# Supplemental Information

## Protein tyrosine phosphatase PRL2 mediates Notch and Kit signals in early T cell progenitors

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## Supplementary Methods

### Real-time PCR

For quantitative reverse-transcription polymerase chain reaction (RT-PCR), total RNA was isolated using the RNeasy Plus Micro Kit (QIAGEN), and then subjected to reverse transcription with random hexamers (SuperScript III kit; Invitrogen). Quantitative real-time PCR was performed with SYBR Green (Qiagen) on an ABI PRISM 7500 system. Gene specific primers were designed to flank introns so that products from cDNA could be distinguished from possible genomic DNA contamination. Primer sequences are listed in Supplementary Table 3.

### In vitro methylcellulose colony-forming cell assay

Clonogenic progenitors were determined in methylcellulose medium (MethoCult GF M3234, StemCell Technologies) with cytokines (SCF, TPO, EPO, IL-3, and GM-CSF) using 500 ETPs per well (6-well plate). Colonies were scored after 7 days of the culture.

### Apoptosis assays

Thymocytes were harvested and cultured in the presence of IL-7 (5ng/ml) for 48hrs with or without 2Gy irradiation. Cell viability was evaluated by PI/Annexin V staining in combination with lineage markers, including CD25, CD44, and CD117.

### Luciferase assay

293 cells were transfected with human PRL2 promoter driven luciferase reporter plasmids containing either RBPJ binding sites or mutant RBPJ binding sites. Luciferase activity was assayed 24 hours after transfection according to the manufacturer's instructions (Promega).

**Supplementary Figure legends**

**Figure S1.** (A) The frequency of $CD4^+$ and $CD8^+$ T cells as well as $B220^+$ B cells in the spleen of $Prl2^{+/+}$ and $Prl2^{-/-}$ mice were quantified by flow cytometry analysis. Data shown are the mean percentage (± SD) of cells in the spleen. No statistical difference was observed. (B) The frequency of DN1, ETP, DN2, DN3, and DN4 thymocytes in $Prl2^{+/+}$ and $Prl2^{-/-}$ mice were quantified by flow cytometry analysis. Frequencies of DN2a, DN2b, and DN3 cells are increased in $Prl2^{-/-}$ thymus compared to $Prl2^{+/+}$ thymus. *$P$<0.05, n=7. (C) The frequency of $B220^+$ and $Gr1/Mac1^+$ cells within the CD4/CD8 DN thymocytes is shown (**$P$<0.01, n=3). (D) Quantitative real-time PCR analysis of DLL and SCF expression in stromal cells from $Prl2^{+/+}$ and $Prl2^{-/-}$ thymus. Data shown are the mean values ± SD (n = 3). (E) Flow cytometry analysis of bone marrow cells from 8 week-old $Prl2^{+/+}$ and $Prl2^{-/-}$ mice to identify hematopoietic stem and progenitor cells. Quantification of LT-HSCs, ST-HSCs, MPPs, LMPPs, CLPs, CMPs, GMPs and MEPs in $Prl2^{-/-}$ mice, presented relative to their abundance in $Prl2^{+/+}$ mice, set as 1. Definition of each cell population is shown in supplementary table 1.

**Figure S2.** (A) The absolute number of donor-derived cells in the thymus of recipient mice repopulated with $Prl2^{+/+}$ or $Prl2^{-/-}$ HSCs.

**Figure S3.** (A) The frequency of donor-derived ETPs, DN2, and DN4 cells in thymus was determined by flow cytometry analysis 18 weeks following HSC transplant. Representative flow cytometry plot is shown. **(**B**)** The frequency of donor-derived cells in the spleen was determined by flow cytometry analysis 18 weeks following HSC transplant. T cell recovery was significantly lower in recipients repopulated with $Prl2^{-/-}$ HSCs compared to that of the $Prl2^{+/+}$ cells. ***$P$<0.001, n=8.

**Figure S4.** (A) Representative flow cytometry plots of T cell cultures with different dose of SCF are shown. (B) The relationship between SCF dosage and T cell growth was evaluated in DLL1-Fc cultures. Total cell count is shown as fold increase at day 12.

**Figure S5.** (A) The structure of mouse and human PRL2 promoter regions is shown. RBPJ and Hes binding sites are indicated. (B) Notch1 directly binds to PRL2 (PTP4A2) in CUTLL1 cells revealed by genome-wide ChIP-seq analysis. Top two tracks are histone acetylation (H3K27ac) and BRD4 that shows active chromatin. Third track is Notch1 and last track is Notch1 when CUTLL1 cells were treated with Notch inhibitor, GSI. It clearly shows that intron 1 is active enhancer and Notch1 binding site is regulated by GSI (Notch signals). (C) The structure of wild type (WT) and mutant (MUT) human PRL2 reporter is shown. Mutation of the RBPJ-binding site is underlined.

**Figure S6.** (A) The structure of mouse and human c-Kit promoter regions is shown. RBPJ and Hes binding sites are indicated. (B) Thymus was harvested from $RBPJ^{fl/fl}$-$Cre^{-}$ (WT) or $RBPJ^{fl/fl}$-$Cre^{+}$ (KO) mice following pI:pC treatment. The frequency of T cell progenitors was determined by flow cytometry analysis. The frequency of c-Kit bright DN2a cells was dramatically reduced in RBPJ-KO thymus compared to that of the WT mice. (C) NOTCH1 does not binds to KIT locus in CUTLL1 cells revealed by genome-wide ChIP-seq analysis.

