Materials and Methods

Generation of the *LRF* mutant mice

We screened a 129/Sv mouse genomic library using mouse *LRF* cDNA as a probe. Exon/intron boundaries of the *Zbtb7a* gene were determined by restriction endonuclease mapping. Schematic representations of the targeting constructs for both conventional (Zbtb7aKO) and conditional (Zbtb7aFlox) knockout *LRF* mutants are described in Fig. S1. To generate a targeting vector for the conventional *LRF* knockout mutant, a loxp site was introduced into the *Bam*HI site in the exon 2 of the *Zbtb7a* gene and a loxp-Neo-loxp hrGFP (hrGFP sequence from pIRES-hrGFP-2a, Stratagene) sequence was introduced into the *Xba* I site in the intron between exon 2 and 3 (Fig. S1A). The targeting construct was linearized and electroporated into CJ7 ES cells. ES cells were subsequently cultured in medium containing neomycin. Individual ES clones were isolated and genotyped by Southern blot. Two correctly recombined clones were subsequently transfected with the pCre-Pac plasmid (a kind gift from Dr. Yagi, Osaka university) by electroporation to delete both exon 2 of the *Zbtb7a* gene and the Neo cassette. Resultant individual ES clones were tested for neomycin sensitivity and genotyped by Southern blot. Two ES clones were independently microinjected into E3.5 C57BL/6 blastocysts and resultant chimeric mice were subsequently crossed with C57BL/6 mice. Germline transmission of the mutant allele was verified by Southern blot and PCR analysis of tail DNA from agouti coat colored F1 offspring ($Zbtb7a^{+/-N1}$). Zbtb7aKO mice were backcrossed to the C57BL/6 strain for 10 generations ($Zbtb7a^{+/-N10}$) prior to use for experiments.

For the conditional *LRF* knockout mutant, we utilized the pKOII vector for gene targeting (a kind gift from Dr. DePinho, Dana-Farber Cancer Institute). A loxp sequence, in conjunction with an artificial *EcoR*I site for Southern blot, was introduced into the *Sph*1 site

in the intron between exon1 and exon2. Linearized targeting vector was electroporated into CJ7 ES cells and subsequently cultured under neomycin selection. Individual ES clones were isolated and genotyped by Southern blot. Chimeric mice were generated as aforementioned and germline transmission of the mutated allele was verified by Southern blot of tail DNA from agouti coat colored F1 offspring (*Zbtb7a*$^{neo/+N1}$). The *Zbtb7a*$^{neo/+N1}$ mice were subsequently bred with the *FLP*-deleter strain (Jackson laboratory) and resultant offspring, in which the Neo cassette was successfully deleted (*Zbtb7a*$^{Flox/+N1}$), were then backcrossed to the C57BL/6 strain for 2 generations (*Zbtb7a*$^{Flox/+N3}$). We then crossed the *Zbtb7a*$^{Flox/+N3}$ mice with the *Mx1-Cre* transgenic mice (Jackson laboratory) to generate *Zbtb7a*$^{Flox/+Mx1cre+}$ double transgenic mice. The *Zbtb7a*$^{Flox/+Mx1cre+}$ mice were further inbred with the *Zbtb7a*$^{Flox/+}$ littermate mice and their offspring were utilized for the initial studies. We also crossed *Zbtb7a*$^{Flox/FloxMx1cre+}$ mice with *Zbtb7a*$^{+/-N10}$ mice and their offspring (*Zbtb7a*$^{Flox/+Mx1cre+}$ and *Zbtb7a*$^{Flox/-Mx1cre+}$) were used for the bulk of this study.

Genotyping of the mutant mice was performed by PCR of tail DNA with the following primers; For Zbtb7aKO mice, PKFW1-CGTCGGGTCTTGGTGTGTC, PKFW2-CAGGGCCATAGGGAGTACTGG, PKXba-CCTGATCCAAGGACTGACTAACTTC, PKGFP CTTGGTGAAGGTGCGGTTG.

For Zbtb7aFlox mice, PCFW1-TCTGAGGCCCGGTGCAT, PCFW2-AGGGTGGTGCTCCCTCTAGAC, PCFW3-ACCGCGGTCTAGGGATCC, PCRV-GCTTGGGCTCCCCATGTAG, PCNeo-GGATGTGGAATGTGTGCGAG. For Cre transgene, OIMR1084CreFW-GCGGTCTGGCAGTAAAAACTATC OIMR1085CreReV-GTGAAACAGCATTGCTGTCACT (Jackson lab).

Flow cytometric analysis and cell sorting

Single cell suspensions were prepared from spleen, thymus and BM (from femoral and tibial bones) by passing cells through 70μm cell strainers (BD). Cells were then re-suspended in PBS containing 2% FBS. After blocking non-specific antibody binding by incubating cells with either FcBlock (BD) or purified Rat IgG (eBioscience), cells were subsequently incubated with fluorochrome-conjugated (or with biotin-conjugated) antibodies for 30 minutes on ice. Full lists of antibodies are described below. For lineage marker labeling, cells were further incubated with fluorochrome-conjugated Streptavidin for 15 minutes. For lineage cell depletion, we incubated biotin-labeled Lin^+ cells with anti-Biotin MicroBeads (Miltenyi Biotec) and subsequently applied the cell suspension onto MACS separation columns (Miltenyi Biotec). We performed FACS analysis by collecting data with FACScan, FACSCalibur, LSRII (BD) or CyAn (DAKO), followed by analysis with FlowJo software (Tree Star). For cell sorting, we used DAPI for live/dead discrimination and subsequently performed cell sorting with MoFlo (DAKO) at the MSKCC Flow Cytometry Core Facility.

