Figure S1

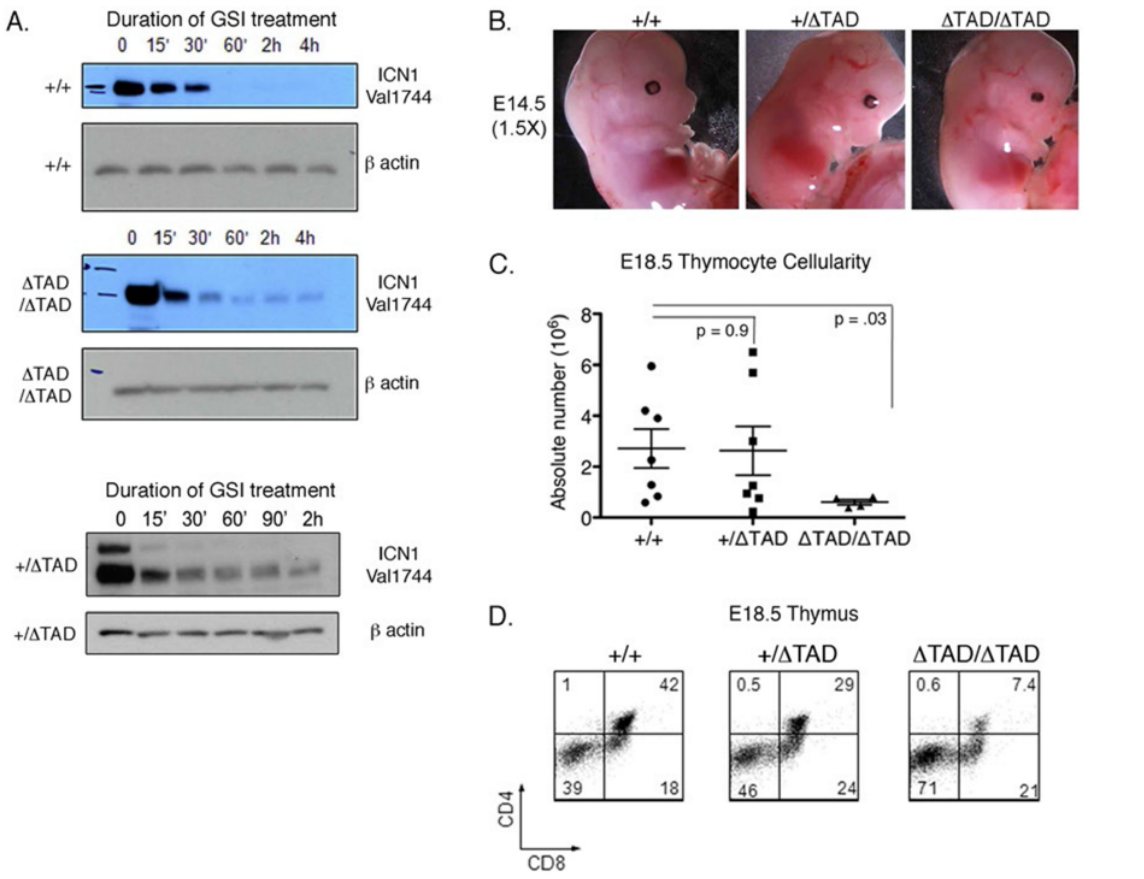

Figure S1. Characterization of ΔTAD protein and gross embryonic development of ΔTAD/ΔTAD embryos.

(A) Deletion of the Notch1 TAD decreases Notch1 turnover. MEFs from +/+, ΔTAD/ΔTAD, or +/ΔTAD were plated in equal numbers and subsequently treated with gamma secretase inhibitors (GSI) or DMSO for 4 hours. Equal amounts of protein isolated from nuclear extracts were used for Western blot to probe for cleaved Notch1 (Val1744). β−actin was the loading control.

(B) Normal gross development of mid-gestation Notch1 ΔTAD/ ΔTAD embryos. Representative images of E14.5 +/+, +/ΔTAD, and ΔTAD/ΔTAD embryos.