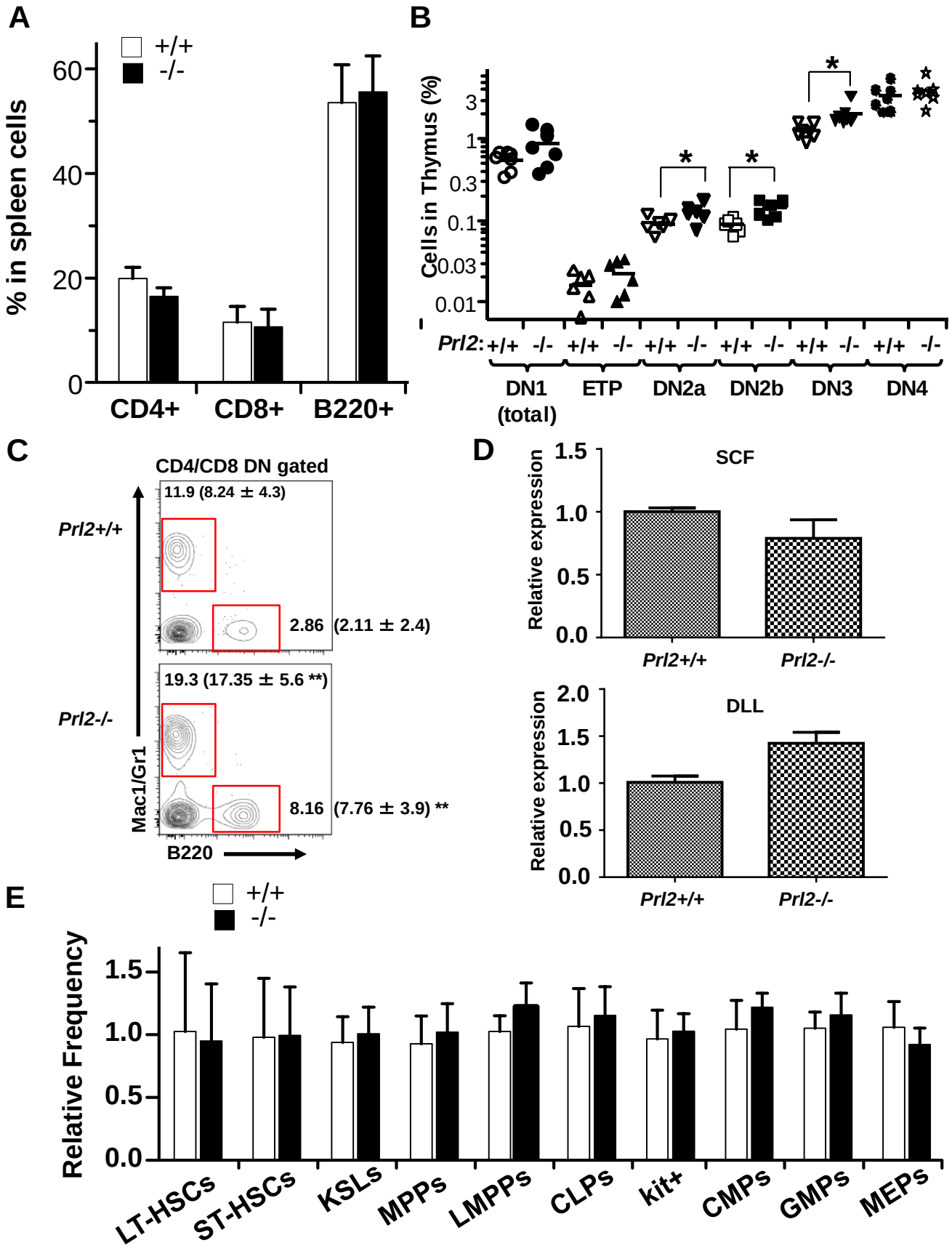
**Figure S7.** C-Kit expression was decreased in DLL1-Fc cultures of *Prl2*$^{-/-}$ LMPPs despite of increased level of c-Kit mRNAs. (A) LMPPs were sorted from *Prl2*$^{+/+}$ and *Prl2*$^{-/-}$ BM cells and cultured with DLL1-Fc. Mean fluorescence intensity of c-Kit was measured at day 12. (B) LMPPs were sorted from *Prl2*$^{+/+}$ and *Prl2*$^{-/-}$ BM cells and cultured with DLL1-Fc. Cells were harvested at each time point and c-Kit mRNA levels were measured by quantitative real-time

PCR. C-Kit mRNA was increased in fresh *Prl2*$^{-/-}$ LMPPs compared to that of the *Prl2*$^{+/+}$ cells (***P*<0.05, n=3). (C) Quantitative real-time PCR analysis of gene expression in sorted ETPs from *Prl2*$^{+/+}$ and *Prl2*$^{-/-}$ mice. Data shown are the mean values ± SD (**P*<0.05, ***p*<0.01, n = 3). (D) Quantitative real-time PCR analysis of *Cebpα* expression in ETPs and LMPPs purified from *Prl2*$^{+/+}$ and *Prl2*$^{-/-}$ mice. Data shown are the mean values ± SD (**P*<0.05, n = 3).

**Figure S8.** (A) Representative flow cytometry plots for apoptosis assays of T-cell progenitors in OP9-DL1 cultures. *Prl2*$^{-/-}$ ETPs showed enhanced apoptosis compared to *Prl2*$^{+/+}$ cells. The frequency of DN1 cells was significantly reduced in *Prl2*$^{-/-}$ LMPPs cultures compared to that of the *Prl2*$^{+/+}$ LMPPs. (B) LMPPs were sorted from *Prl2*$^{+/+}$ and *Prl2*$^{-/-}$ BM cells and cultured with DLL1-Fc. The survival of different T cell population was measured by Annexin V and PI staining. Only *Prl2*$^{-/-}$ ETPs showed decreased survival compared to *Prl2*$^{+/+}$ ETPs (***P*<0.01, n=3).

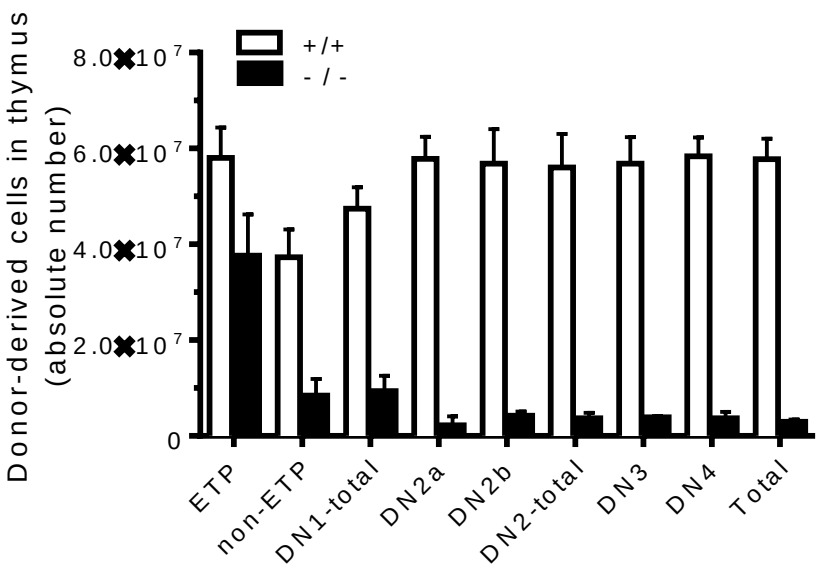
**Supplementary Table 1 – 3** List for definition of each population used (Table 1), antibodies (Table 2), and primers (Table 3) are shown.