Antibodies for FACS

The following biotin-conjugated antibodies are all purchased from eBioscience; Biotin-CD3 (145-2C11), Biotin-CD4 (L3T4), Biotin-CD8 (CT-CD8b), Biotin-B220 (RA3-6B2), Biotin-CD19 (MB19-1), Biotin-IgM (II/41), Biotin-Gr1 (6y-6G), Biotin-CD11b (M1/70), Biotin-NK1.1 (PK136), Biotin-TER119 and Biotin-CD31 (390). Biotin-Ly-6C (AL-21) was purchased from BD. The fluorochrome-conjugated antibodies that were used in this study were as follows: APC-AA4.1 (AA4.1), PE-Cy7-IgM (R6-60.2), APC-Cy7-B220 (RA3-6B2), PE-Cy7-CD4 (L3T4), PE-IL-7Rα (A7R34), APC-Cy7-cKit (2B8), Sreptavidin-PE-Cy7, APC-CD25 (PC61.5), APC-Alexa750-CD8 (53-6.7) and Sreptavidin-APC-Cy7 from

eBioscience. PE-CD19 (1D3), FITC-IgM (R6-60.2), APC-B220 (RA3-6B2), FITC-CD45.2 (104), PE-CD45.1 (A20), APC-CD8 (53-6.7), FITC-CD44 (IM7), PE-CD25 (PC61), FITC-CD43 (S7), FITC-Sca-1 (E13-161.7), PE-Gr1 (RB6-8C5), FITC-CD11b (M1/70) and Streptavidin-PE-TexasRed from BD. Pacific-Blue-CD4 (GK1.5) is from Biolegend. For the analysis of FL lymphopoiesis, we used fluorochrome-conjugated anti-B220, CD19, AA4.1 and biotin-conjugated lineage markers (CD3, CD4, CD8, Gr1, TER119, NK1.1 and Ly6-C), followed by incubation with PE-Cy7-Streptavidin. For the analysis of BM lymphopoiesis, BM cells were stained with fluorochrome-conjugated anti-B220, CD19, IgM, CD43 and lineage markers (CD3, CD4, CD8, Gr1, CD11b, TER119, NK1.1 and Ly6-C), followed by incubation with APC-Cy7-Streptavidin. For the analysis of *in vitro* propagated B and T cells, we use FITC-NK1.1 (PK136), PE-CD19 (1D3), PE-Cy7-CD11b (M1/70), APC-CD11c (HL3), APC-Cy7-CD25 (PC61), PE-TexasRed-B220 (RA3-6B2), PerCP-CD4 (RM4-5), Pacific-Blue-CD8 (53-6.7) (all from BD) and Alexa-700-CD44 (IM7, Biolegend).

B and T cell expansion *in vitro*

OP9-GFP control and OP9-DL1 cells were provided by Dr. Zuniga-Pflucker (University of Toronto). Flow-sorted HSCs or PreProB cells were cultured on either OP9-GFP or OP9-DL1 stromal cells for indicated days as previously described (*S1*). 3.0 x 10^5 OP9-DL1 (or OP9-GFP) cells were plated onto a 6 well plate (50,000 cells per well) the day before starting co-culture. Cells were maintained with lymphocyte expansion (LE) medium {alpha-MEM medium supplemented with 20% FBS, 5ng/ml mIL-7 and 5ng/ml mFlt3 ligand (Peprotech)}. Every fourth day, cells were collected by forceful pipetting, filtered through a 70μm cell strainer (BD), and seeded onto a new tissue culture plate containing fresh medium and cytokines.

Peripheral Blood (PB) analysis

Mice were anesthetized with isoflurane and peripheral blood samples were collected from the retroorbital sinus with heparinized capillary tubes (Fisher Scientific). 25μl of PB was diluted with 225μl of PBS (1:10 dilution, total 250 μl) and blood counts performed with an Adivia 120 hematology analyzer (Bayer). For FACS analysis, 30-50 μl of PB was stained with antibodies as described above and washed once with PBS. We subsequently eliminated red blood cells (RBC) by incubating samples with FACS Lysing Solution (BD) according to manufacture's instruction.

Transplantation experiments and retroviral transduction

The congenic strain that carries the CD45.1 antigen (B6.SJL-Ptprc^{a}Pepc^{b}/BoyJ) was purchased from Jackson laboratory and bred with C57BL/6 mice (CD45.2^{+}) to generate CD45.1^{+}CD45^{+} double positive mice. Resulting CD45.1^{+}CD45.2^{+} mice were utilized as recipients for the BM reconstitution assays (Fig. S3A). Both *Zbtb7a*$^{+/+}$ and *Zbtb7a*$^{-/-}$ FL cells were obtained from 12.5 d.p.c embryos by intercrossing *Zbtb7a*$^{+/-}$ mice. We pooled two FLs for each genotype (*Zbtb7a*$^{+/+}$ and *Zbtb7a*$^{-/-}$) from single litter and subsequently injected 1x10^{5} FL cells (Tester, CD45.2) along with 0.5x10^{5} CD45.1^{+}BM mononuclear cells (Competitor, CD45.1) into lethally irradiated recipient mice (CD45.1^{+}CD45.2^{+}).

Retrovirus constructs, pMSCV-IRES-GFP and pMSCV-EBF1-IRES-GFP, were kind gifts from Dr. Kondo (Duke University). PIG and PIG-LRF retrovirus constructs were previously described (*S2*). The MIGR1-Flag-LRF construct was generated by inserting mouse LRF cDNA from pSG5-Flag-Pokemon (*S2*) into the BglII site of the MSCV-MIGR1 vector. For *in vivo* rescue experiments (Fig. S5B), the viral supernatant was prepared as previously

described (*S2*). FL cells were collected and pooled from five 13.5 d.p.c. FLs for each genotype (*Zbtb7a*$^{+/+}$ and *Zbtb7a*$^{-/-}$). Cells were subsequently lineage-depleted (Lin=CD3, CD4, CD8, B220, CD19, IgM, NK1.1, Gr-1 and TER119) with MACS separation columns (Miltenyi Biotec). The resultant Lin^{-} cells were further incubated in 12-well plates (4 x 10^5 cells per well) with 2 ml of HSC expansion medium {X-VIVO 15 medium (CAMBREX) supplemented with 1% BSA (A8806, Sigma), 10ng/ml hIL-11, 50ng/ml mSCF and 10ng/ml mTPO (PeproTech)} for 12 hours. Cells were then mixed with 2 ml of viral supernatant containing polybrene (at a final concentration of 10 μg/ml) and subsequently spin-infected for 2h at 2000 rpm. After 24 hours, we evaluated transduction efficiency based on the GFP positivity by FACS (50-60% of the cells were GFP positive). Approximately, 5x10^4 GFP positive cells were injected retroorbitally into a sub-lethally irradiated *Rag1*$^{-/-}$ recipient mouse. For exogenous expression of LRF in HSCs, 10,000 HSCs from pooled WT mice BMMNCs were flow-sorted into individual wells of a U-bottom 96-well plate containing 100μl of HSC expansion medium and subsequently incubated for 12 hours. Cells were then mixed with 100μl of viral supernatant containing polybrene (at a final concentration of 10 μg/ml) and subsequently spin-infected for 2h at 2000 rpm. 24 hours after infection, cells were harvested, washed once with PBS and then seeded on OP-DL1 stromal cell layers with LE medium.