(C) Thymuses from E18.5 embryos were isolated from +/+ (n=11), +/ΔTAD (n=18), and ΔTAD/ΔTAD (n=7). Absolute number of thymocytes in E18.5 embryos

(**D**) Representative FACS plots showing percentages T-cells labeling with CD4 and/or CD8 in the +/+, +/ΔTAD, or ΔTAD/ΔTAD thymus.

Figure S2

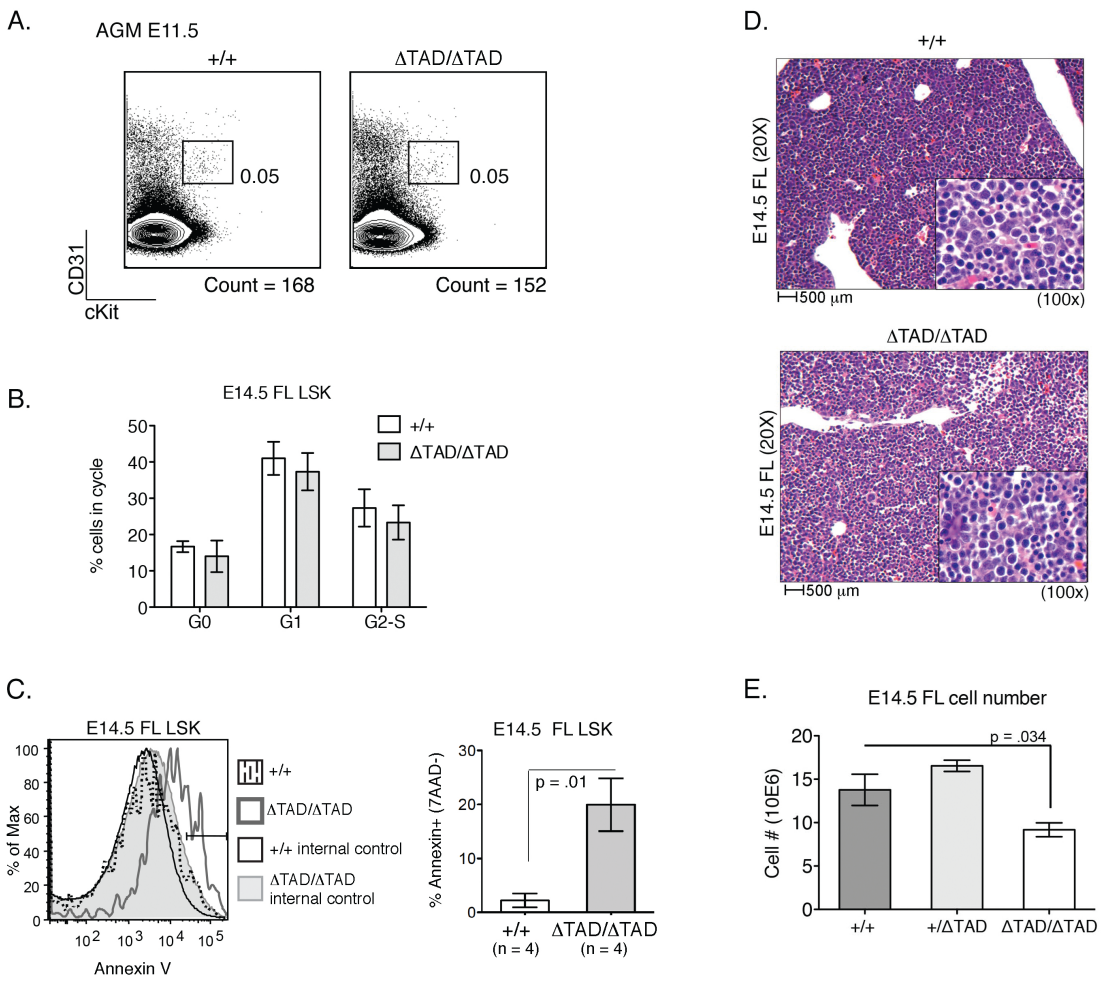

Figure S2. Characterization of E11.5 ΔTAD/ΔTAD AGM cells and E14.5 FL cells.

