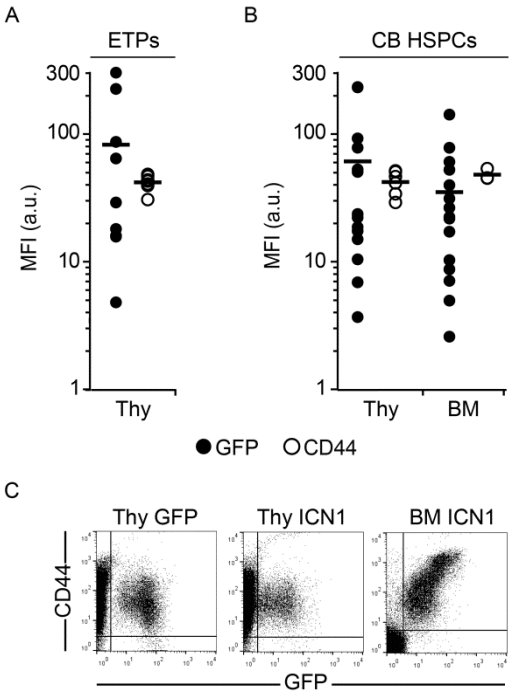
Table S1. Primers used for PCR amplification of CSL-binding sites in ChIP assays

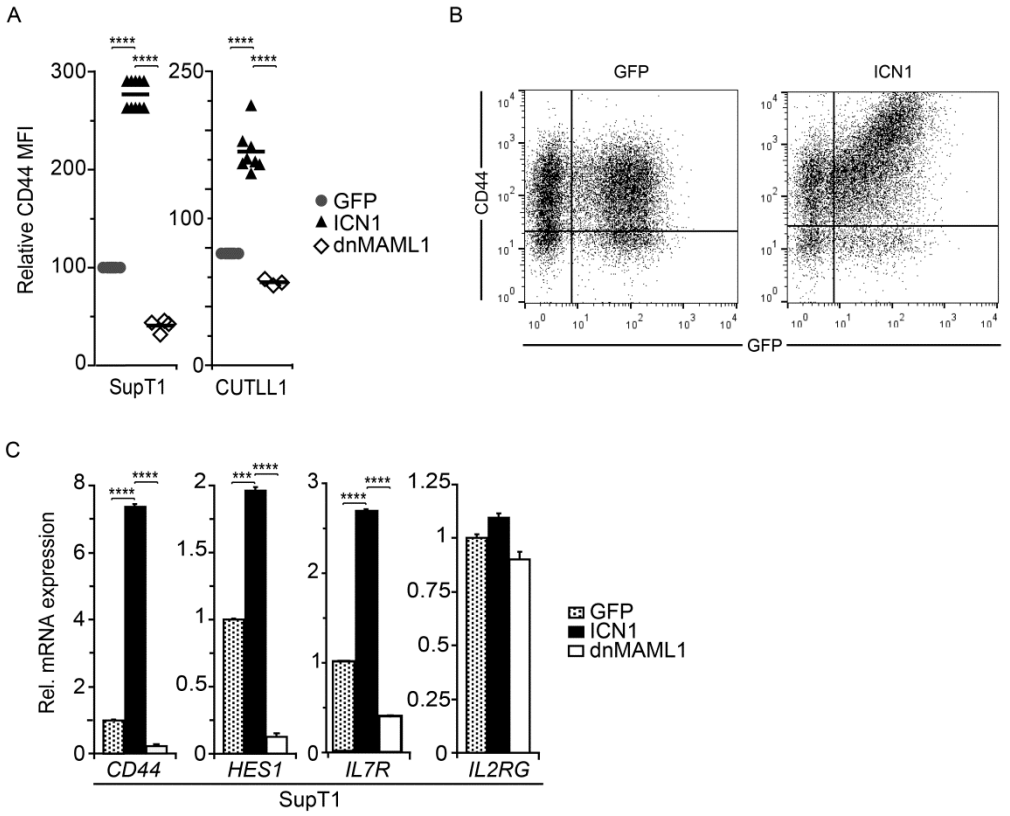
		Primer sequence (5´→3´)
CD44 site 1	forward	GCGTAAAACCCATTTTGTGG
	reverse	AAACCAGTAGCTGGGAAGCA
CD44 site 2	forward	TGCTTCCCAGCTACTGGTTT
	reverse	ATGCAGCAGGTCACAGACTC
CD44 site 3	forward	GCACACCAGGAAATGGTCTT
	reverse	AATTCAATCAGCAGCCTTGG
CD44 site 4	forward	CCTCTGATAAGGGGGAAAGC
	reverse	CACCACTGGTCACACAAACC
CD44 site 5	forward	CCTCTGATAAGGGGGAAAGC
	reverse	CACCACTGGTCACACAAACC
c-*myc*	forward	GAGGAGCAGCAGAGAAAGG
	reverse	TCCCCCACGCCCTCTGC