For inducible *LRF* inactivation in the BM-reconstituted recipient mice, either *Zbtb7a*$^{Flox/+\ Mx1cre+}$ or *Zbtb7a*$^{Flox/-\ Mx1cre+}$ donor BM cells (CD45.2^{+}) were obtained from two mice (for each genotype), pooled and, after red blood cell lysis, transplanted into lethally irradiated recipient mice (CD45.1^{+}). We injected 4x10^6 BMMNCs per recipient mouse via the retroorbital sinus. Engraftment of the donor-derived cells was confirmed by analyzing isotypes (CD45.2 positivity) of PB mononuclear cells on day21 post transplantation. We

subsequently treated recipient mice with pIpC as previously described (*S3*) and examined their BM 14 days after the last pIpC administration by FACS. For inducible *LRF* inactivation in the BM-reconstituted recipient athymic nude mice, *Zbtb7a*$^{Flox/-\ Mx1cre+}$ donor BM cells were obtained from two mice, pooled and, after red blood cell lysis, transplanted into lethally irradiated recipient NCr-*Foxn1*$^{nu/nu}$ mice (Taconic). Recipient mice were lethally irradiated (8.5 Gy, single dose) and subsequently transplanted with 4x10^6 BMMNCs per mouse.

***In vivo* GSI treatment**

GSI (MRK-003) was obtained from Merck & Co., Inc. MRK-003 was orally administered at a dose of 100 μg/g (in 0.5% methylcellulose), once daily, using a 3-Days ON, 4-Days OFF intermittent dose schedule. The first dose was given 4 days after the first pIpC injection and a total of 9 doses of MRK-003 were administered.

Immunohistochemical/fluorescent analysis

All tissues were fixed in phosphate-buffered 10% formalin solution (Fisher Diagnostic) and embedded in paraffin. Antibodies directed against CD3 (Dako) and Pax5 (Pharmingen) were used to identify T cells and B cells, respectively. Species-specific secondary antibodies were used for double staining (*S4*). Immunofluorescent analysis on BM sections was performed as previously described (*S5*) using antibodies against PU-1 (SC-5949, Santa Cruz Biotechnology) and CD3 (Dako).

Real time PCR assay

Cells were flow-sorted directly into 2ml Eppendorf tubes containing 1ml of TRIZOL (Invitrogen). RNA was extracted according to the manufacturer's specifications and

subsequently treated with DNase I (Invitrogen) to eliminate residual genomic DNA. cDNA was synthesized using the SuperScript™ III First-Strand Synthesis System (Invitrogen). Real-time PCR was performed using QuantiTect SYBR Green RT-PCR Kit (Qiagen) and a Light Cycler (Roche). We measured relative mRNA expression levels of the target gene and corresponding control Hprt gene in each sample. The target mRNA expression level was normalized by dividing its relative mRNA amount with the corresponding Hprt mRNA amount. q-PCR was performed in duplicate or triplicate and the normalized-standard deviation was calculated using the formula described below.

Normalized standard deviation

$$= (\text{MeanX} / \text{MeanH}) \times \sqrt{(\text{StdvX} / \text{MeanX})^2 + (\text{StdvH} / \text{MeanH})^2}$$

MeanX= Mean value of gene X (gene of interest)
StdvX=Standard deviation of gene X
MeanH= Mean value of Hprt
StdvH= Standard deviation of Hprt

All primer sequences, except for E2A (*S6*), LRF, pTCRα and Hprt, were obtained from the PrimerBank (http://pga.mgh.harvard.edu/primerbank/index.html) (*S7*).

Primer sequences and their PrimerBank IDs are as follows:

LRF FW-GAGAAGAAAATCCGGGCCAAG, LRF RV-GCAGCTATCGCACTGGTATGG

HPRT FW-CACAGGACTAGAACACCTGC, HPRT RV-GCTGGTGAAAAGGACCTCT

E2A_E12FW-GTGGCCGTCATCCTCAGC, E2A_E12 RV-GCTGCTTTGGGGTTCAGG

E2A_E47FW-GCCGAAGAGGACAAGAAGG, E2A_E47 RV-CTTCTCCTCCAGGGACAG

pTCRα FW-ACCATCAGGCATCGCTGGC, pTCRα RV-CGAGGACCAGGCAAACCACC

TdT FW-AGAGACCTTCGGCGCTATG (6678273a1)

TdT RV-TGACAGTCTTCCCCTTAGTCC

Rag1 FW-ACCCGATGAAATTCAACACCC (6679623a1)

Rag1RV-CTGGAACTACTGGAGACTGTTCT

Rag2 FW-AAGGCTGGCCTAAGAGATCCT (6677661a1)

Rag2 RV-GATAACGAAGAGGTGGGAGGTA

Ig_alpha FW-TCTTCTTGTCATACGCCTGTTTG (6680892a1)

Ig_alpha RV-GATGTTAGACTGAAGGCTGAACC

Ig_beta FW-CGAGGTTTGCAGCCAAAAAG (6680375a1)

Ig_beta RV-CACAATGCGTCCCTCTTCTG

VpreB1 FW-GCTGCTGGCCTATCTCACAG (8850238a1)

VpreB1 RV-CCAATGTTATGGTCGTTGCTCA

EBF1 FW-GCATCCAACGGAGTGGAAG (6681253a1)

EBF1 RV-GATTTCCGCAGGTTAGAAGGC

Pax5 FW-CCATCAGGACAGGACATGGAG (6679213a1)

Pax5 RV-GGCAAGTTCCACTATCCTTTGG

STAT5a FW-CGCCAGATGCAAGTGTTGTAT (6755672a1)

STAT5a RV-TCCTGGGGATTATCCAAGTCAAT

Notch1 FW- CCCTTGCTCTGCCTAACGC (31543332a1)

Notch1 RV- GGAGTCCTGGCATCGTTGG

Notch2 FW- ATGTGGACGAGTGTCTGTTGC (33859592a1)

Notch2 RV- GGAAGCATAGGCACAGTCATC

Notch3 FW- TGCCAGAGTTCAGTGGTGG (6679096a1)

Notch3 RV- CACAGGCAAATCGGCCATC

Nrarp FW- AAGCTGTTGGTCAAGTTCGGA (16751909a1)

Nrarp RV- CGCACACCGAGGTAGTTGG

Deltex FW- ATCAGTTCCGGCAAGACACAG (11611467a1)

Deltex RV- CGATGAGAGGTCGAGCCAC

Hes1 FW- ATAGCTCCCGGCATTCCAAG (6680205a2)

