Figure S1. Notch-dependent murine T-ALL cells are resistant to Notch1 inhibitory antibodies
(A) Growth of the indicated murine T-ALL cell lines was monitored over a 3-day period in the presence of non-specific human IgG (hIgG), a humanized IgG specific for the Notch1 ligand binding region (LBR, WC613), a humanized IgG specific for the Notch1 negative regulatory region (NRR, WC75), or the γ-secretase inhibitor compound E (1 μM). Antibodies were used at a dose (10 mg/ml) that produces strong inhibition of ligand-mediated Notch1 activation.[1] Each time point was determined in triplicate. The results shown are representative of two independent experiments.RLU, relative luminescence units produced by the Cell-Titer Glo assay for cell growth. (B) The cell line 330 was transduced with empty MigRI virus or with human ICN1 and then treated for 72 hr with vehicle alone (DMSO) or vehicle containing the γ-secretase inhibitor (GSI) compound E (1 μM). Cells were then harvested, stained with propridium iodide, and analyzed by flow cytometry. The histograms show the DNA content of cells from the indicated treatment groups.

Figure S2. Detection of activated Notch1 in T-ALL cell lines
Whole cell lysates were subjected to SDS-PAGE and transferred to PVDF membranes, which were then stained with antibodies specific for i) the neoepitope created by γ-secretase cleavage of Notch1 at residue V1744 (V1744) or ii) β-actin. The presence or absence of PEST deletions in the cell lines is also indicated.

Figure S3. Differential 3′ polyadenylation of *Notch1* transcripts
Upper panel: position of a probe specific for a region 3′ of a major polyadenylation site identified at position 7979 (relative to the 5′ ATG start codon). Lower panel: results of Northern blot analysis with this probe on poly-A RNA prepared from the indicated cell lines.

Figure S4. Detection of small Ikaros isoforms in a subset of murine T-ALLs
Western blots of cell lysates were stained with an antibody specific for the N-terminus of Ikaros or an antibody against β-actin.

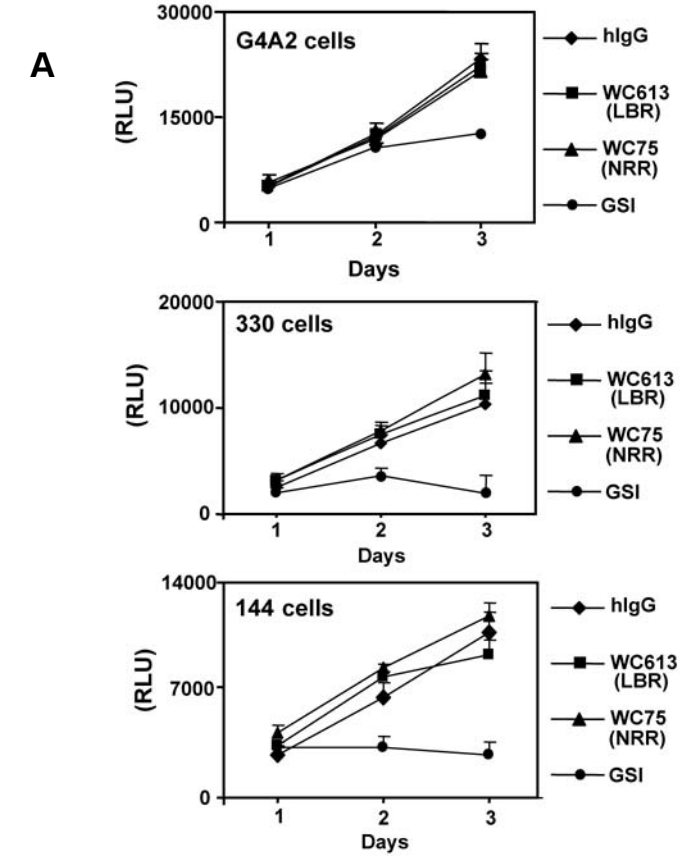
Figure S5. Sequence of the *TCRB-NOTCH1* fusion transcript expressed by the human T-ALL cell line CUTLL1

Figure S6. Alignment of ectopic RAG signal sequences near murine *Notch1* with the corresponding sequences of human *NOTCH1*
The murine and human Notch1 genes were aligned with ClustalW2. The murine RAG signal sequences are shown in red. Residues that match the RAG signal sequence consensus are underlined. Residues conserved between man and mouse are denoted with an asterisk.

REFERENCES

1. Aste-Amezaga M, Zhang N, Lineberger JE, et al. Characterization of notch1 antibodies that inhibit signaling of both normal and mutated notch1 receptors. *PLoS One*. 2010;5:e9094.
2. Ji Y, Resch W, Corbett E, et al. The in vivo pattern of binding of RAG1 and RAG2 to antigen receptor loci. *Cell* 2010;141:419-31.