# Figure S1

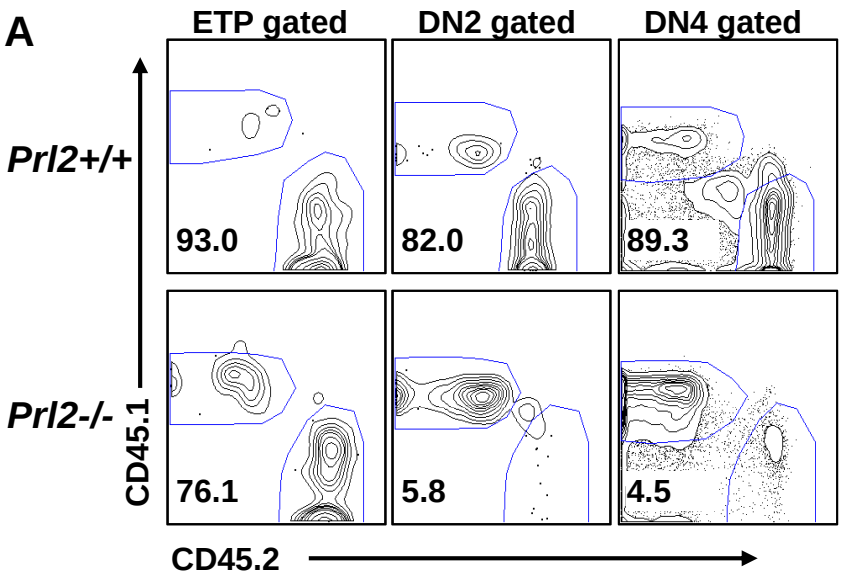

A
+/+
-/-
% in spleen cells
60
40
20
0
CD4+
CD8+
B220+
B
Cells in Thymus (%)
3
1
0.3
0.1
0.03
0.01
*
*
*
Prl2: +/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-
DN1 (total)
ETP
DN2a
DN2b
DN3
DN4
C
CD4/CD8 DN gated
Prl2+/+
11.9 (8.24 ± 4.3)
2.86 (2.11 ± 2.4)
Prl2-/-
19.3 (17.35 ± 5.6 **)
8.16 (7.76 ± 3.9) **
Mac1/Gr1
B220
D
SCF
Relative expression
1.5
1.0
0.5
0.0
Prl2+/+
Prl2-/-
DLL
Relative expression
2.0
1.5
1.0
0.5
0.0
Prl2+/+
Prl2-/-
E
+/+
-/-
Relative Frequency
1.5
1.0
0.5
0.0
LT-HSCs
ST-HSCs
KSLs
MPPs
LMPPs
CLPs
kit+
CMPs
GMPs
MEPs

# Figure S2

**A**


Donor-derived cells in thymus
(absolute number)
8.0✖10 7
6.0✖10 7
4.0✖10 7
2.0✖10 7
0
+/+
- / -
ETP
non-ETP
DN1-total
DN2a
DN2b
DN2-total
DN3
DN4
Total

# Figure S3


A
ETP gated
DN2 gated
DN4 gated
Prl2+/+
Prl2-/-
93.0
82.0
89.3
76.1
5.8
4.5
CD45.1
CD45.2