Hes1 RV- GCGCGGTATTTCCCCAACA

Gata3 FW- CTCGGCCATTCGTACATGGAA (6679951a1)

Gata3 RV- GGATACCTCTGCACCGTAGC

Western blot analysis

BMMNCs were isolated, stained with antibodies and subsequently flow-sorted into individual wells of a U-bottom 96-well plate containing 50μl of 2x protein sample buffer per well (15,000-30,000 cells were sorted onto one well). Protein lysate was then boiled for 10 min. The protein lysate from approximately 15,000 PreProB cells were used for one Western blot (WB). The following antibodies were used: anti-EBF1 (C-20, Santa Cruz Biotechnology), anti-STAT5 (C-17, Santa Cruz Biotechnology), anti-HSP90 (#610419, BD) and anti-LRF (13E9) (*S2*). Protein was visualized by SuperSignal western blotting kit (PIERCE). The intensity of the signal was measured by using ImageJ 1.34S software (http://rsb.info.nih.gov/ij/). Relative protein expression levels for Ebf-1 and Stat5 were normalized by signal intensity of the corresponding HSP90 protein.

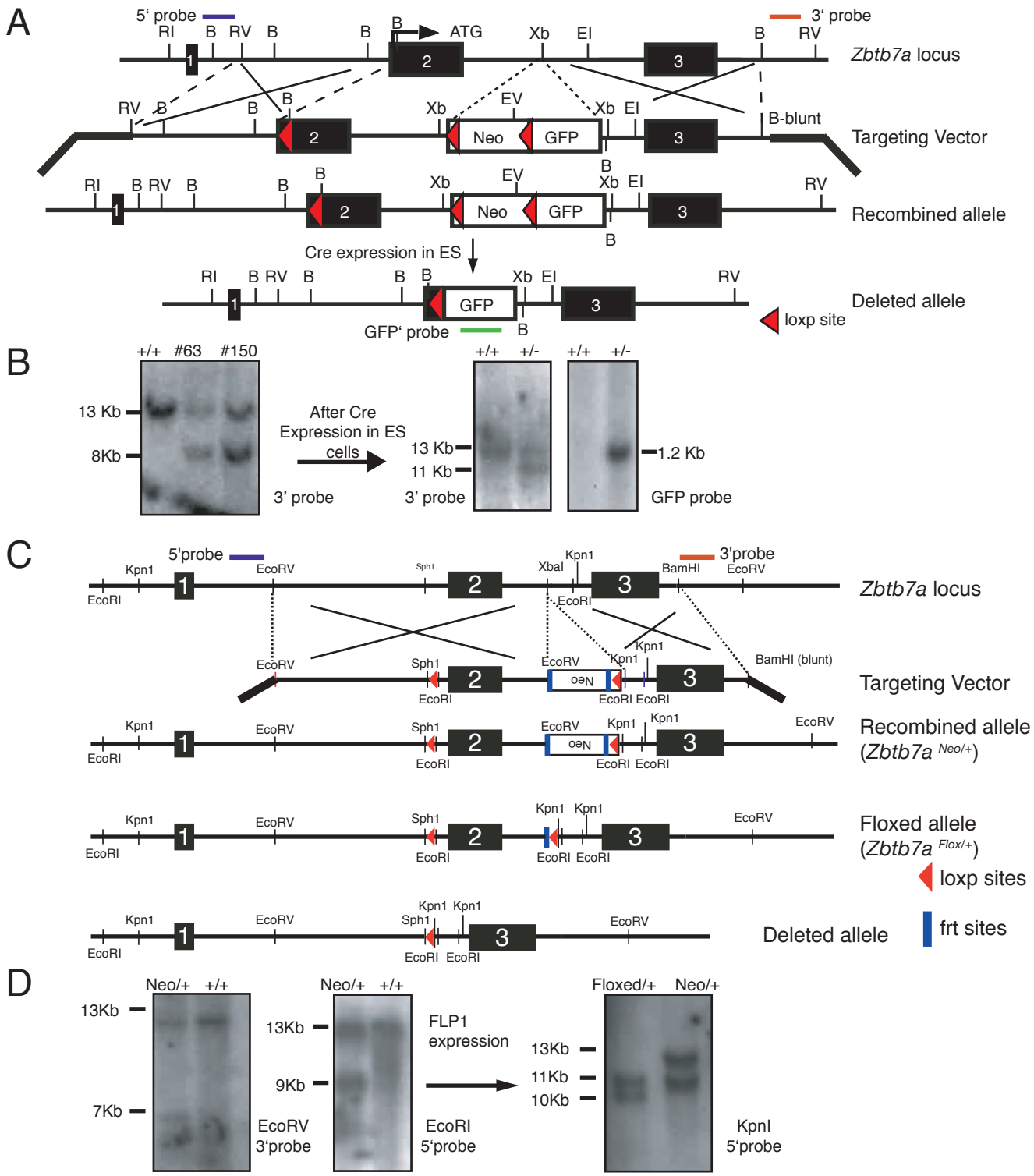

Fig. S1. (A) Schematic representations of the LRF knockout strategy. A loxp site was introduced into the *Bam*HI site of exon 2 and a loxp-neo-loxp GFP cassette was introduced into the *Xba* I site in the intron between exon 2 and 3. Both exon2 and the Neo cassette were excised upon transient Cre expression in ES cells. Although a loxp-GFP sequence was introduced into exon 2 in frame immediately following the translation start codon, we did not observe any GFP fluorescence possibly due to misfolding of the GFP protein *(S8)*. **(B)** Southern blot analysis of two independent recombined ES clones (left) and the ES clone after transient Cre expression (right). **(C)** Schematic representations of the conditional LRF knockout strategy. A loxp site was introduced into the *Sph*1 site in the intron between exon 1 and 2. An frt-Neo-frt-loxp cassette was positioned at the *Xba*1 site in the intron between exon 2 and 3. Deletion of the Neo cassette was performed in vivo by taking advantage of the FLP1-deleter mice. **(D)** Southern blot analysis of the correctly recombined ES clones (2 left panels). *Zbtb7a*$^{Neo/+}$ mice were subsequently crossed with the FLP1-deleter mice *(S9)* and resulting offspring were genotyped by Southern blot (right). Expected band sizes in the Southern blot are indicated.

