Divergent effects of enhanced Notch signals on leukemia stem cells and hematopoietic stem cells

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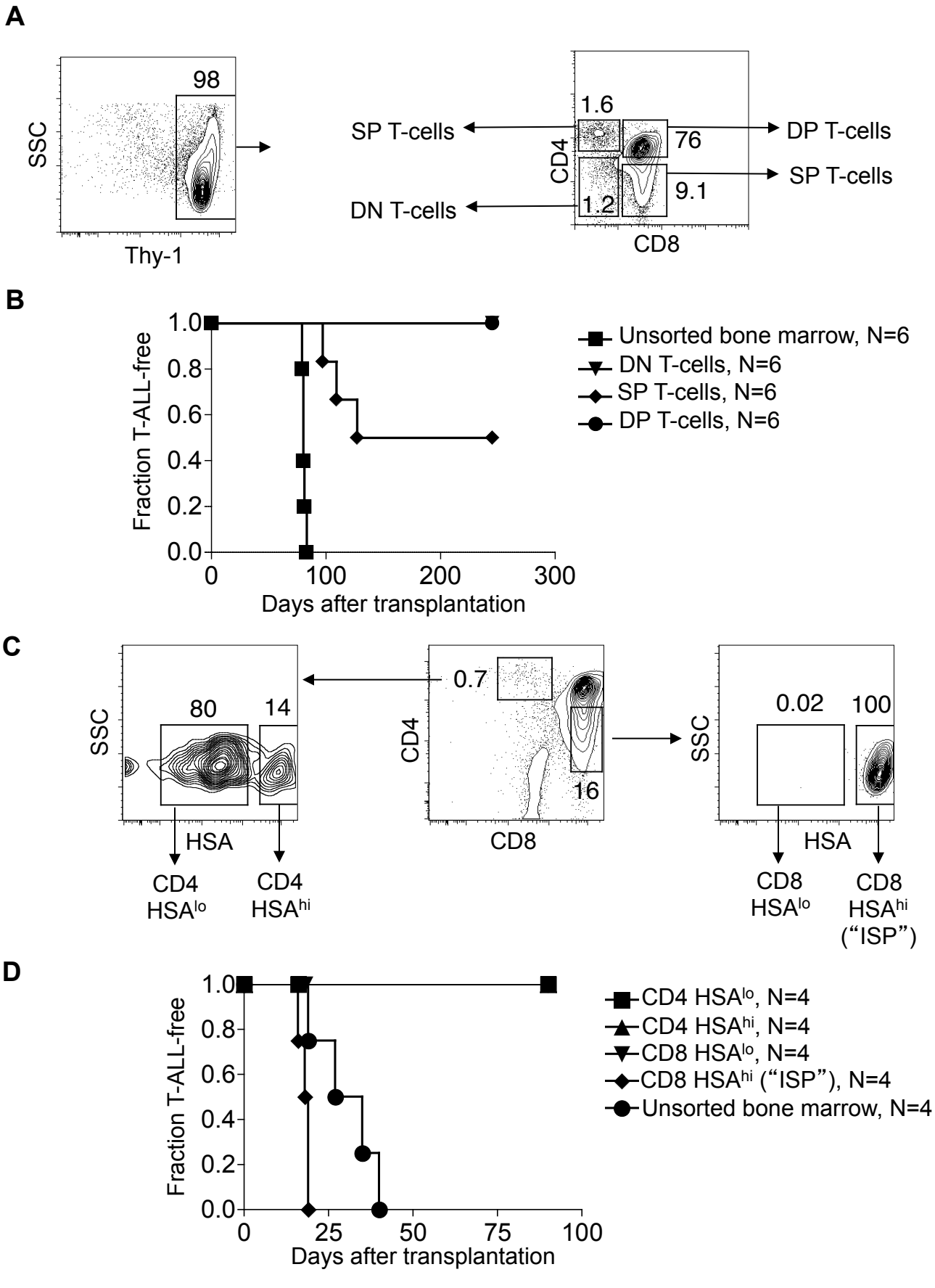
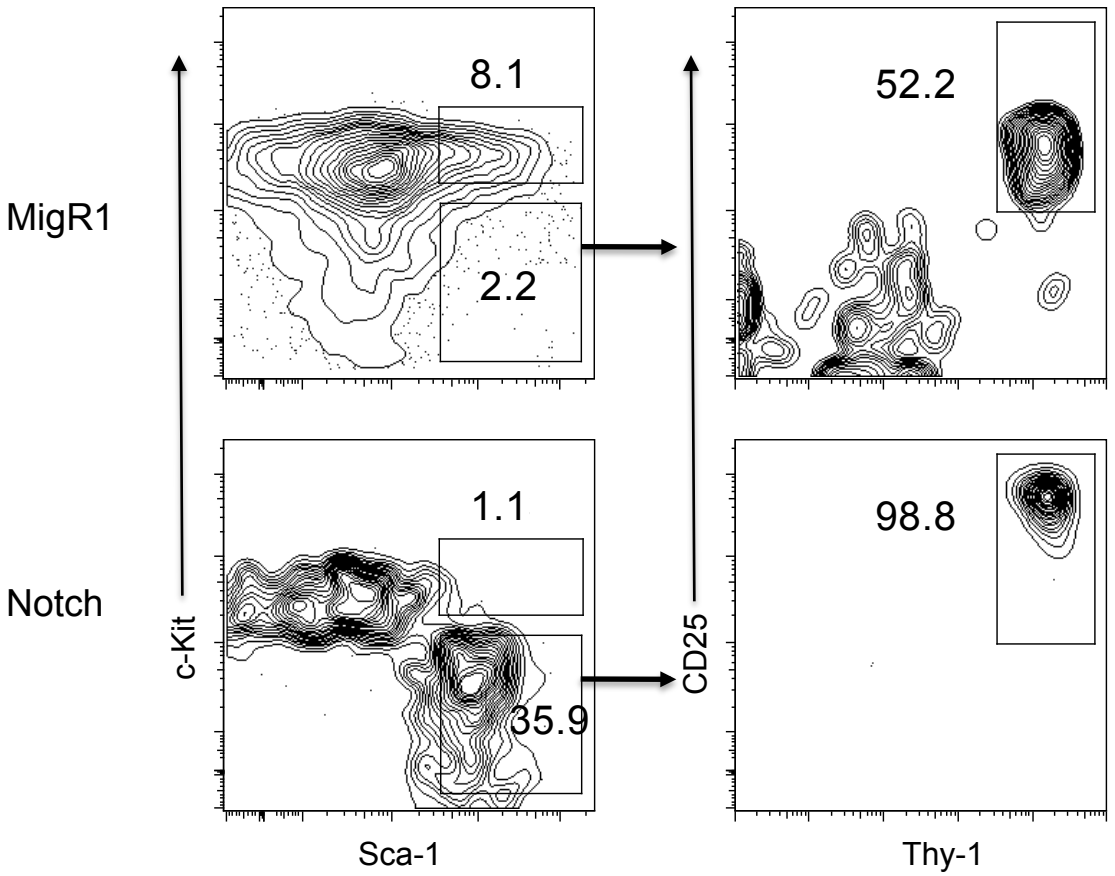
Supplemental Fig 1.