Table S2. Primers used for mutagenesis of putative CSL-binding sites

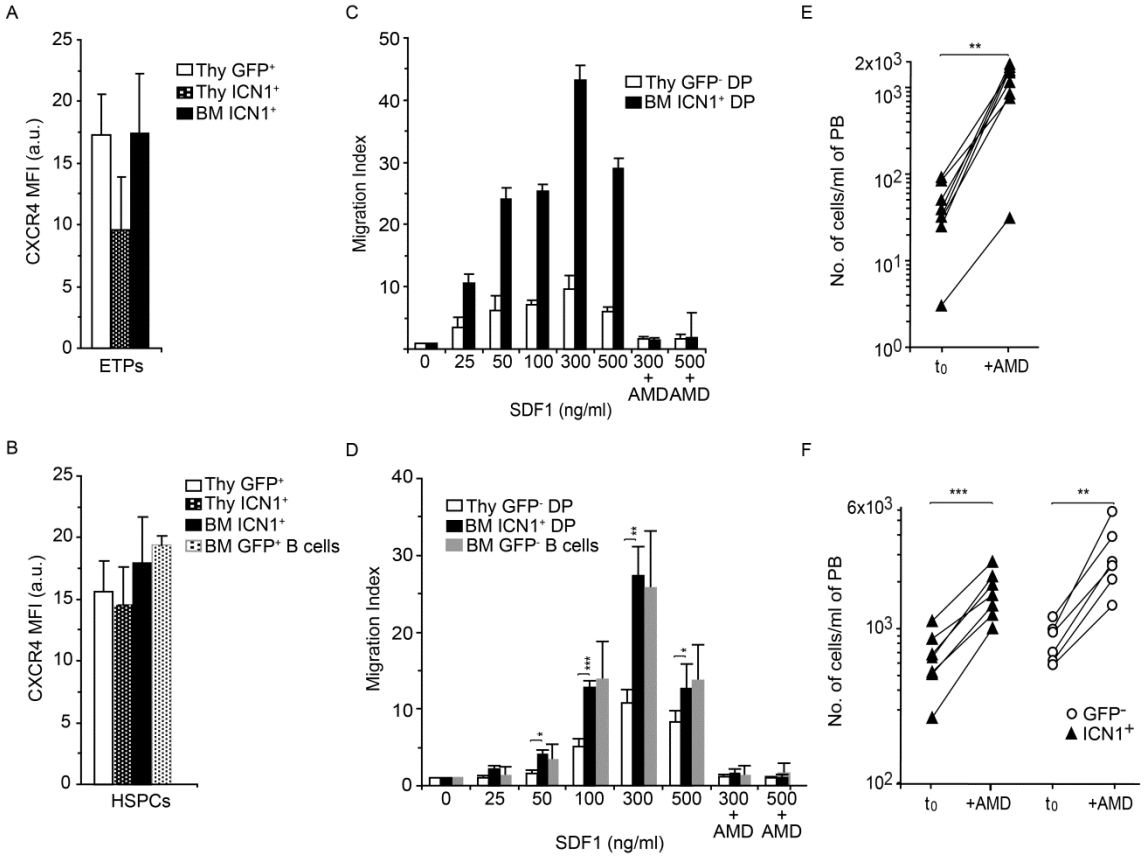
		Primer sequence (5´→3´)
site1	forward	TTGCATCTCTCCCCTGTACCCTTCCAGGGTGCAGC
	reverse	GCTGCACCCTGGAAGGGTACAGGGGAGAGATGCAA
site2	forward	AGATCATTCCTTTTCGTACCAGCTGTCCCGTGAAA
	reverse	TTTCACGGGACAGCTGGTACGAAAAGGAATGATCT
site3	forward	ACTCATAATAAACTAGTACCAAGGTCTGATTACCC
	reverse	GGGTAATCAGACCTTGGTACTAGTTTATTATGAGT
site4	forward	GTTTCCTTTCCAGTTGTACCCATCCCATGGTTTGG
	reverse	CCAAACCATGGGATGGGTACAACTGGAAAGGAAAC
site5	forward	AAGCATCTAAGAGTTGTACCAGGGTTTGTGTGACC
	reverse	GGTCACACAAACCCTGGTACAACTCTTAGATGCTT