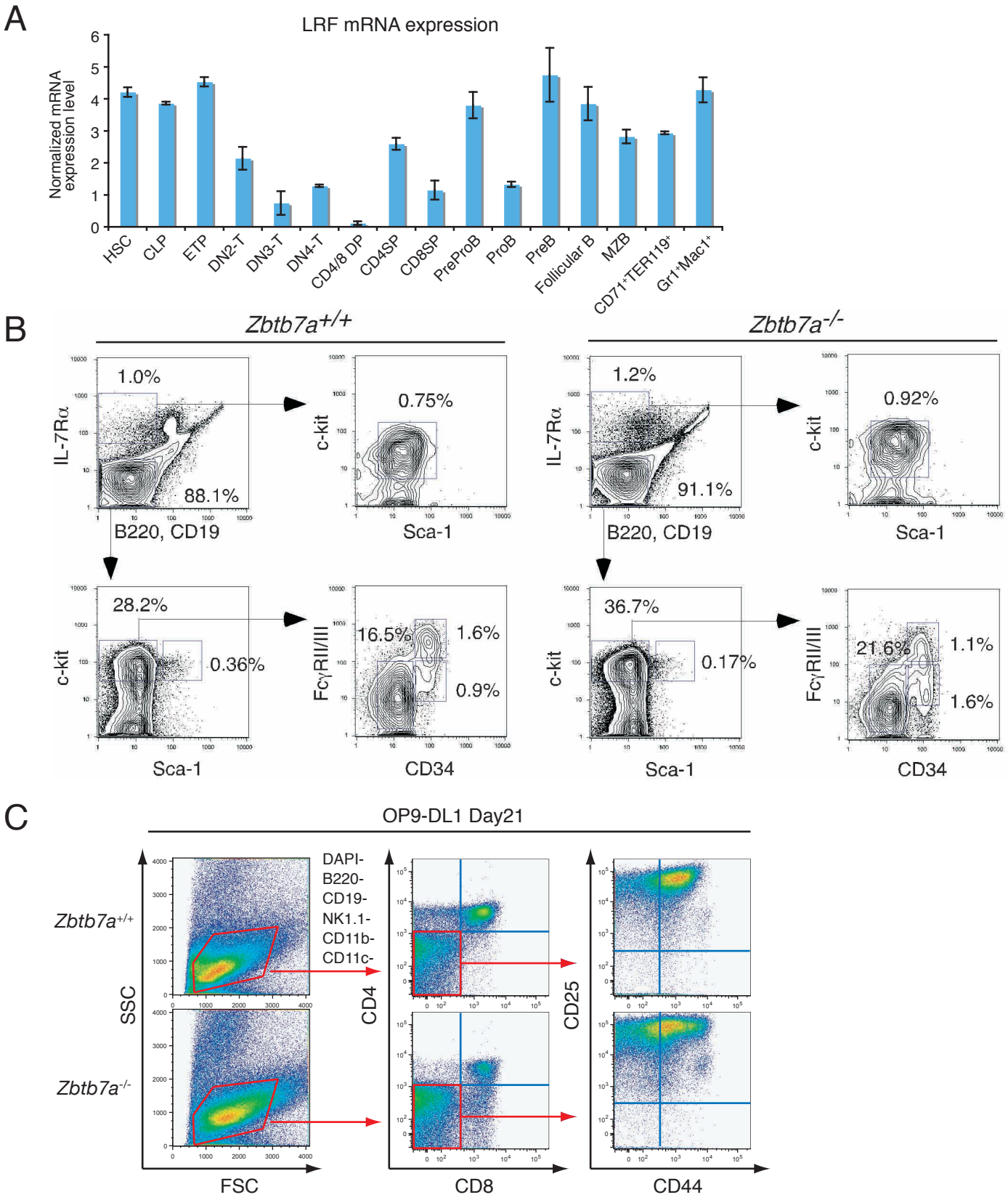

Fig. S2. (A) LRF mRNA expression in multiple hematopoietic lineage cells. Each compartment was flow-sorted and q-RT-PCR analysis was performed as described in Fig. 3A. **(B)** Representative FACS profiles for FL hematopoietic stem cell (FL-HSC) and progenitors in 14.5 d.p.c. embryos. Analysis was performed as previously described *(S10)*. **(C)** 14.5 d.p.c. FL-HSCs were flow-sorted and subsequently cultured on OP9-DL1 stromal cell layers in the presence of IL-7 and Flt3 ligand for 21 days. Propagated cells were analyzed by FACS using indicated antibodies plus DAPI.