(A) Representative flow cytometry plot of cKit and CD31 in E11.5 AGM cells from +/+ and ΔTAD/ΔTAD embryos. HSCs in the E11.5 AGM are found in the $CD31^{+}cKit^{+}$ population

(B) Cell cycle analysis (using Ki67 and DAPI) of E14.5 FL LSKs from +/+ (n = 4) and ΔTAD/ΔTAD (n = 4)

(C) Increased apoptosis in ΔTAD/ΔTAD E14.5 FL LSKs. Representative flow cytometry plots of Annexin V^{+} cells from +/+ (dotted line) and ΔTAD/ΔTAD (bold gray line) E14.5 FL LSKs. Annexin V expression on internal control Lin^{+} cells of +/+ (solid black line) and

ΔTAD/ΔTAD (light grey shading) was used to determine the positive gate for Annexin V staining. Bar graph represents the normalized percentage of Annexin V^+ $7AAD^-$ cells from E14.5 +/+ and ΔTAD/ΔTAD FL LSKs (n = 4). Values were determined by subtracting the mean percentage of +/+ Annexin V^+ Lin^+ cells (calculated as % Annexin V^+ cells ± SEM, which was 1.600 ± 0.147, n=4) from the mean percentage of Annexin V^+ +/+ LSKs, and by subtracting the mean percentage of ΔTAD/ΔTAD Annexin V^+ Lin^+ cells (4.025 ± 0.728, n=4) from the mean percentage of Annexin V^+ ΔTAD/ΔTAD LSKs.

(D) H & E staining of +/+ and ΔTAD/ΔTAD FL. E14.5 FLs were fixed in 4% PFA and embedded in paraffin prior to sectioning. Images are 20x and inset is 100x.

(E) Decreased cellularity in ΔTAD/ΔTAD E14.5 FL. Single cell suspensions were made from individual RBCs lysed E14.5 FLs. All cell counts were performed on a hemocytometer.