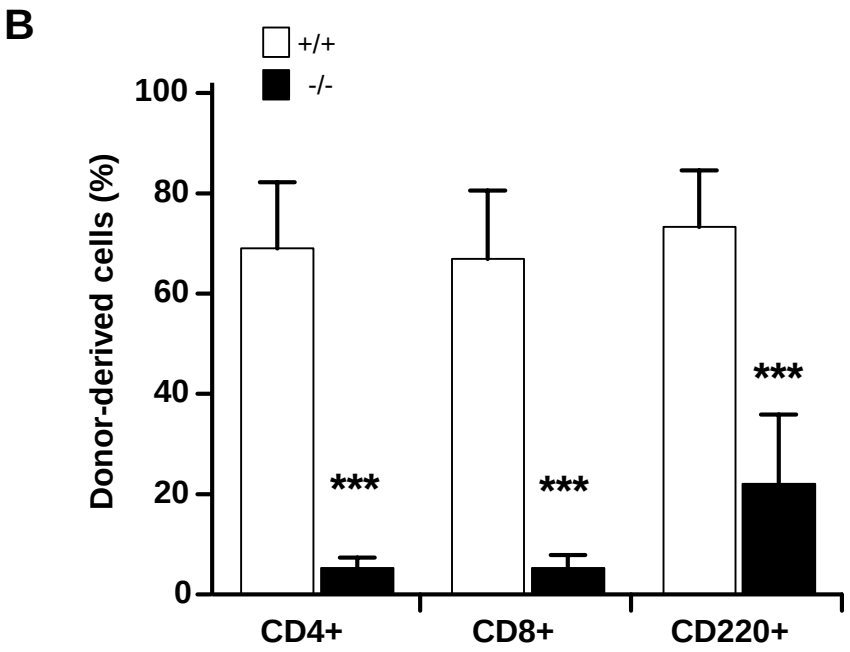
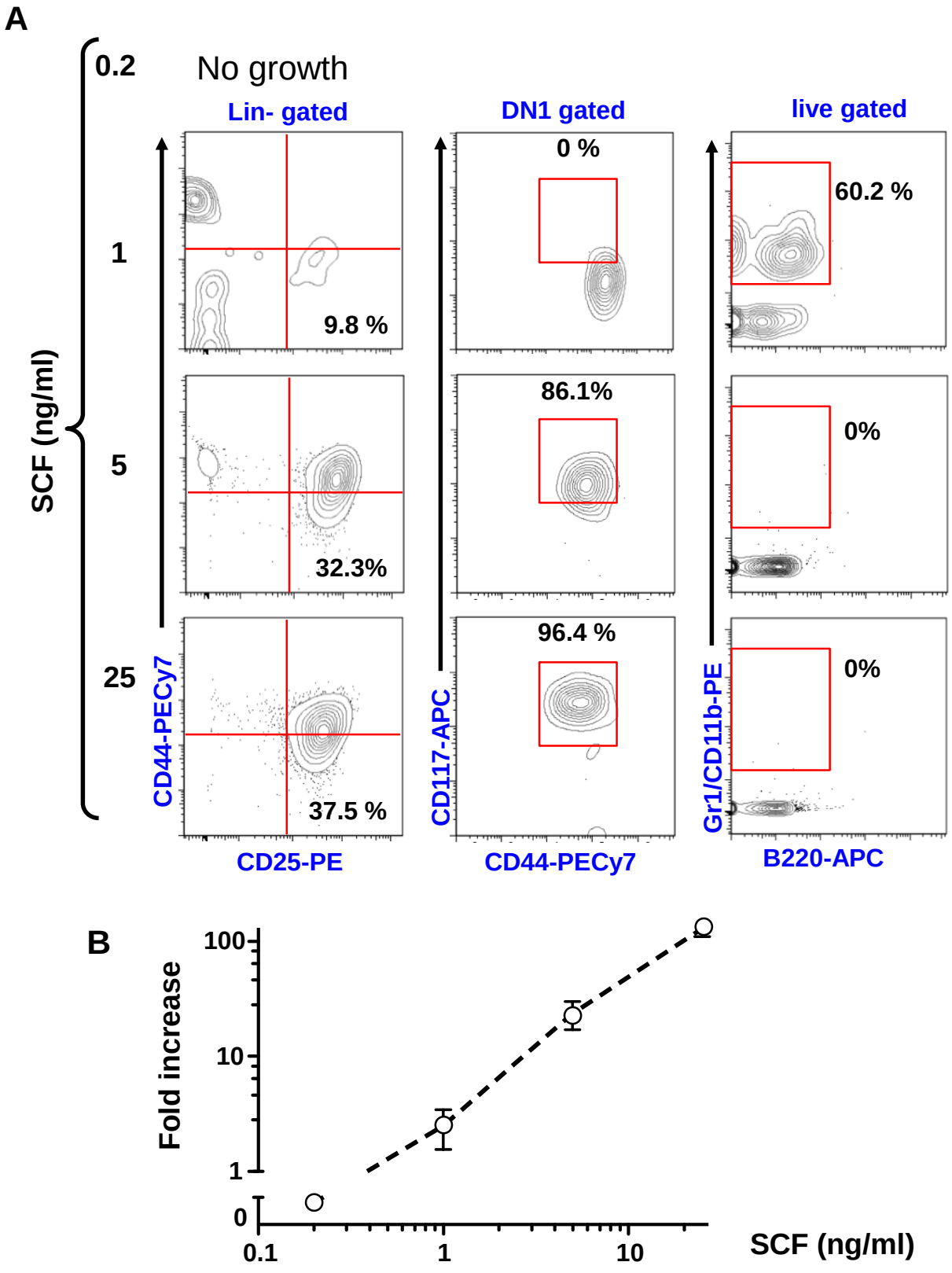
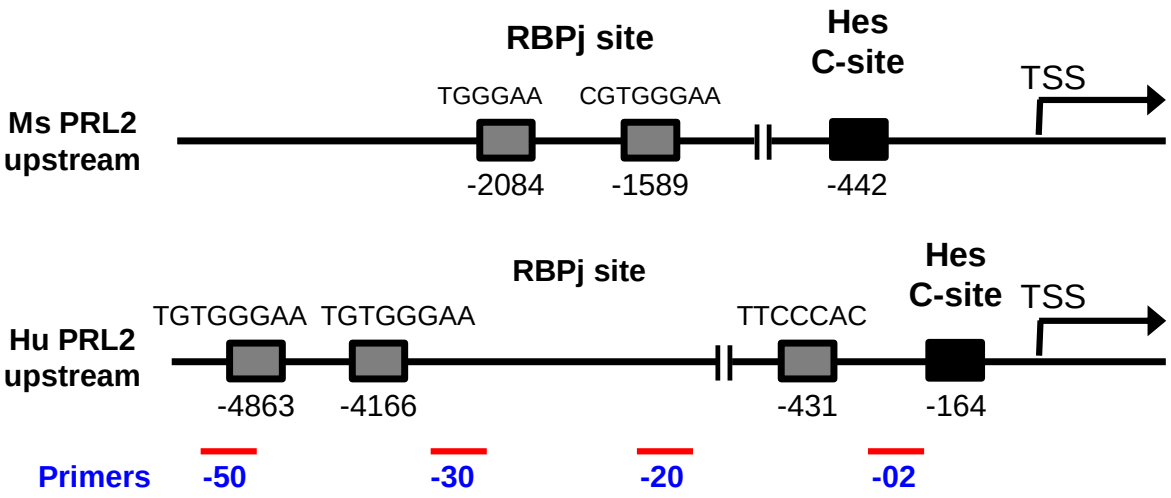