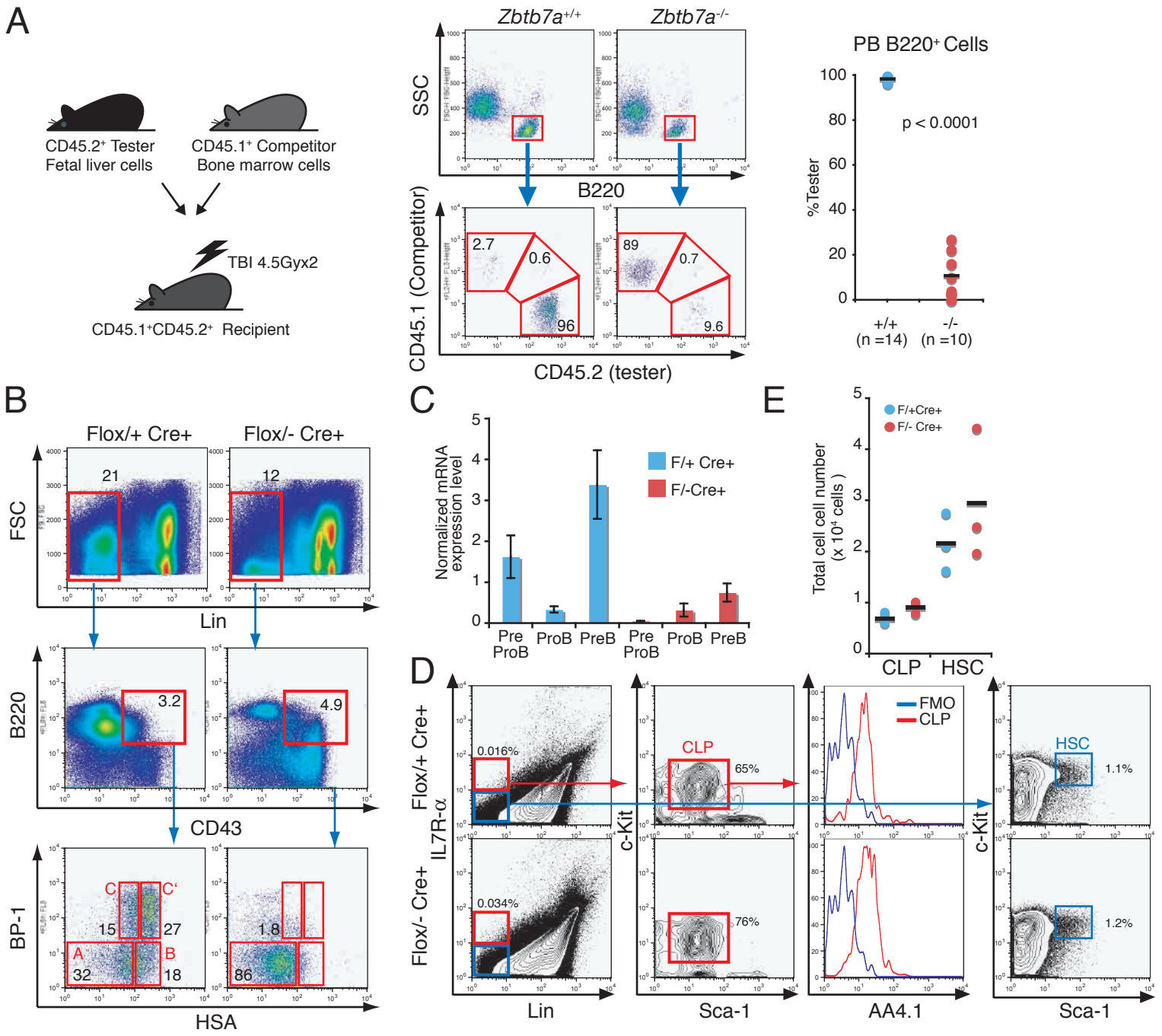

Fig. S3. (A) Schematic representations of the BM reconstitution assay (left) and representative FACS profiles of the PB B cell compartment two months after transplantation. *Zbtb7a*$^{+/+}$ or *Zbtb7a*$^{-/-}$ FL cells (1x10^5 Tester cells, CD45.2$^+$) were transplanted into lethally irradiated recipient mice (CD45.1$^+$CD45.2$^+$) together with WT BM cells (0.5 x10^5 cells, CD45.1$^+$). Isotypes of the repopulating B cells in the PB were analyzed by FACS two month after transplantation (middle). Dot graph demonstrates proportions of tester-derived B cells over total donor derived-B cells in the PB (right). **(B)** The BM B cell compartment was characterized by FACS according to Hardy's classification *(S11)* one month after the last pIpC injection. Lineage positive cells were labeled as described in Fig. 1D. **(C)** LRF mRNA expression levels in the early B cell compartment one month after the last pIpC injection. RNA was extracted from flow-sorted PreProB, ProB and PreB cells. After cDNA synthesis, q-RT-PCR for both LRF and Hprt was performed as described in Fig. 3A. The bar graph shows normalized amounts of LRF mRNA. **(D)** BMMNCs were collected from pIpC-treated animals (one month after the last pIpC injection) and subsequently stained with fluorochrome-conjugated anti-Sca-1, IL-7-Rα, c-Kit, AA4.1 and biotin-conjugated lineage markers (CD3, CD5, CD4, CD8, B220, CD19, IgM, Gr1, CD11b, TER119, NK1.1 and Ly6-C), followed by incubation with APC-Cy7-Streptavidin. Representative FACS profiles for HSCs (Lin$^-$, IL-7-Rα$^-$, Sca-1$^+$, c-Kit$^+$) and CLPs (Lin$^-$, IL-7-Rα$^+$, Sca-1^{low}, c-Kit^{low}, also positive for AA4.1) are demonstrated. *FMO (Fluorescence Minus One control). **(E)** Absolute numbers of HSCs and CLPs from each genotype (3 animals per each genotype) are shown in the dot graph.