Figure S1


A
G4A2 cells
330 cells
144 cells
(RLU)
Days
hIgG
WC613 (LBR)
WC75 (NRR)
GSI
30000
15000
0
20000
10000
14000
7000
1
2
3

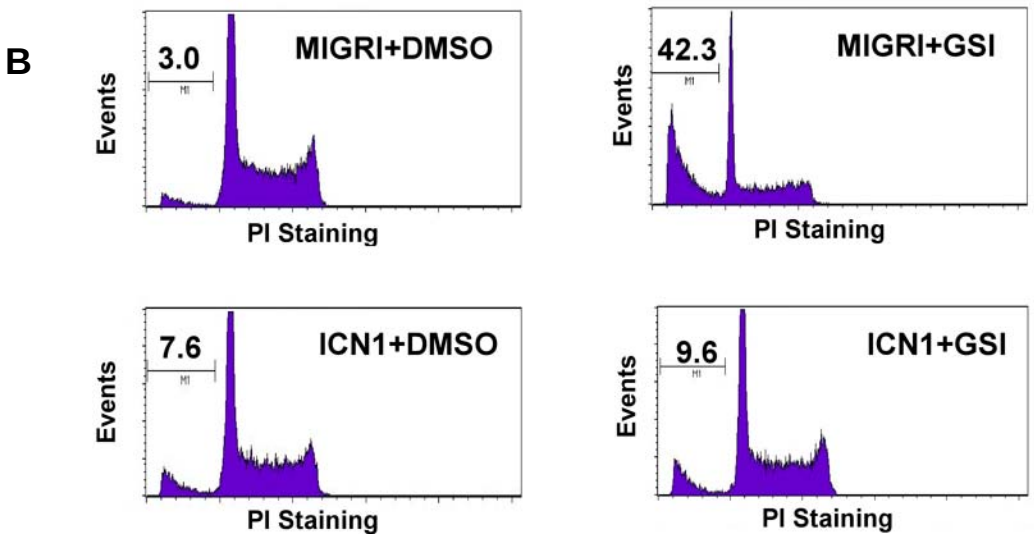

B
3.0
MIGRI+DMSO
42.3
MIGRI+GSI
7.6
ICN1+DMSO
9.6
ICN1+GSI
Events
PI Staining

Figure S2

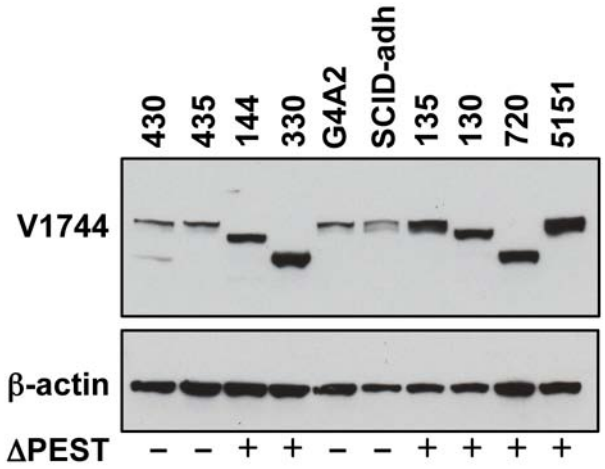

430
435
144
330
G4A2
SCID-adh
135
130
720
5151
V1744
β-actin
ΔPEST
– – + + – – + + + +

Figure S3

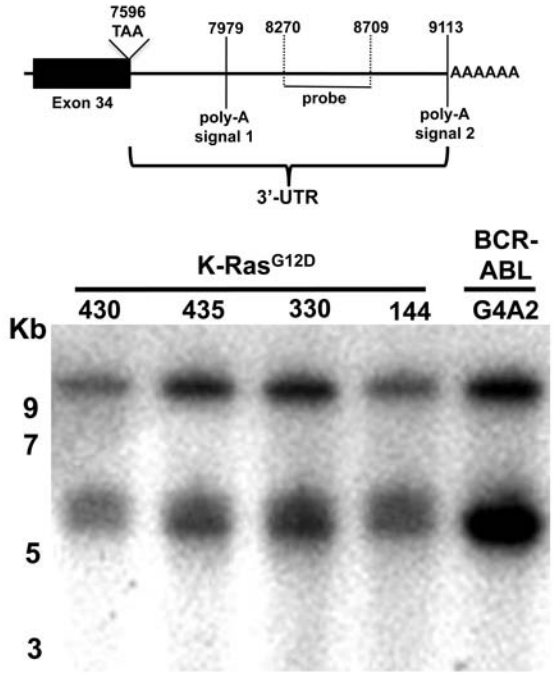

7596
TAA
7979
8270
8709
9113
AAAAAA
Exon 34
probe
poly-A
signal 1
poly-A
signal 2
3'-UTR
K-Ras^{G12D}
BCR-
ABL
430
435
330
144
G4A2
Kb
9
7
5
3