B
+/+
-/-
Donor-derived cells (%)
100
80
60
40
20
0
***
***
***
CD4+
CD8+
CD220+

Figure S4

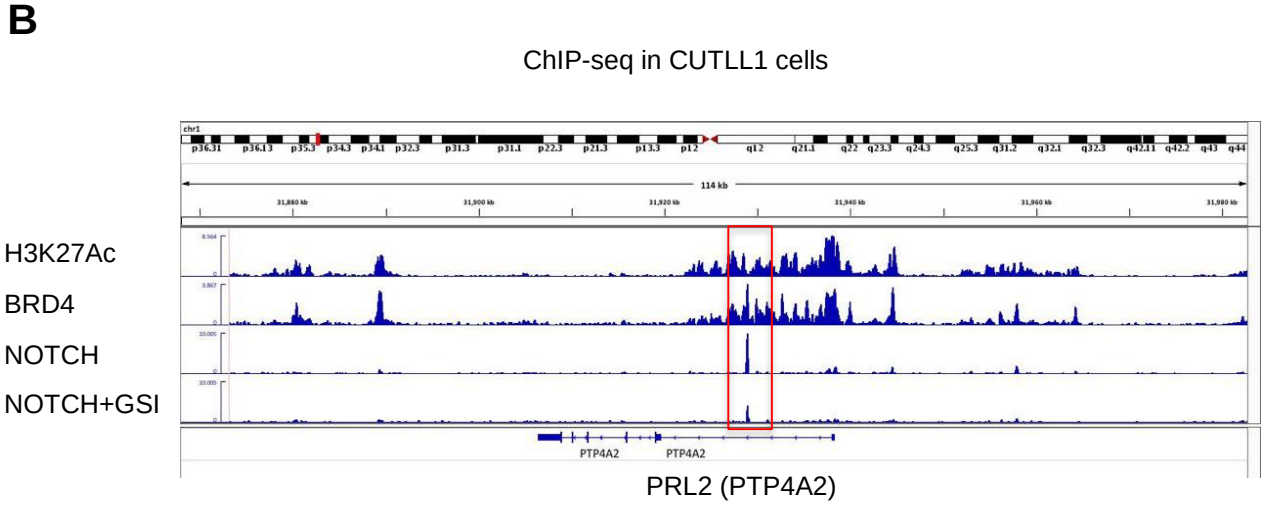

A
No growth
Lin- gated
DN1 gated
live gated
SCF (ng/ml)
0.2
1
5
25
9.8 %
0 %
60.2 %
32.3%
86.1%
0%
37.5 %
96.4 %
0%
CD44-PECy7
CD117-APC
Gr1/CD11b-PE
CD25-PE
CD44-PECy7
B220-APC
B
Fold increase
100
10
1
0
0.1
1
10
SCF (ng/ml)