Supplemental Figure 1. Correlation between ICN1 and CD44 expression in human CB HSPC-derived cells engrafting the BM. (**A, B**) Expression of GFP and CD44 by control GFP^{+} CD45^{+} human cells that infiltrate the BM and thymus (Thy) of RAG-2$^{-/-}$ x γc$^{-/-}$ mice transplanted with either ETPs (A) or CB HSPCs (B) transduced with a control retrovirus encoding GFP. Cells were obtained at 2-6 and 9 weeks post-transplant, respectively. Note that no BM engraftment of ETP-derived cells was observed. Mean Fluorescence Intensity (MFI) data were combined from 3 independent experiments, with a total of 8 to 14 mice/group. (**C**) FACS histograms showing the correlated expression of GFP reporter vs CD44 by human ICN1-transduced CB HSPCs that infiltrate the thymus (Thy) and BM of RAG-2$^{-/-}$ x γc$^{-/-}$ mice at 9 weeks post-transplant. Control GFP-transduced CB HSPCs infiltrating the thymus are shown as control. Background staining for CD44 was determined with an irrelevant isotype-matched mAb. Results are representative of at least 3 independent experiments.

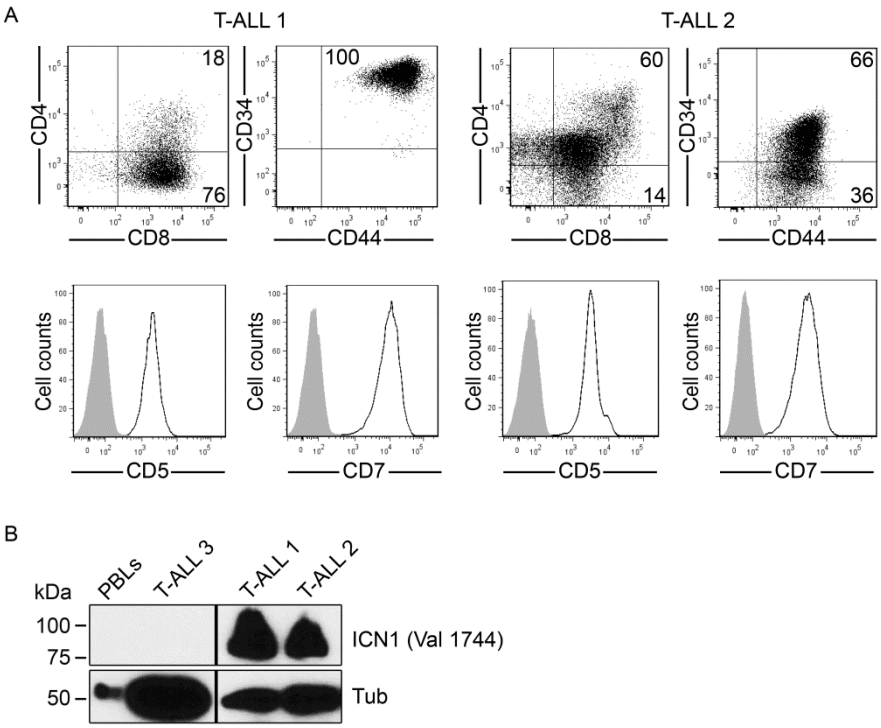


Supplemental Figure 2. Enforced Notch signaling induces *CD44* gene transcription and protein upregulation in human T-ALL cell lines. (**A**) Relative surface expression of CD44 on SupT1 and CUTLL1 T-ALL cells transduced with retrovirus encoding either GFP and ICN1, or GFP and dnMAML1, or GFP alone as control. Results are shown as relative MFI normalized to MFI values of GFP-transduced control cells ± SEM (n≥ 4). (**B**) FACS histograms showing the correlated expression of GFP reporter vs CD44 by SupT1 T-ALL cells transduced with retrovirus encoding ICN1 and GFP or GFP alone as control. Results are representative of 3 independent experiments. (**C**) Relative mRNA expression of *CD44, HES1*, *IL7R* and *IL2RG* analyzed by Q-PCR and normalized to *GAPDH* expression in SupT1 cells in (A). Bars represent mean ± SEM (n=3).