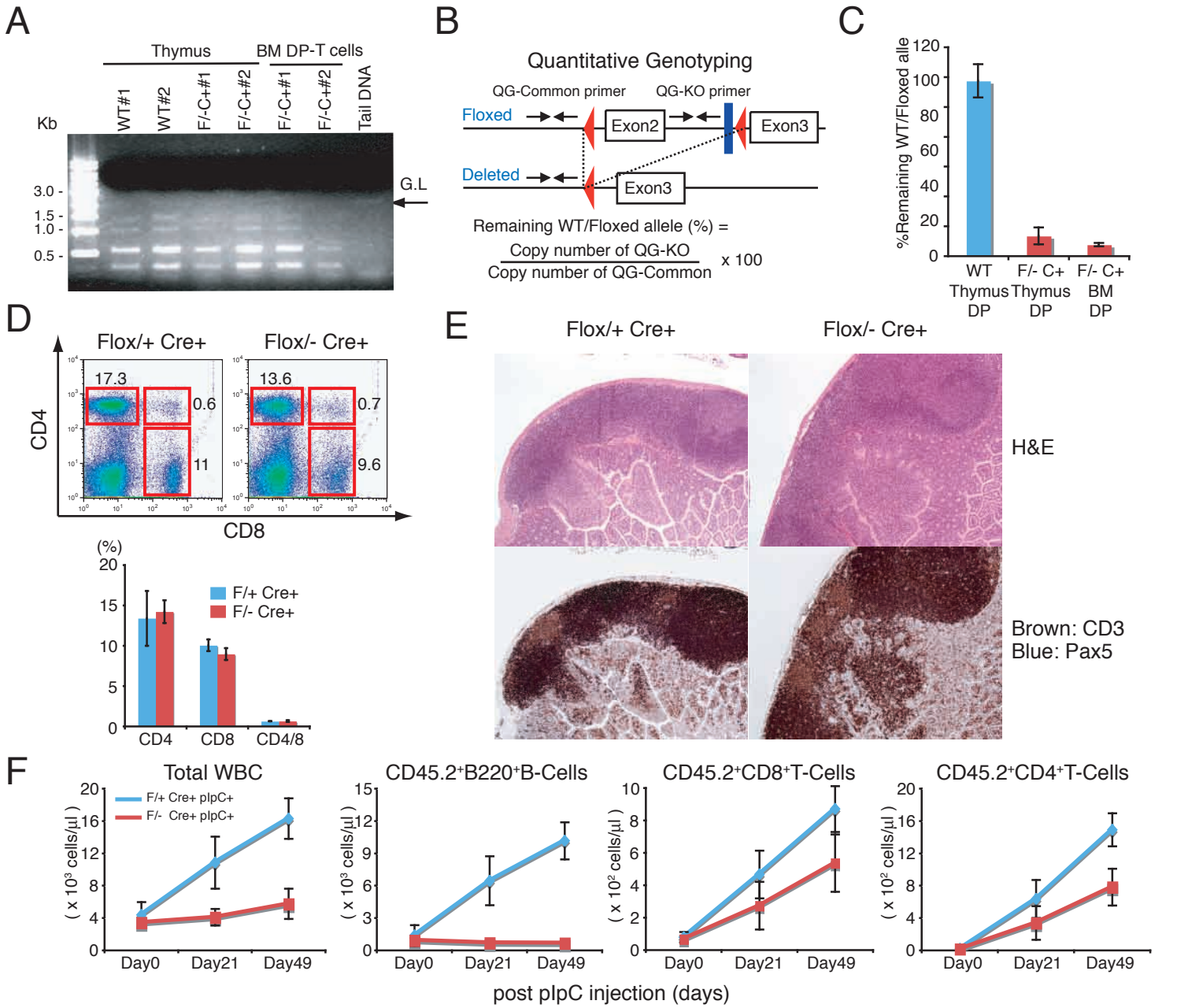

Fig. S4. (A) DNA was extracted from flow-sorted thymic and extrathymic BM DP-T cells one month after pIpC treatment. Image demonstrates analysis of Dβ1-to-Jβ1 rearrangement at the TCRβ locus by PCR, performed as previously described *(S2)*. Thymic and BM DP-T cells were analyzed together with tail DNA control. Arrow denotes the germline configuration. **(B)** Schematic representations of the primer design for quantitative measurement of Zbtb7a gene dosage. Two primer sets were used for PCR, QG-Common and QG-KO, as indicated. Upon PCR reaction, QG-Common primer set amplifies an intronic sequence, which remains intact regardless of recombination events, while the QG-KO primer set amplifies an intronic sequence that is deleted upon recombination. We monitored *Zbtb7a* gene status by measuring relative copy numbers of each amplicon with by the indicated formula. **(C)** We calculated percent remaining WT or Floxed allele of the *Zbtb7a* gene as described in (B). Bar graph demonstrates percent remaining WT or Floxed allele in thymic and extrathymic BM DP-T cells in WT and *Zbtb7a*$^{Flox/-}$ *Mx1cre*$^{+}$ mice one month after the last pIpC injection. **(D)** Representative FACS profiles for splenic T cell compartments one month after pIpC treatment (top). Average percent positivity of three mice for each genotype is demonstrated with a bar graph (bottom). **(E)** Immunohistochemical analysis on sections from the Peyer's patches collected one month after pIpC treatment. Representative images of H&E staining (top) and CD3/Pax5 double staining are presented. **(F)** Time-course follow-up of recipients' PB counts after pIpC injections. Each group consists of 6 animals. Blue and red lines demonstrate PB cell counts of recipient mice, which were transplanted with *Zbtb7a*$^{Flox/+}$ *Mx1cre*$^{+}$ or *Zbtb7a*$^{Flox/-}$ *Mx1cre*$^{+}$ BMMNCs, respectively. We measured PB WBC counts with a hematology analyzer and subsequently calculated total numbers of B and T cells as described in Fig. 1C. The average cell count of 6 animals was plotted for each time point with standard deviations.