# Figure S5

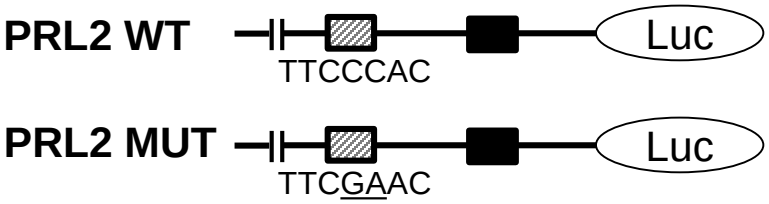
**A**



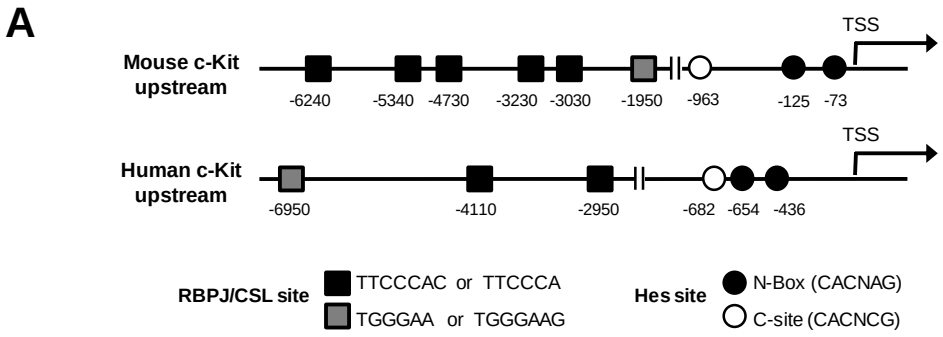

**B**

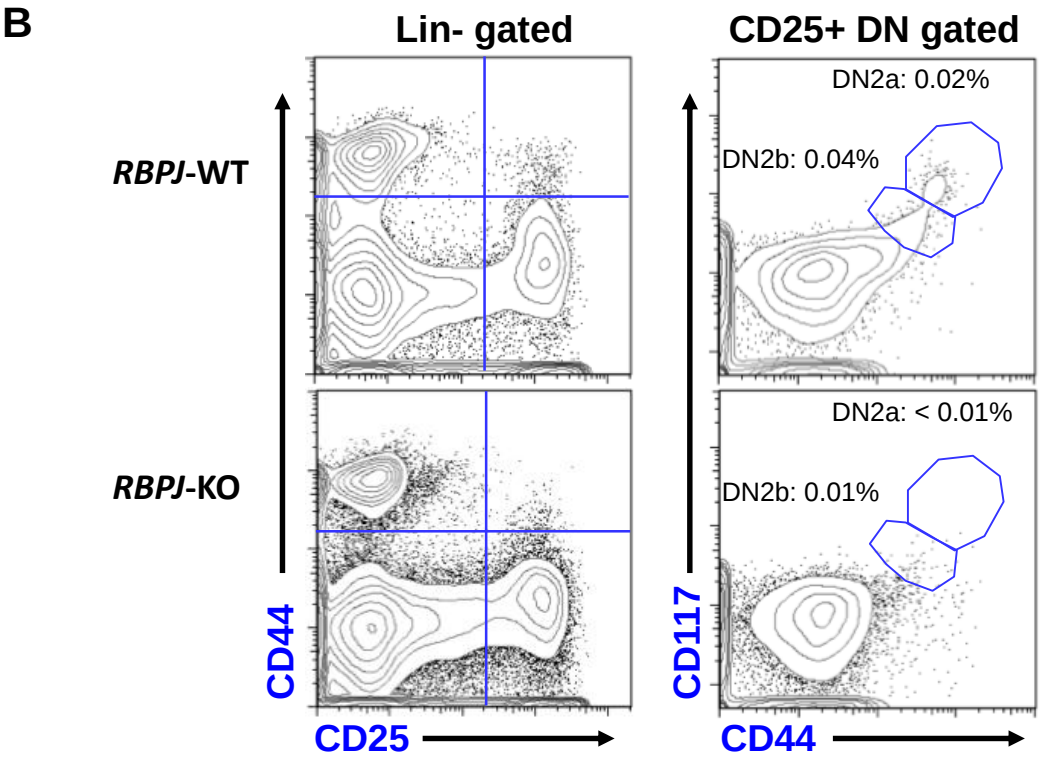


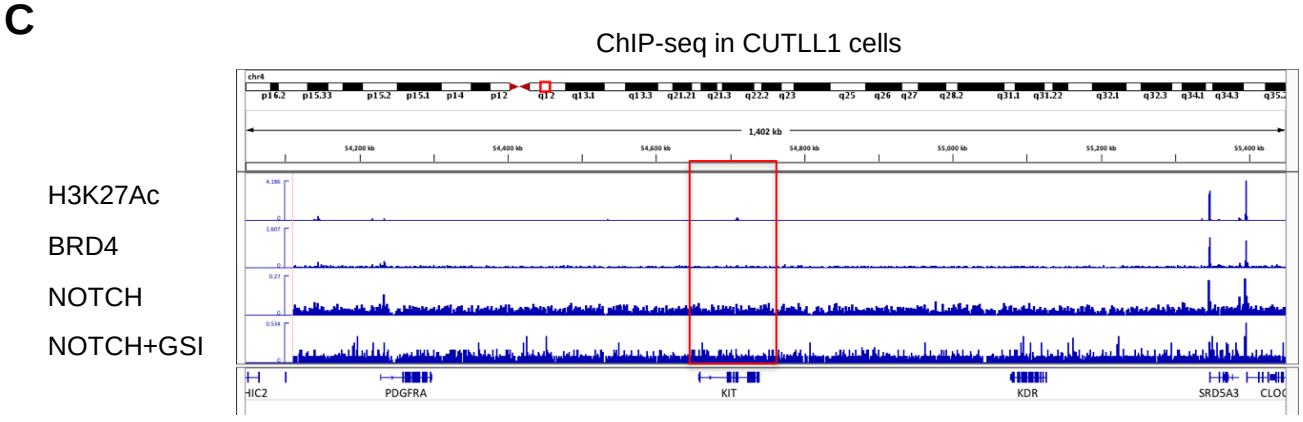

**C**



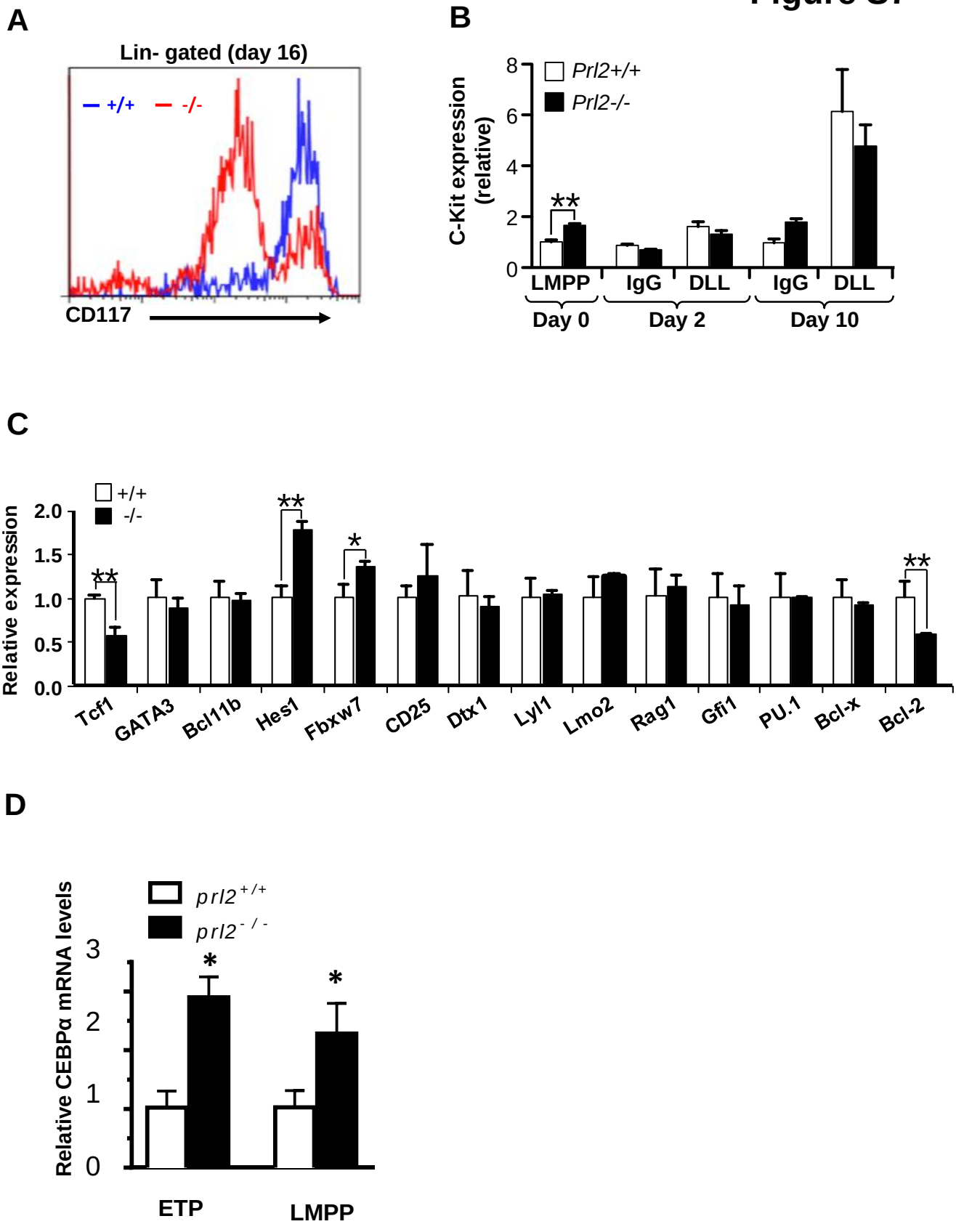
# Figure S6


A
Mouse c-Kit upstream
TSS
-6240
-5340
-4730
-3230
-3030
-1950
-963
-125
-73
Human c-Kit upstream
TSS
-6950
-4110
-2950
-682
-654
-436
RBPJ/CSL site
TTCCCAC or TTCCCA
TGGGAA or TGGGAAG
Hes site
N-Box (CACNAG)
C-site (CACNCG)