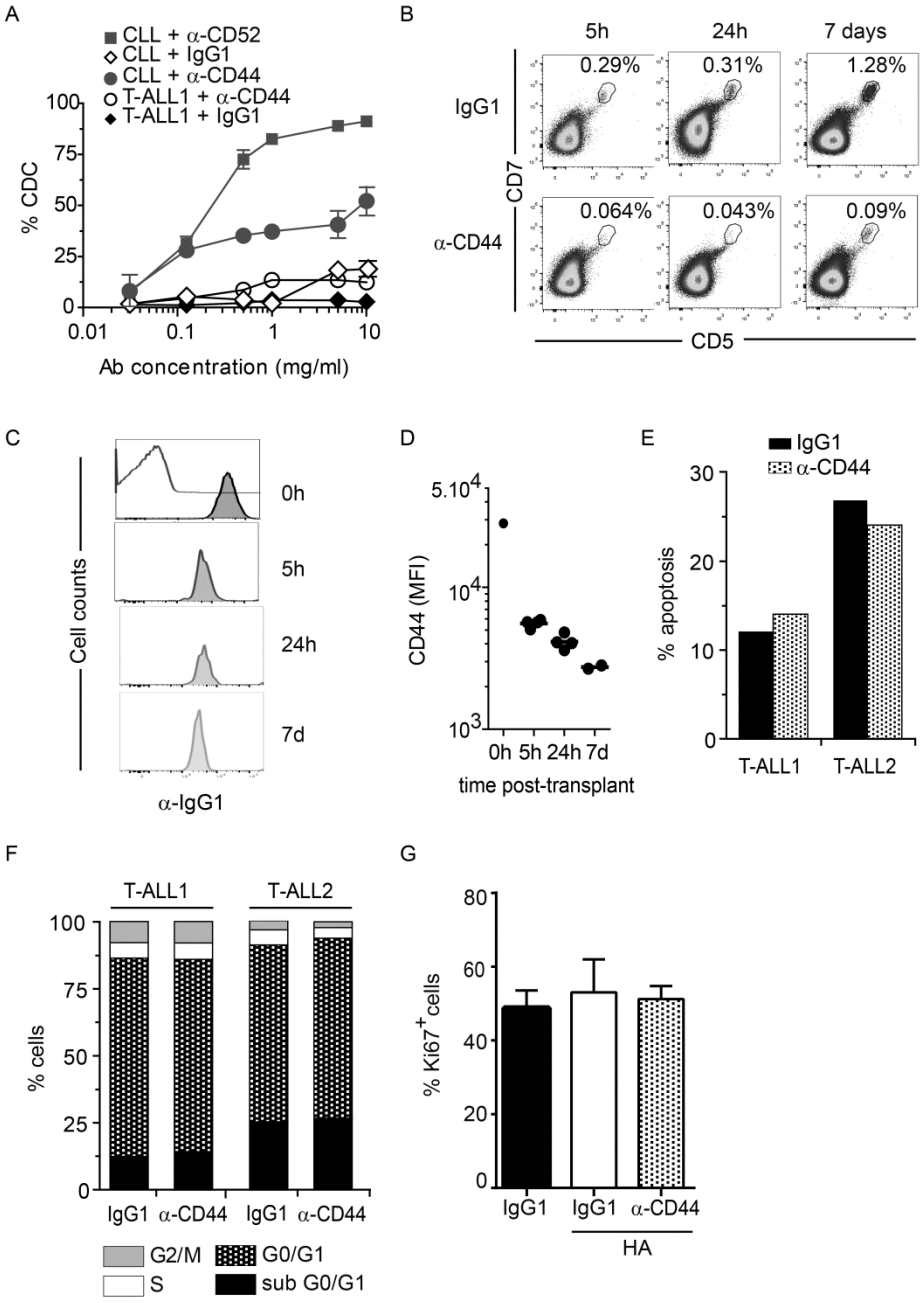


Supplemental Figure 3. BM engraftment of pre-leukemic T cells induced by ectopic Notch signaling involves CXCR4 function.