Supplemental Fig 1.

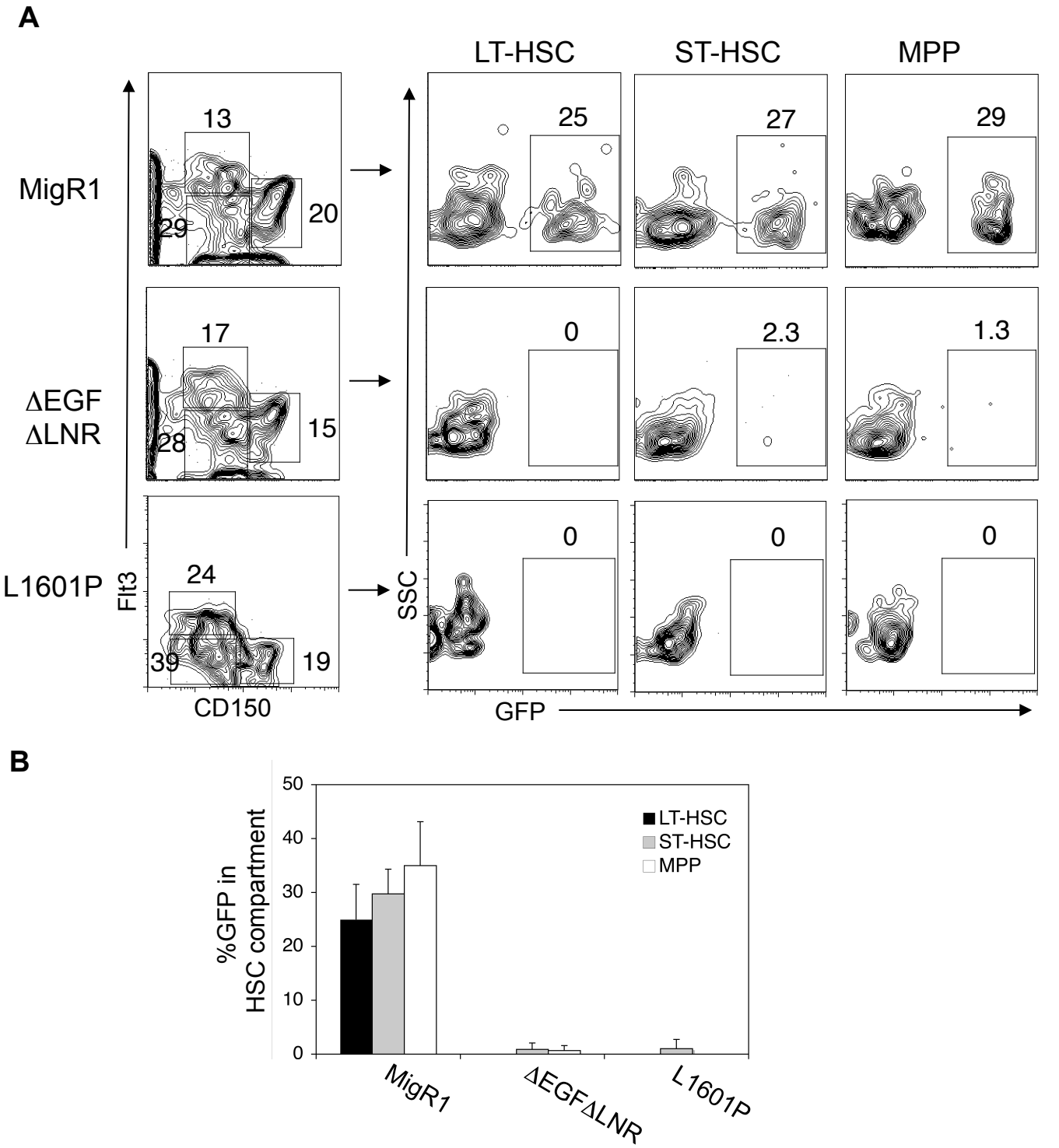
Supplemental figure 1. LSC activity resides predominantly in the Immature Single Positive (ISP) compartment in Notch-induced T-ALL. 12 lethally irradiated mice were reconstituted with 5FU-treated donor BM cells transduced with activated Notch1. After 6 weeks, bone marrow from leukemic mice was sorted into three GFP^+ subpopulations – DN T-cells ($Thy\text{-}1^+CD4^-CD8^-$), SP T-cells ($CD4^+CD8^-$ and $CD4^-CD8^+$), and DP T-cells ($CD4^+CD8^+$). (A) Unsorted cells along with all cells of each subpopulation were then equally transferred into 6 lethally irradiated mice along with 200,000 syngeneic normal BM cells. Absolute numbers of transferred cells per mouse: Unsorted cells (1,500,000 cells), DN T-cells (2500 cells), SP T-cells (30,000 cells), and DP T-cells (500,000 cells). Each mouse receiving DN T-cells received all the DN T-cells from 2 leukemic mice. Each mouse receiving SP T-cells received all the SP T-cells from 2 leukemic mice. Each recipient mouse receiving DP T-cells received all the DP T-cells from 2 leukemic mice. (B) Kaplan-Meier graphs show the fraction of secondary recipient mice without T-ALL as a function of time. (C) 16 lethally irradiated mice were reconstituted with 5FU-treated donor BM cells transduced with activated Notch1 ($\Delta EGF\Delta LNR\Delta P$). After 6 weeks, bone marrow from leukemic mice was sorted into four GFP^+ subpopulations -- $CD4^+HSA^{lo}$, $CD4^+HSA^{hi}$, $CD8^+HSA^{lo}$, and $CD8^+HSA^{hi}$ (ISP-like). Unsorted cells and all the cells of each subpopulation were then equally transferred into 4 lethally irradiated mice along with 200,000 syngeneic normal BM cells. Absolute numbers of transferred cells per mouse: Unsorted (3,000,000 cells), $CD4^+HSA^{lo}$ (30,000 cells), $CD4^+HSA^{hi}$ (1,000 cells), $CD8^+HSA^{lo}$ (300 cells), and $CD8^+HSA^{hi}$ ISPs (600,000 cells). (D) Kaplan-Meier graphs show the fraction of secondary recipient mice without T-ALL as a function of time.