Figure S3

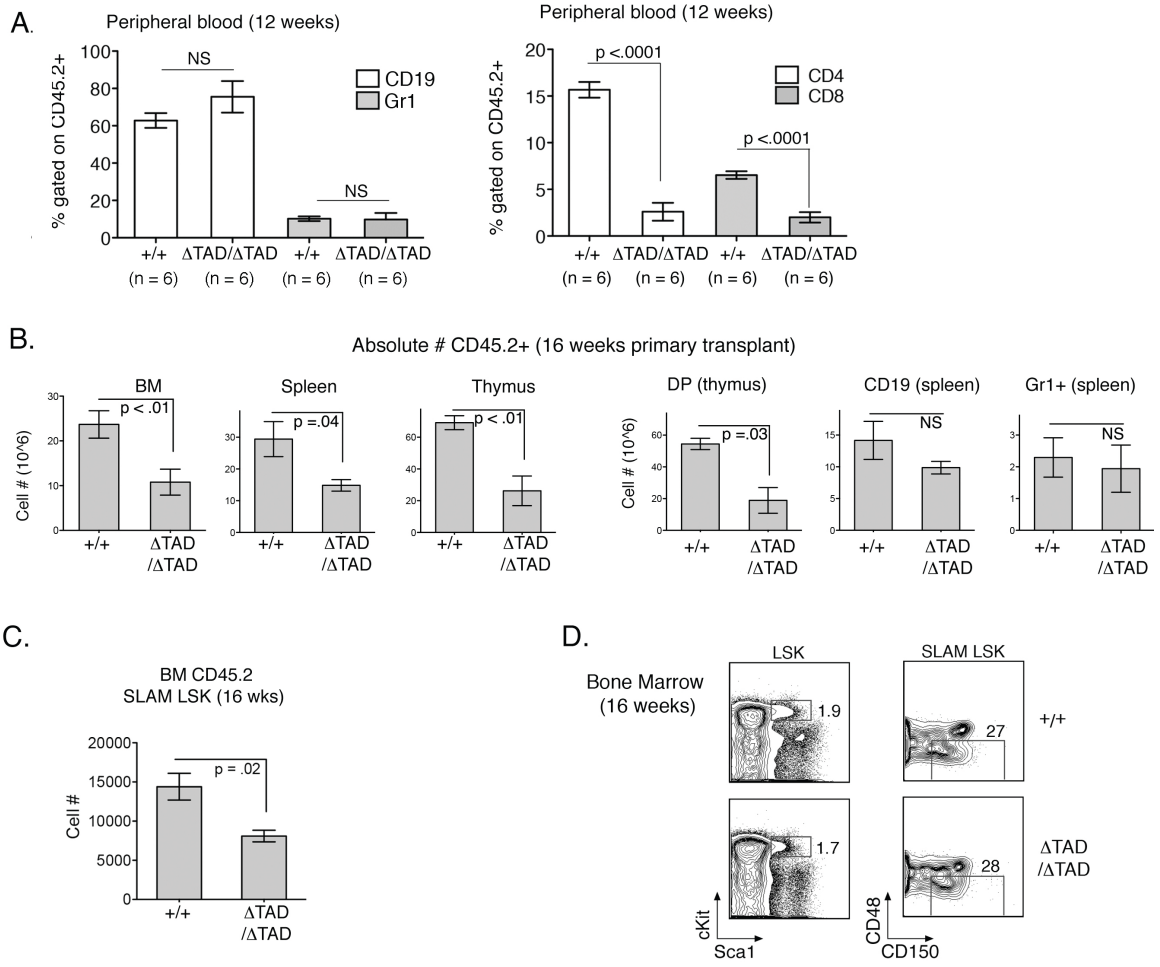

Figure S3. Hematopoietic development from E14.5 ΔTAD/ΔTAD FL cells.

(A) Multi-lineage reconstitution by CD45.2$^+$ cells measured by expression of CD4, CD8, CD19, Gr1, and CD11b in the peripheral blood at 16 weeks post-transplant of ΔTAD/ΔTAD E14.5 FL cells.

(B) Absolute numbers of CD45.2$^+$ cells in the BM, Spleen and thymus of primary transplant recipients (16 weeks). Absolute numbers of DP (CD4$^+$CD8$^+$) CD45.2$^+$ cells in the thymus and CD19$^+$CD45.2$^+$, Gr1$^+$CD45.2$^+$ cells in spleen of primary transplant recipients 16 weeks post-transplant of ΔTAD/ΔTAD E14.5 FL cells.

(**C**) Absolute numbers of CD45.2$^+$ SLAM-LSKs in the bone marrow of primary transplant recipients 16 weeks post-transplant with ΔTAD/ΔTAD E14.5 FL cells.

(D) Presence of phenotypic HSCs, but no aberrant accumulation of SLAM-LSKs in the

BM following transplant of ΔTAD/ΔTAD FL cells. Representative FACS plots of LSK and SLAM-LSK populations in the BM at 16 weeks post-transplant of ΔTAD/ΔTAD E14.5 FL cells.