(**A, B**) CXCR4 expression of human DP cells infiltrating the BM and thymus (Thy) of RAG-2$^{-/-}$ x γc$^{-/-}$ mice transplanted with either ETPs (A) or CB HSPCs (B) transduced with GFP and ICN1 or with GFP-only vectors, recovered at 4-6 weeks or 9 weeks post-transplant, respectively. No BM engraftment of GFP-transduced ETPs was observed. CXCR4 expression of BM-resident B cells derived from control GFP$^+$ HSPCs is shown in (B). MFI ± SEM data of 3-4 experiments with a total of 8-14 mice/group are shown; a.u; arbitrary units. (**C, D**) SDF1-dependent *in vitro* migration of human ICN1$^+$ DP T cells infiltrating the BM of mice in (A) and (B), respectively. Triplicates of human cells depleted of mouse cells were seeded in 5μm-pore 96-well Boyden chambers (Chemo Tx system, Neuro Probe) containing the indicated SDF1 concentrations, with or without 50ng/ml of the CXCR4 antagonist AMD3100 (Sigma Aldrich). Migrating cells were collected after 12h and cell numbers were quantified by FACS using Calibrite Beads (BD Biosciences). Thymus-resident DP cells derived from non-transduced (GFP$^-$) ETPs (C) or HSPCs (D) and BM-resident B cells derived from non-transduced HSPCs (D) were analyzed in parallel. Mean data ± SEM of relative migration normalized to basal migration in the absence of SDF1 are shown (n=3-4). (**E, F**) *In vivo* mobilization of human ICN1$^+$ DP T cells resident in the BM of mice in (A) and (B), respectively. BM-resident B cells generated from non-transduced (GFP$^-$) HSPCs were analyzed in (F). Cell mobilization to the PB was analyzed at 4-6 weeks or 9 weeks post-transplant for (E) and (F), respectively, either before (t=0) or one hour after subcutaneous injection of 5μg/g of AMD3100. Absolute numbers of human cells/ml of PB are shown (n=6-8).



Supplemental Figure 4. Characterization of primary human T-ALL cells. (**A**) Flow cytometry analysis of CD44, CD34, CD8, CD7, CD5 and CD4 expression in primary T-ALL cells obtained from BM samples of two patients (T-ALL1 and T-ALL2) at the time of diagnosis. Numbers in quadrants in upper panels indicate percentages of positive cells. Shaded histograms in bottom panels show background MFI determined with irrelevant isotype-matched mAb controls. The same control was used for CD5 and CD7 expression of T-ALL1 cells. (**B**) Western blot analysis of active NOTCH1 expression in human T-ALL1 and T-ALL2 cells. Peripheral blood lymphocytes (PBLs) from a control donor and primary T-ALL cells lacking active NOTCH1 (T-ALL3) were analyzed in parallel. Expression of α-tubulin is shown as loading control.



Supplemental Figure 5. Persistent blockage of CD44 by mAb treatment reduces T-ALL leukemia burden independently of complement-dependent cytotoxicity. (A) Complement-dependent cytotoxicity (CDC) of primary T-ALL1 cells ($2x10^5$) incubated with increasing concentrations of anti-CD44 HP2/9 mAb or control IgG1 (37°C, 30 min.), washed and incubated with 25% rabbit complement (Serotec) for 190 min. As positive control, $CD44^+$ chronic lymphocytic leukemia (CLL) cells were incubated with either anti-CD44 or anti-CD52 mAb (Genzyme). Mean percentages ± SEM of non-viable cells determined by 7-aminoactinomycin-D (7-AAD) (BD Biosciences) FACS staining are shown (n=3). **(B)** BM engraftment in $Rag2^{-/-}$ x $\gamma c^{-/-}$ mice of anti-CD44 HP2/9 mAb or IgG1 pre-treated T-ALL1 cells, shown as percentages of electronically-gated $CD7^+$ $CD5^+$ cells from one out of 9-10 mice/group at distinct times post-transplant. **(C, D)** *In vivo* persistence of HP2/9 mAb at the surface of anti-CD44-pre-treated T-ALL1 cells in (B), determined by reactivity with PE-labelled anti-mouse IgG1. (C) shows results from a representative mouse, including background staining (unshaded histogram) and basal CD44 expression before transplantation (shaded histogram, 0h) determined by HP2/9 mAb reactivity. (D) shows CD44 MFI values of cells recovered from the BM of 10 mice at the indicated times. **(E, F)** Apoptosis (E) and cell cycle analysis (F) of T-ALL1 and T-ALL2 cells cultured for 32h or 48h, respectively, onto OP9 stromal cells (ATCC CRL-2749) in MEM medium (Lonza) containing 10% FCS, 200 IU/ml IL-7, 50 IU/ml FLT3-L, 100 IU/ml SCF (NIBSC), and 6µg/ml of anti-CD44 HP2/9 or IgG1. **(G)** Percentages of cycling cells, determined by anti-Ki67-PE intracellular FACS staining (BD Pharmigen), among T-ALL1 cells pre-treated (4ºC, 1h) with anti-CD44 HP2/9 mAb or IgG1 (100µg/ml) and then cultured on HA-coated (5 mg/ml; HR&D systems, GLR002) tissue plates for 72h. IgG1-pre-treated T-ALL1 cells cultured on uncoated plates were assayed as controls.