Supplemental Fig. 2



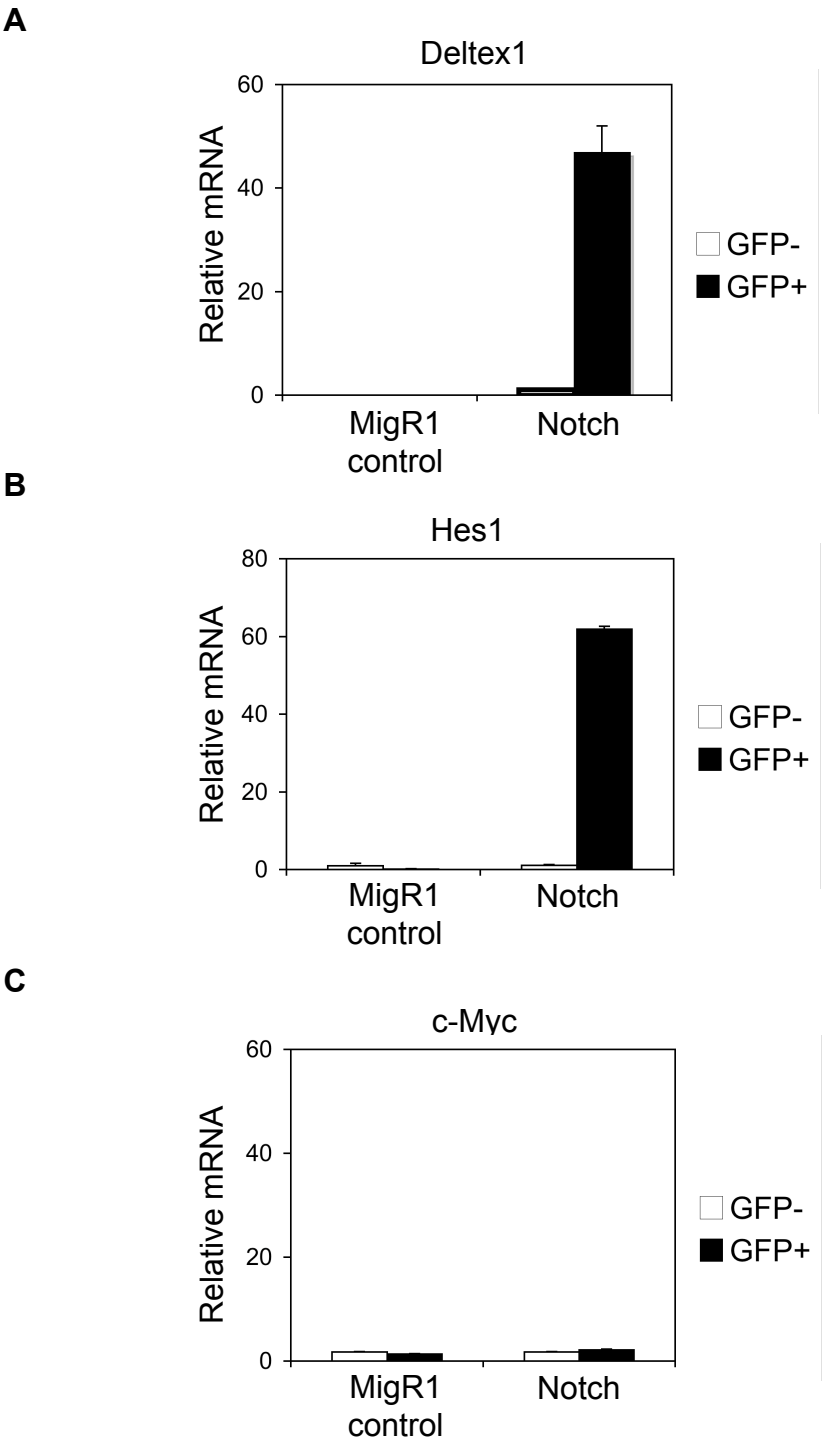
Supplemental figure 2. Notch activation promotes the outgrowth of Sca-1^{+}Lineage^{-} T-cells. MigR1 control and activated Notch were engineered into mice using bone marrow transplantation as described in Fig. 2. 13 weeks after transplantation, the Lineage^{-}GFP^{+} (donor-derived) compartment was analyzed for HSPCs (Lineage^{-}Sca-1^{+}c-Kit^{+}) and DN T-cells (Sca-1^{+}c-Kit^{-} Thy-1^{+}CD25^{+}).