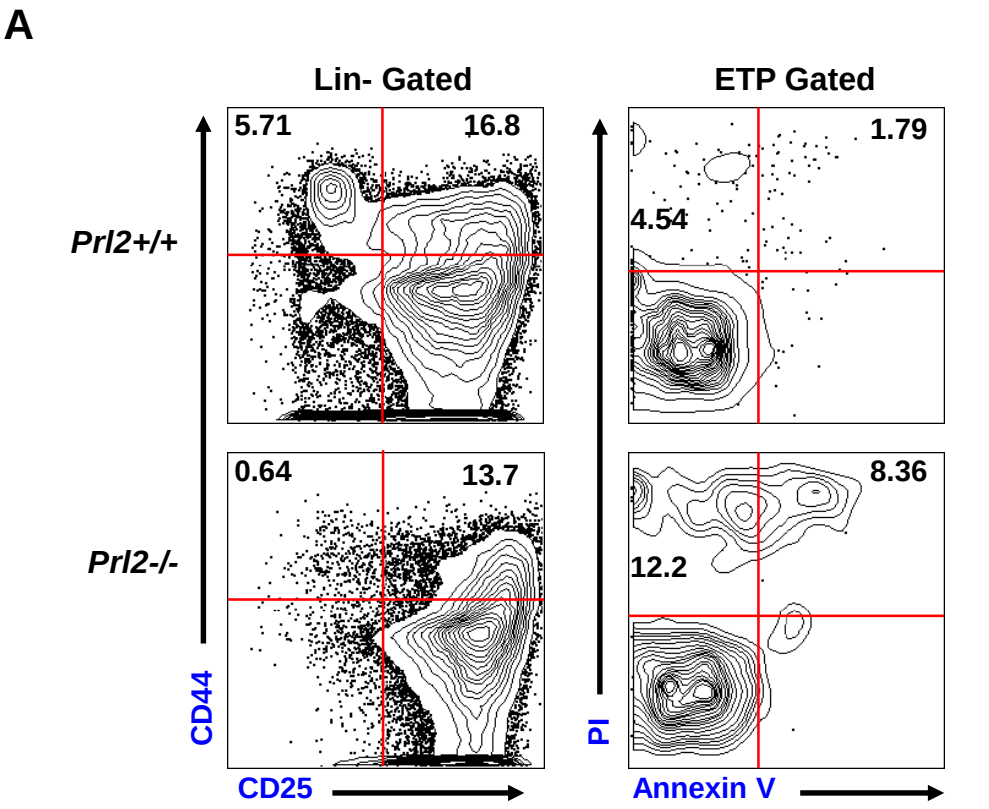

B
Lin- gated
CD25+ DN gated
RBPJ-WT
RBPJ-KO
DN2a: 0.02%
DN2b: 0.04%
DN2a: < 0.01%
DN2b: 0.01%
CD44
CD25
CD117
CD44

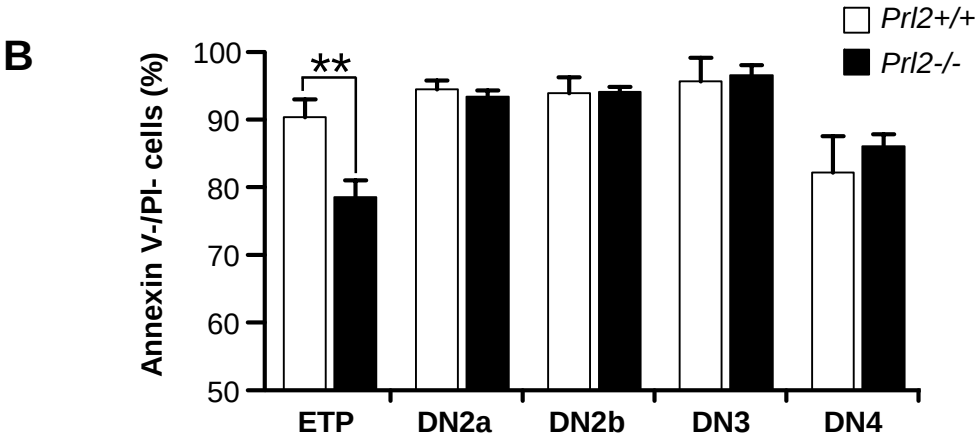

C
ChIP-seq in CUTLL1 cells
H3K27Ac
BRD4
NOTCH
NOTCH+GSI
PDGFRA
KIT
KDR
SRD5A3

# Figure S7


A
Lin- gated (day 16)
+/+
-/-
CD117
B
C-Kit expression (relative)
Prl2+/+
Prl2-/-
**
LMPP
IgG
DLL
IgG
DLL
Day 0
Day 2
Day 10
C
Relative expression
+/+
-/-
**
**
*
**
Tcf1
GATA3
Bcl11b
Hes1
Fbxw7
CD25
Dtx1
Lyl1
Lmo2
Rag1
Gfi1
PU.1
Bcl-x
Bcl-2
D
Relative CEBPα mRNA levels
prl2+/+
prl2-/-
*
*
ETP
LMPP

# Figure S8


A
Lin- Gated
ETP Gated
Prl2+/+
Prl2-/-
5.71
16.8
1.79
4.54
0.64
13.7
8.36
12.2
CD44
CD25
PI
Annexin V


B
Prl2+/+
Prl2-/-
Annexin V-/PI- cells (%)
100
90
80
70
60
50
**
ETP
DN2a
DN2b
DN3
DN4

# Supplementary Table 1

## Definition of population

| | |
|---|---|
| LT-HSCs | CD34-/CD135-/Sca-1+/CD117+/Lin- |
| | CD150+/CD48-/Sca-1+/CD117+/Lin- |
| ST-HSCs | CD34+/CD135-/Sca-1+/CD117+/Lin- |
| MPPs | CD150-/CD48+/Sca-1+/CD117+/Lin- |
| LMPPs | CD34+/CD135+/Sca-1+/CD117+/Lin- |
| CLPs | CD127+/Sca1+(dim)/CD117+(dim)/Lin- |
| CMPs | CD34+/CD16_32w-/Sca-1-/CD117+/Lin- |
| GMPs | CD34+/CD16_32w+/Sca-1-/CD117+/Lin- |
| MEPs | CD34-/CD16_32w-/Sca-1-/CD117+/Lin- |
| Lin | CD3e,Gr1,CD11b,Ter-119,B220 |
| | |
| TSPs | CD135+/CD117+/CD127-/CD44+/CD25-/Lin- |
| ETPs | CD117+/CD127-/CD44+/CD25-/Lin- |
| DN2a | CD117+(bright)/CD44+/CD25+/Lin- |
| DN2b | CD117+(dim)/CD44+/CD25+/Lin- |
| DN3 | CD117-/CD44-/CD25+/Lin- |
| DN4 | CD117-/CD44-/CD25-/Lin- |
| Lin | CD4,CD8a,TCR-b,NK1.1,Gr1,CD11b,Ter-119,B220 |

