, Clone: NP4D6; RRID:AB_2745545
https://www.thermofisher.com/antibody/product/CD203c-antibody-clone-np4d6-monoconal-ma5-28586

Anti-Human FcRhiLA-FITC Biologend Cat# 334608, Clone: AER-37 [CRA-1]; RRID:AB_1227653
https://www.biologend.com/en-us/products/fc-anti-human-fc-receptor-like-a-antibody-5059

Anti-Human CD56 (NCAM-1)-AF700 BD Biosciences Cat# 557919, Clone: B159; RRID:AB_396940
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alex-fluor-700-mouse-anti-human-cd56-ncam-1.557919

Anti-Human CD56 (NCAM-1)-FITC BD Biosciences Cat# 562794, Clone: B159; RRID:AB_2737799
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fictr-mouse-anti-human-cd56-ncam-1.562794

Anti-Human CD56-BV421 BD Biosciences Cat# 562751, Clone: NCAM16.2; RRID:AB_2732054
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv421-mouse-anti-human-cd56-562751

Anti-Human CD127-APC BD Biosciences Cat# 558598, Clone: HIL-7R-M21; RRID:AB_647113
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alex-fluor-647-mouse-anti-human-cd127-558598

Anti-Human CD117-PE BD Biosciences Cat# 555714, Clone: YB5.B8; RRID:AB_396058
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-mouse-anti-human-cd117-555714
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  C1498 was purchased at ATCC.

Authentication  Cell lines were not independently authenticated, beyond the identity provided from the supplier (e.g., ATCC).

Mycoplasma contamination  Cell line is mycoplasma-free.

Commonly misidentified lines (See iCLAC register)  No commonly misidentified cell 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 CS7BL/6 J, Rag2−/−, Ifn−/−, TNF−/−, and CD45.1 mice were purchased from the Jackson laboratory. MIIIPTD/WT: Flt3ITD/ITD and Il−15 transgenic mice were generated by our group. Both male and female mice were utilized in this study. MIIIPTD/WT: Flt3ITD/ITD mice were used at 18-25 weeks of age. Other mice were used at 6-12 weeks of age. Mice were housed in City of Hope Animal Facility with light cycle: A 12-light/12-dark cycle and temperatures of 65-75°F (-18-23°C) with 40-60% humidity.

Wild animals No wild animals were used in this study.

Field-collected samples The study did not involve samples collected from the field.

Ethics oversight All animal procedures used in this study were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of City of Hope under the protocol number 20003 and 18108.

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

Human research participants

Policy information about studies involving human research participants.

Population characteristics Human donor samples were collected at City of Hope National Medical Center (COHNMC). AML patient samples were also obtained at COHNMC. All available patient characteristics are provided in Supplementary Table1.

Recruitment All patients were recruited through regular visits at COHNMC. There were no biases for the recruitment of human research participants.

Ethics oversight Human specimens were collected from patients registered at City of Hope National Medical Center who had consented to the Institutional Review Board approved protocol IRB# 18067; healthy donor specimens were collected from patients consented to IRB# 06229.

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 Cells were collected, washed with 1% FBS + DPBS, stained with different fluorescence-conjugated antibodies at 4°C for 30 min, 1% FBS + DPBS twice, and then analyzed using BD LSRSFortessaTM X20 and BD FACSAriaTM Fusion.

Instrument Cells were either analyzed on BD LSRSFortessaTM X20 or sorted by BD FACSAriaTM Fusion.

Software Flow Cytometry data were analyzed by FlowJo V10 (Treestar) and Novoexpress (Agilent).

Cell population abundance The purity of sorted cells was detected via FACSAriaTM Fusion and samples with purity >90% were used.

Gating strategy ILC1s from human peripheral blood were identified using surface staining with a live/dead cell viability cell staining kit and the following monoclonal antibodies: lineage (anti-CD3, anti-CD4, anti-CD8, anti-CD14, anti-CD15, anti-CD16, anti-CD19, anti-CD20, anti-CD33, anti-CD34, anti-CD203c, and anti-FcεRI), anti-CD56, anti-CD127, anti-CD45R, and anti-c-Ki. ILC1s or NK cells from mice were identified using live/dead cell viability dyes and the following monoclonal antibodies: lineage (anti-CD3 and anti-CD19), anti-NK1.1, anti-NKp46, anti-CD49b, and anti-CD49a. Human LSCs were identified by lineage (anti-CD2, anti-CD3, anti-CD4, anti-CD8, anti-CD14, anti-CD19, anti-CD20, anti-Mac-1, anti-CD36, and anti-CD257a), anti-CD45, anti-CD34, and anti-CD38. Mouse LSCs were identified by lineage (anti-CD3, anti-CD19, anti-B220, anti-Ly6G/C, anti-Mac-1 and anti- Ter119), anti-ScA-1, and anti-c-Ki. Human ILC1s were gated by Lin− (CD56−) CD127+ [CD38−] [CD11b−] [c-Ki+]. Mouse ILC1s were gated by Lin− (NK1.1+ [NKp46+ [CD49b−] [CD49a+−])− Mouse ILC1s were gated by Lin− [NK1.1+ [NKp46+ [CD49b+ [CD49a−])− Mouse ILC1s were gated by Lin− (NK1.1− [NKp46+ [CD49b+ [CD49a−])− Mouse LSCs were gated by CD45dimLin− (CD34+ [CD38−])− Mouse LSCs were gated by Lin− (Sca−1− [c-Ki+].

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