Supplemental Fig. 3



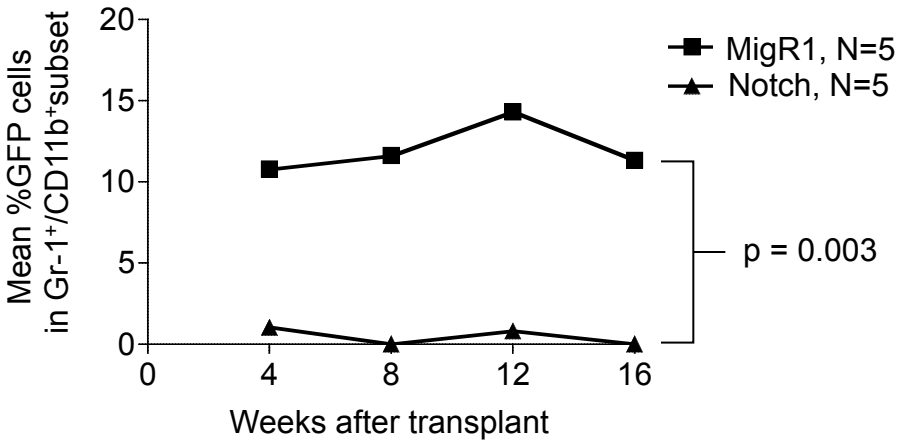
Supplemental figure 3. Notch depletes HSC numbers in p16$^{-/-}$ mice. (A) Lethally irradiated mice were reconstituted with 5FU-treated donor p16$^{-/-}$ BM cells transduced with MigR1 control or activated Notch1 alleles (ΔEGFΔLNR or L1601P). The long-term HSC (LT-HSC, Lineage^{-}Sca-1^{+}c-Kit^{+}CD150^{+}Flt3^{-}), short-term HSC (ST-HSC, Lineage^{-}Sca-1^{+}c-Kit^{+}CD150^{-}Flt3^{-}), and Multipotent Progenitor (MPP, Lineage^{-}Sca-1^{+}c-Kit^{+}CD150^{-}Flt3^{+}) compartments were measured for donor (GFP^{+}) reconstitution by MigR1 control and Notch-activated HSCs at 13 weeks post-transplantation. (B) Bar graph analysis of donor-derived (GFP^{+}) LT-HSCs, ST-HSCs, and MPPs in MigR1 control and activated Notch mice at 13 weeks after transplantation.