Figure S4

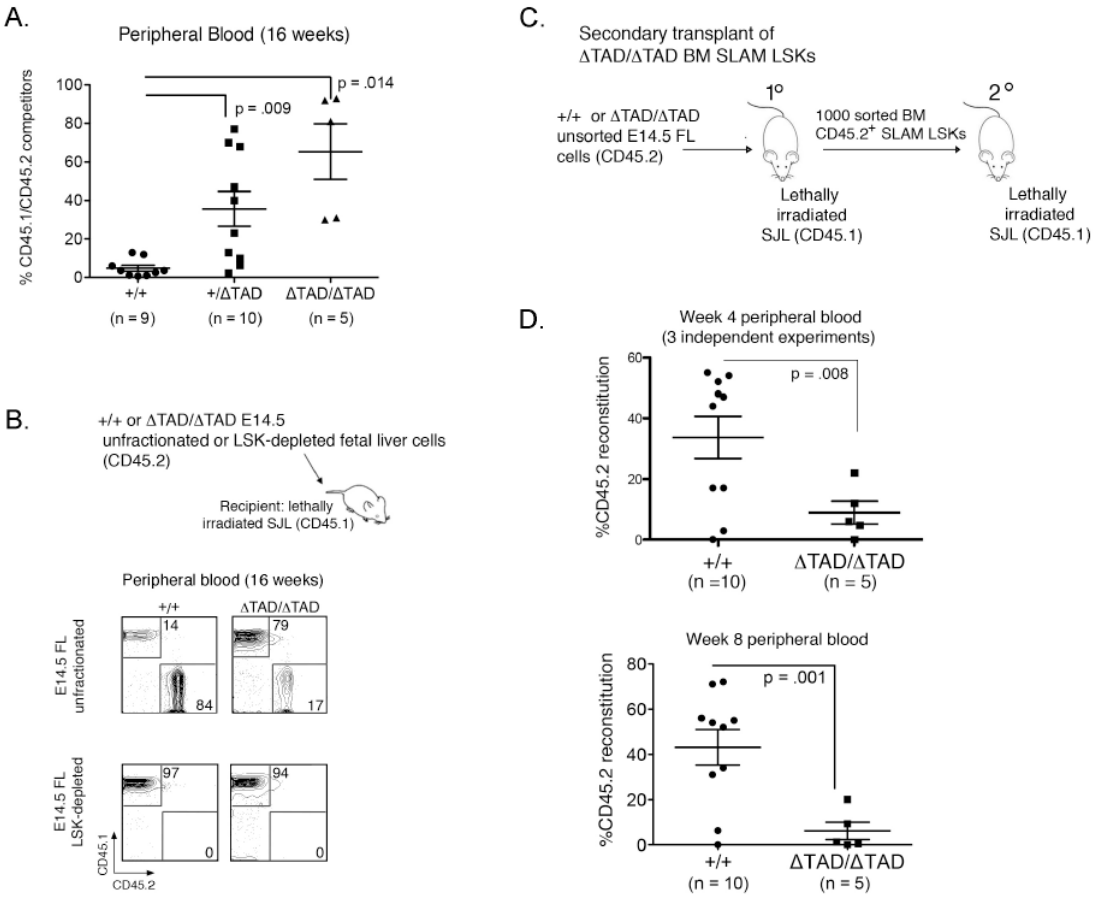

Figure S4. ΔTAD/ΔTAD E14.5 FL HSCs are impaired in primary and secondary transplants.

(A) Reconstitution of competitor F1 ($CD45.1^+CD45.2^+$) cells in peripheral blood (16 weeks) of recipients receiving 500 sorted E14.5 FL SLAM-LSKs from +/+, +/ΔTAD or ΔTAD/ΔTAD **i**n competition with 500 sorted BM SLAM-LSKs from F1 competitors

(B) Unfractionated or LSK-depleted (by FACS sorting) E14.5 FL cells from +/+ and ΔTAD/ΔTAD embryos were transplanted into lethally irradiated CD45.1 recipients. Long-term reconstitution, measured by flow cytometry of $CD45.2^+$ cells in peripheral blood was observed in recipients +/+ or ΔTAD/ΔTAD cells. $Lin^-Kit^-Sca1^-$ (LSK-depleted) FL cells were unable to reconstitute irradiated recipients (bottom panel).

(C) Schematic for non-competitive secondary transplant of +/+ or ΔTAD/ΔTAD derived

BM SLAM-LSKs. 1000 sorted donor derived CD45.2^{+} SLAM-LSKs were harvested from the BM of mice 16 weeks after receiving a primary transplant of 2 x10^{6} +/+ or ΔTAD/ΔTAD unsorted FL cells (donor cells expressed CD45.2)

(D) Reconstitution (measured by % of CD45.2) of the peripheral blood (4 weeks and 8 weeks) of secondary transplant recipients from 3 independent experiments.