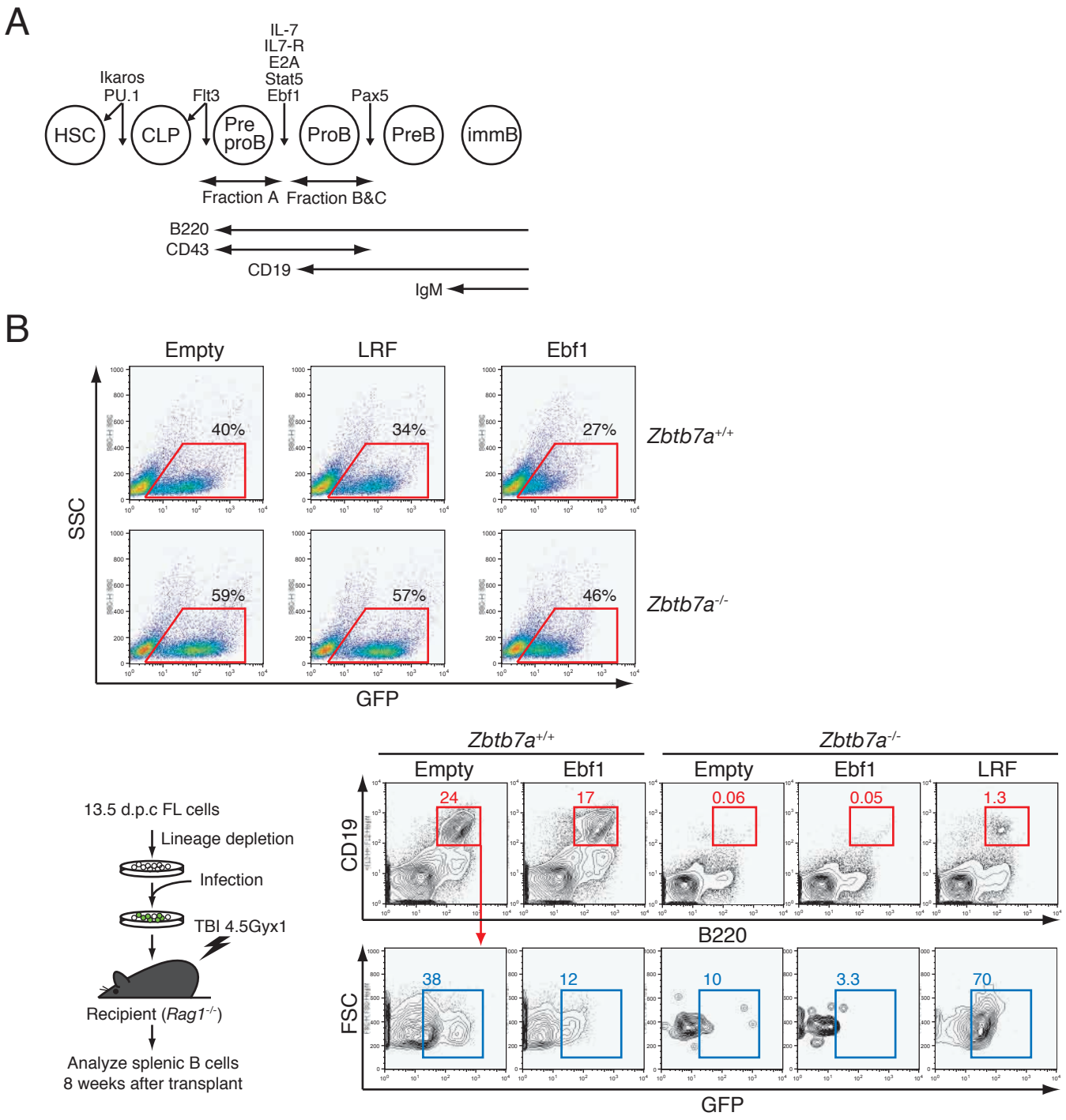

Fig. S5. (A) Schematic representation of early B cell development with critical genes involved in this process indicated. Surface marker expression in the early B cell compartment (plus Hardy's classification) is also demonstrated. **(B)** Lineage depleted 13.5 d.p.c. FL cells (*Zbtb7a*$^{+/+}$ *or Zbtb7a*$^{-/-}$) were transduced with retroviruses encoding either GFP only, EBF1-IRES-GFP or LRF-IRES-GFP and were subsequently transplanted into sub-lethally irradiated *Rag1*$^{-/-}$ recipient mice. Infection efficiency (GFP positivity) was examined by FACS before transplantation (top). Schematic representations of lymphoid lineage reconstitution experiment (bottom left). Recipient mice were sacrificed 8 weeks after transplant and analyzed for splenic B cell fractions by FACS. Representative FACS profiles are presented. Percentage positivity of B220^{+}CD19^{+} B cells in the spleens is indicated in red and percentage positivity for GFP in the splenic B cell population is depicted in blue (bottom right).

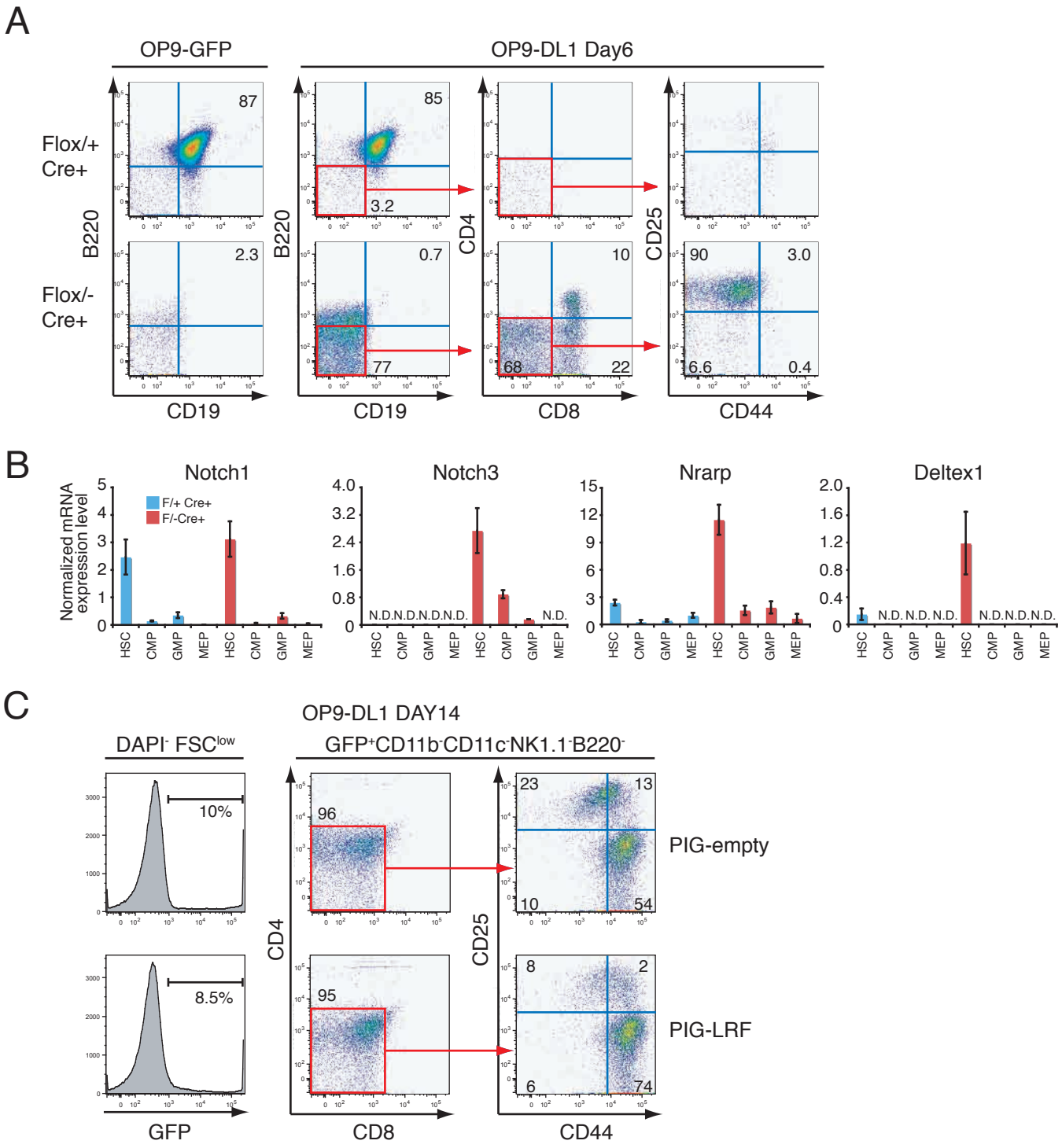

Fig. S6. (A) PreProB cells of the pIpC-treated mice were flow-sorted and subsequently cultured on either OP9-GFP control cells or OP9-DL1 cells with cytokines (IL-7 and Flt3 ligand). Representative FACS profiles of the propagated cells upon 6 days of culture are shown. **(B)** Notch signature in Myeloid progenitor compartments. HSC, CMP (Common Myeloid Progenitor), GMP (Granulocyte/Macrophage lineage-restricted Progenitors) and MEP (Megakaryocyte/Erythrocyte lineage-restricted Progenitors) compartments were flow-sorted from pIpC-treated animals one month after pIpC treatment. RNA was extracted and cDNA synthesis was subsequently performed. q-RT-PCR analysis was performed as described in Fig. 3A. **(C)** Flow-sorted HSCs were retrovirally infected with either GFP-empty vector (PIG-empty) or LRF-IRES-GFP vector (PIG-LRF) and cultured on OP9-DL1 cells for 14 days in the presence of IL-7 and Flt3 ligand. GFP positive fractions of propagated cells were further analyzed for the indicated surface markers.

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