Supplemental Fig. 4



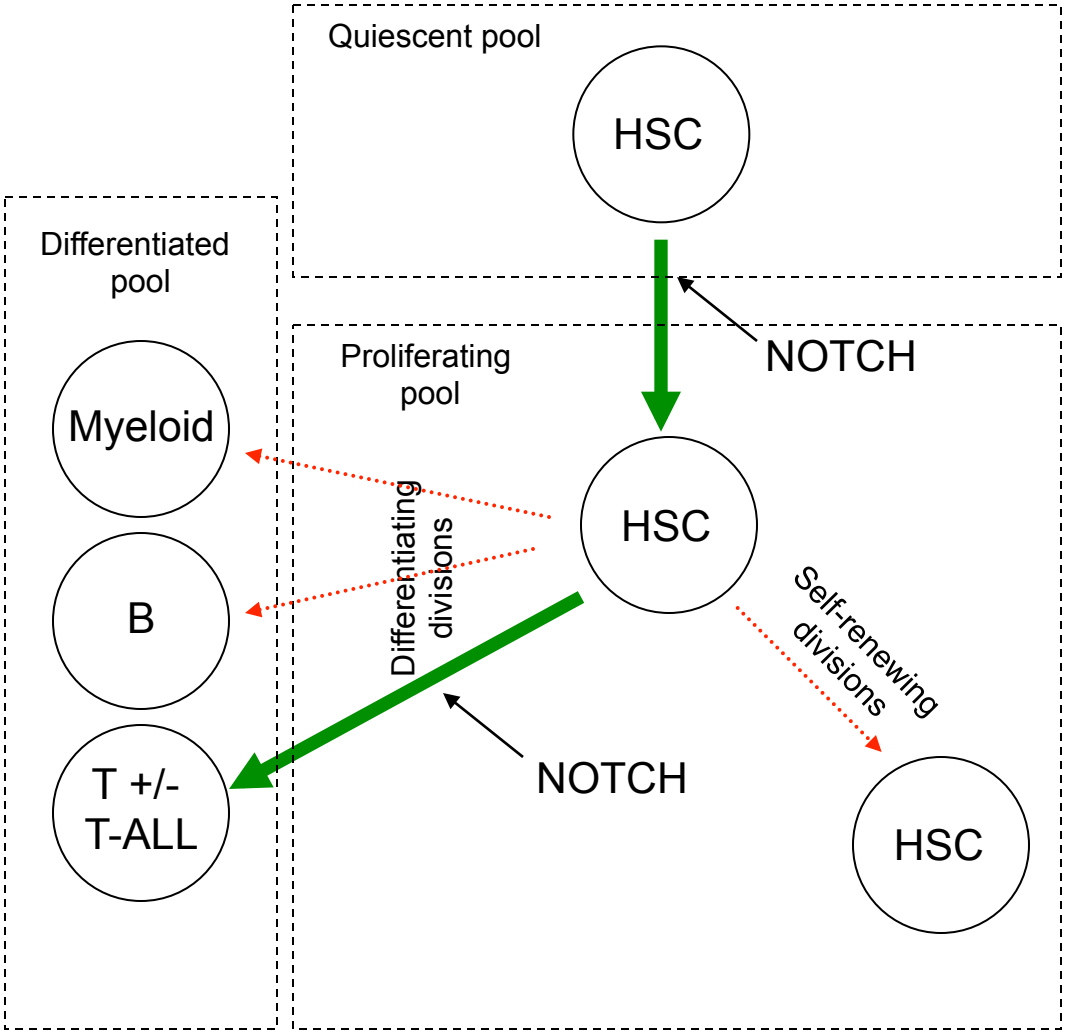
Supplemental figure 4. Notch signaling in HSPCs induces Notch targets Deltex1 and Hes1 but not c-Myc. Lethally irradiated mice were reconstituted with 5FU-treated donor Rag1$^{-/-}$ BM cells transduced with MigR1 control or activated Notch1. At six weeks after transplant, the donor-derived HSPC compartment (GFP^{+}Lineage^{-}Sca-1^{+}c-Kit^{+}) was sorted and analyzed for indicated Notch target gene expression by quantitative real-time PCR. Gene expression levels were quantified relative to a standard generated using cDNA from the murine T-ALL leukemia cell line, SCID-ADH, and normalized to 18S expression.

Supplemental Fig. 5



Supplemental figure 5. Competitive transplantation of Notch-activated HSPCs leads to impaired myeloid reconstitution. As described in Fig. 5, 500 Rag1$^{-/-}$ HSPCs (GFP^{+}CD45.2^{+}Lineage^{-}Sca-1^{+}c-Kit^{+}) retrovirally transduced with MigR1 control or Notch were transferred to lethally irradiated mice with 200,000 CD45.1^{+} whole BM competitors. Mice were bled every 4 weeks to measure donor (GFP^{+}) reconstitution of the myeloid compartment (CD11b^{+}Gr-1^{+}). Experiment was performed three times.

Supplemental Fig. 6



Supplemental figure 6. **Model of how Notch may progressively deplete the HSC compartment over time.** Notch signaling drives quiescent HSCs into cell cycle and directs them into differentiating divisions toward the T-cell lineage at the expense of self-renewing divisions that would preserve HSC numbers.

Supplemental Table 1

Supplemental Table 1. Summary of transfer experiments of various T-cell subsets from Notch-induced leukemia into lethally irradiated syngeneic recipients.

T-cell subset transferred	Cell dose (per mouse)	Outcome (Fraction T-ALL[A])	LSC frequency	95% C.I.
Double-negative DN T-cells[B]	2,500	0/6		
	500	0/2		
CD4 HSA^{lo} T-cells	150,000	0/6		
	5,000	0/6		
CD4 HSA^{hi} T-cells	6,000	0/6		
	1,000	0/6		
CD8 HSA^{lo} T-cells	500	0/4		
	150	0/6		
CD8 HSA^{hi} T-cells (ISP)	300,000	7/7	1:953	1:443 - 1:2051
	30,000	4/4		
	3,000	8/8		
	300	2/10		
	30	0/10		
Double-positive DP T-cells	3,000,000	6/6	1:229482	1:126400 -1:416629
	300,000	11/13		
	30,000	0/19		
	3,000	0/18		

[A]T-ALL defined by flow cytometrically-defined T-cell lymphoblasts >20% of BM cells and/or diffuse lymphadenopathy and splenomegaly.
[B]Thy-1^{+}CD4^{-}CD8^{-} BM cells