Figure S4

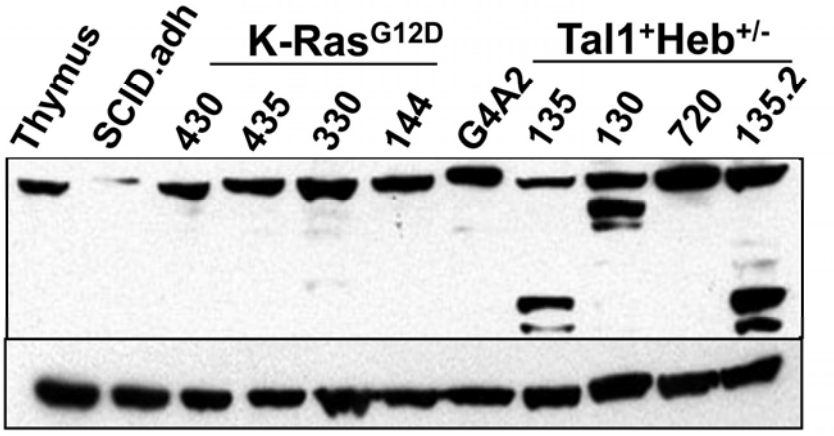
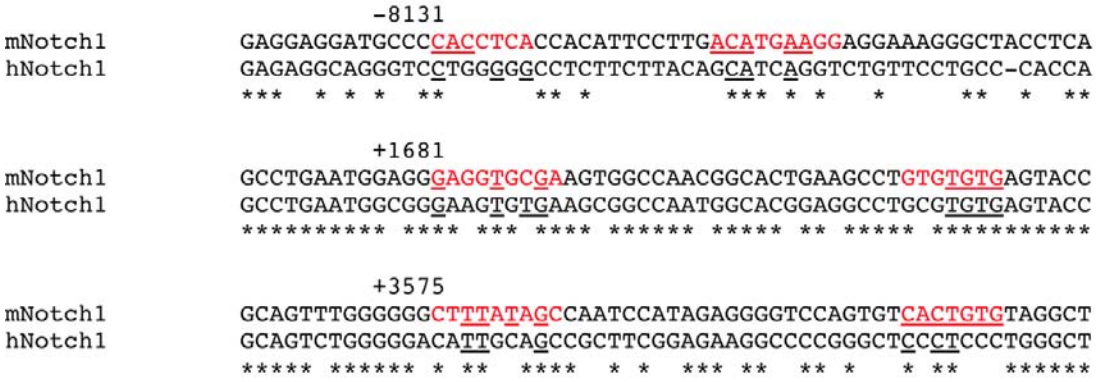

Thymus
SCID.adh
K-Ras^{G12D}
Tal1^{+}Heb$^{+/-}$
430
435
330
144
G4A2
135
130
720
135.2

Figure S5


Sequence of unknown origin
Jβ2.4
TTTTGTTGACCAGCCCGTGGGCTTTTTCTTCTGTGGGCCTGAGGGACATTCAGTACTTCGGCGCCGGGACCCGGCTCTCAGTGCTGG
L L T S P W A F S S V G L R D I Q Y F G A G T R L S V L
NOTCH1 (Exon 28)
GTGAGACCGTGGAGCCGCCCCCGCCGGCGCAGCTGCACTTCATGTAC
G E T V E P P P P A Q L H F M Y

Figure S6


-8131
mNotch1 GAGGAGGATGCCCCACCTCACCACATTCCTTGACATGAAGGAGGAAAGGGCTACCTCA
hNotch1 GAGAGGCAGGGTCCTGGGGGCCTCTTCTTACAGCATCAGGTCTGTTCCTGCC-CACCA
+1681
mNotch1 GCCTGAATGGAGGGAGGTGCGAAGTGGCCAACGGCACTGAAGCCTGTGTGTGAGTACC
hNotch1 GCCTGAATGGCGGGAAGTGTGAAGCGGCCAATGGCACGGAGGCCTGCGTGTGAGTACC
+3575
mNotch1 GCAGTTTGGGGGGCTTTATAGCCAATCCATAGAGGGGTCCAGTGTCACTGTGTAGGCT
hNotch1 GCAGTCTGGGGGACATTGCAGCCGCTTCGGAGAAGGCCCCGGGCTCCCTCCCTGGGCT