Figure S5

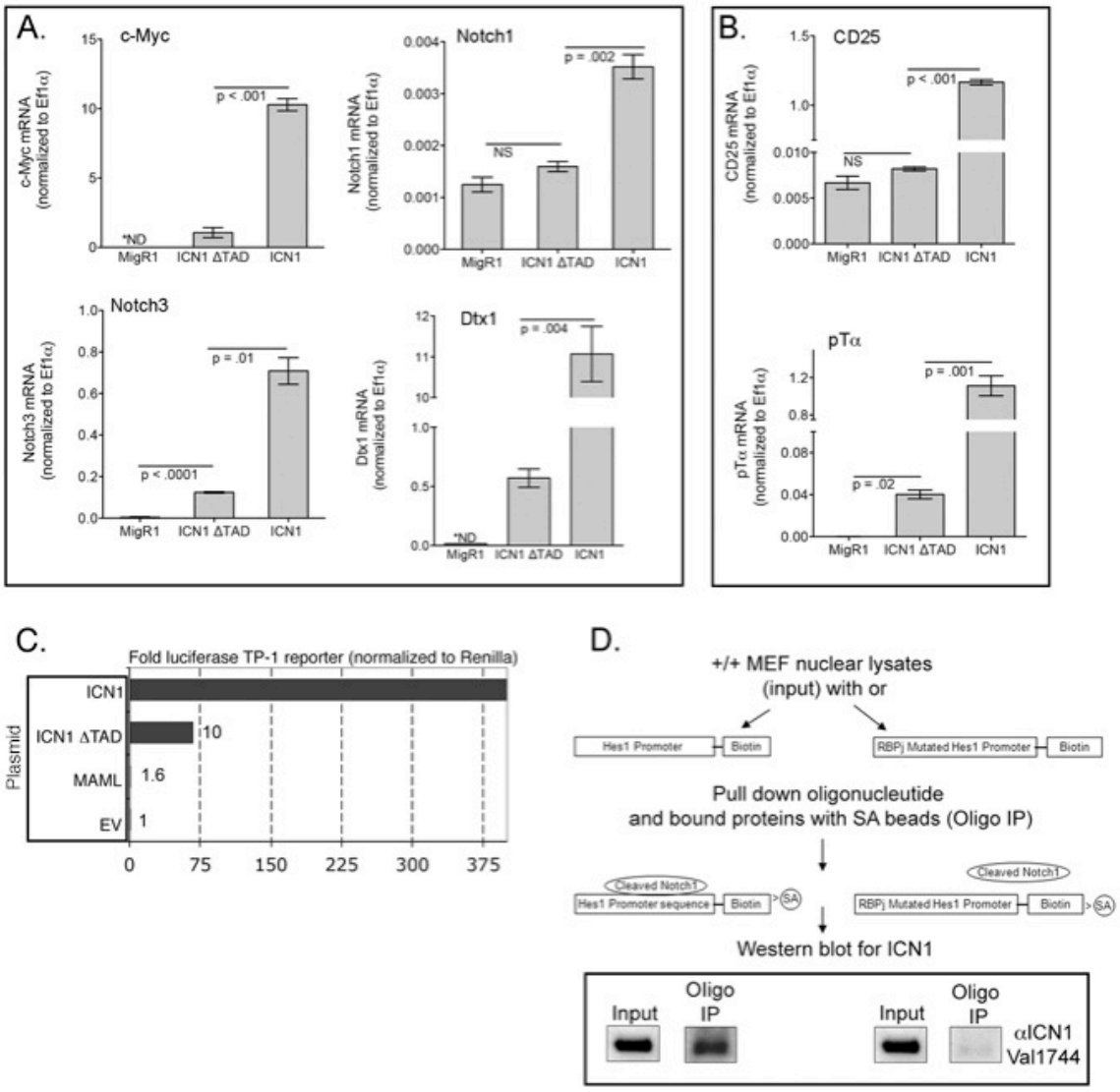

Figure S5. Notch1 TAD loss impairs transcription and transcriptional complex formation in vitro.