# Supplementary Table 2

## Antibody List

| Antibody | Clone | Supplier | Conjugation |
|---|---|---|---|
| CD3ε | 145-2C11 | Biolegend | FITC, PE, PE-Cy7, Biotin |
| CD4 | RM4-5 | eBioscience | FITC, PE, Biotin |
| CD8α | 53-6.7 | eBioscience | APC, Biotin |
| Mac-1 (CD11b) | M1/70 | Biolegend | FITC, PE, APC, Biotin |
| FcγRII/III (CD16/32w) | 93 | Biolegend | PE-Cy7 |
| CD34 | RAM34 | BD bioscience | FITC |
| CD45.1 (Ly5.1) | A20 | BD bioscience | PE, PE-Cy7, APC-Cy7 |
| CD45.2 (Ly5.2) | 104 | BD bioscience | FITC, PerCP-Cy5.5, APC |
| CD48 | HM48-1 | eBioscience | FITC, PE, APC |
| CD150 | TC15-12F12.2 | Biolegend | PE, PerCP-Cy5.5, APC |
| B220 (CD45R) | RA3-6B2 | Biolegend | FITC, PE, PE-Cy7, APC, Biotin |
| c-Kit (CD117) | 2B8 | BD bioscience | FITC, PE, APC, PE-Cy7, APC-Cy7 |
| Sca-1 (Ly-6A/E) | D7 | BD bioscience | PE, PerCP-Cy5.5, PE-Cy7, Pacific Blue |
| Ter119 | Ter119 | Biolegend | FITC, PE, Biotin |
| Gr1 | RB6-8C5 | Biolegend | FITC, PE, APC, Biotin |
| Ki-67 | B56 | BD bioscience | PE |
| Streptavidin | | Biolegend | APC-Cy7 |
| Annexin-V | | BD bioscience | FITC |
| CD25 | PC61.5 | BioLegend | FITC, PE, APC |
| CD44 | IM7 | BioLegend | PerCP-Cy5.5, PE-Cy7 |
| TCR-b | H57-597 | BioLegend | FITC, PE |
| TCR-gd | GL-3 | eBioscicence | APC |
| NK1.1 | PK136 | BD bioscience | FITC, PE |
| FcE-R | MAR-1 | Biolegend | PE, APC |
| CD135 | A2F10 | eBioscicence | PE |
| CD127 | A7R34 | Biolegend | PE, APC |
| c-Kit (human) | 104D2 | BioLegend | APC |
| | | | |
| c-KitY703 | D13A2 | Cell Signaling | |
| Actin | | Cell Signaling | |

# Supplementary Table 3

## Primer & oligonucleotide List

### qPCR primer

| | | |
|---|---|---|
| PRL2 | GGCTGTAACAGGGTGGAAGA | GCCACCAACATCTGGGTACT |
| c-Kit | GTGCCAACCAAGACAGACAAGA | GCCAGCTCGTCATCTTCCATG |
| Tcf1 | GGAGATGAGAGCCAAGGTCA | TGCGGGCCAGTTCATAGTA |
| GATA3 | GAAGGCAGGGAGTGTGTGAAC | CTTGATAAGGGGCCGGTTCTG |
| Bcl11b | CTACTGTCACCCACGAAAGGC | TGGGAAGAGGAGGCAGCTATG |
| Lmo2 | ACGGAAATTGTGCAGGAGAG | CTTTGTCTTTCACCCGCATC |
| Hes1 | GAGTGCATGAACGAGGTGACC | GGGTAGGTCATGGCGTTGATC |
| Dtx1 | GGTGGCCATGTACTCCAATGG | GTCTGGGTGTCTGCAAAACCA |
| Fbxw7 | AGTGATAGAGCCCCAGTTCCA | TCCTCAGCCAAAATTCTCCA |
| CD25 | CGTGGAAGGACAGAGTGTTCA | GCCAGAAATCGGTGGTGTTCT |
| Rag1 | CCCACAAATCAAATTTTCCGAGTG | CTTCTCTGGAACTACTGGAGACTG |
| Lck | CAAGATCTCGCTGCCCATCC | TAACTGTGCAGGGCGATAACC |
| Lyl-1 | GCAGAAGCGCAGACCAAG | CACACGGCTGTTGGTGAA |
| Bcl-2 | TGAGTACCTGAACCGGCATCT | TCAAACAGAGGTCGCATGCTG |
| Bcl-X | TGCAGGTATTGGTGAGTCGGA | TGCTGCATTGTTCCCGTAGAG |
| Ebf1 | CTATGTGCGCCTCATCGACTC | ACTCGGCACATTTCAGGGTTC |
| PU.1 | GGATCTGACCAACCTGGAG | CTACTACTCCTTCGTGGGCAG |
| Gfi1 | GCTCCCGAATTTCCAGCCTC | TGTTTGGACCCTCGGATACTC |
| HPRT | TCCTCCTCAGACCGCTTTT | CCTGGTTCATCATCGCTAATC |
| ActB | CCTAAGGCCAACCGTGAAAAG | CAGAGGCATACAGGGACAGCA |

### ChIP primer

| | | |
|---|---|---|
| huPRL2/ChIP/-200 | AGGCCACGTTCTGCCTTC | CTCCTCCTCCTCGTGGTTC |
| huPRL2/ChIP/-400 | CAGTTCTTAGGGTGGGTGTGTG | TGGGGATTTCCAACTCAGAAG |
| huPRL2/ChIP/-1000 | AATCTGCGTCGTTAAAGGTGA | ACCTGGGCGACAGAGTGAGA |
| huPRL2/ChIP/-2000 | GGGAAGGGTCATTCACTTGG | TGTTTTCAGATTCTCCCCTGCT |
| huPRL2/ChIP/-3000 | CCGCCTTCTGGGTTCAAGT | TAGCCGAGAGTGGTGGTAGG |
| huPRL2/ChIP/-4000 | CAGGGCACTTATGCGTGAAAC | CTCTCACCCCGACATCCAA |
| huPRL2/ChIP/-5000 | TCCCCAAGTCTACATGACAACA | TGAAGCCCTCAGAAAAGTGG |