(A) Expression of Notch1, Notch3, c-Myc and Dtx1 in 8946 cells transduced with MigR1 control, ICN1, or ICN1 ΔTAD. Data is representative of 2 independent experiments 8946 cells were treated with doxycycline for 24hrs prior to mRNA preparation to turn off the human c-Myc transgene.

(B) Additional experiment showing expression of CD25 and pTa in 8946 cells.

(C) Activation of TP-1 luciferase reporter (12 RBPj binding sites) by ICN1, ICN1 ΔTAD, MAML1 or empty vector (EV). U2OS cells were transduced with the indicated virus or

empty vector. Luciferase activity was measured 48hrs post transduction and normalized to Renilla.

(D) Lack of cleaved Notch1 binding to Hes1 oligonucleotide with mutated RBPj binding sites (Hes1 mutated IP). Nuclear lysate from +/+ MEFs was incubated with either the Hes1 oligonucleotide or the Hes1 mutated oligonucleotide. Cleaved Notch1 expression was observed in +/+ MEFs (left panel) and was pulled down with the Hes1 oligonucleotide, but not with Hes1 mutated oligonucleotide (right panel).

Table S1. Primer and oligonucleotide sequences

qPCR primers	
Hes1 Forward	5' GAA AGA TAG CTC CCGGCA TT 3'
Hes1 Reverse	5' GTC ACC TCG TTC ATG CAC TC 3'
Ef1 alpha Forward	5' CAC TTG GTC GCT TTG CTG TT 3'
Ef1 alpha Reverse	5' GGT GGC AGG TGT TAG GGG TA 3'
Notch1 Common Forward Primer	5' AAG GGC TGG CTT GTG GTA G 3'
Notch1 Reverse +/+	5' CGA GGC CAC ATC TGA CAA GT 3'
Notch1 Reverse DTAD/DTAD	5' CCG AGC TGA GAA TTC CGA GG 3'
Jag1 F	5' GGTAACACCTTCAATCTCAAGGC 3'
Jag1 R	5' GGTCCTACACTTTGCTGGTGG 3'
Itgal F	5' GCAAAGTCGACCTGGTGTTT 3'
Itgal R	5' ACATCGGGGTTCTTGTTCTG 3'
pTα F	5'-CAGGCTCTACCATCAGGCAT-3'
pTα R	3'-ACCAGACAGGGTTGTCAAGG-5
Dtx1, c-Myc, CD25, Notch1, Notch3	TaqMan primers were purchased from Applied Biosystems
Oligonucleotides	
Hes1 promoter biotinylated oligo	5'-biotin-GTGTCTCTTCCTCCCATTGGCTGAAAGT
	TACTGTGGGAAAGAAAGTTTGGGAAGTTTCACAC
	GAGCCGTTCGCGTGCAGTCCCAGATATA
	5'-biotinTATATCTGGGACTGCACGCGAACGGCTCGT
	GTGAAACTTCCCAAACTTTCTTTCCCACAGTAACT
	TTCAGCCAATGGGAGGAAGAGACAC
Hes1 promoter mutated oligo	5'-biotin-GTGTCTCTTCCTCCCATTGGCTGAAAGTT
	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	CCGTTCGCGTGCAGTCCCAGATATA
	5'-biotin-TATATCTGGGACTGCACGCGAACGGTTT
	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAACT

	TTCAGCCAATGGGAGGAAGAGACAC
Primers for site directed mutagenesis of TAD (including from human ICN1	
Sense	CCGTGGACTCCCTGAGCTCAGCAGC
Anti sense	CTGGCTGCTGAGCTCAGGGAGTCCA
Genotyping primers	
Notch1^{in32} Forward	5' TCT AAG TGC TCC GAG GAG ATC A 3'
Notch1^{in32} (WT allele) Reverse	5' CAG GGG TTG GAG AGA CAT TCA 3'
Notch1^{in32} (Mutant allele) Reverse	5' TCG CCT TCT ATC GCC TTC TTG 3'
TAD forward	5' GTT GTA CAT CTG CCT GAC TGG GG 3'
TAD reverse	5' GTG GTA GCA AGG AAG CTA AGG 3'