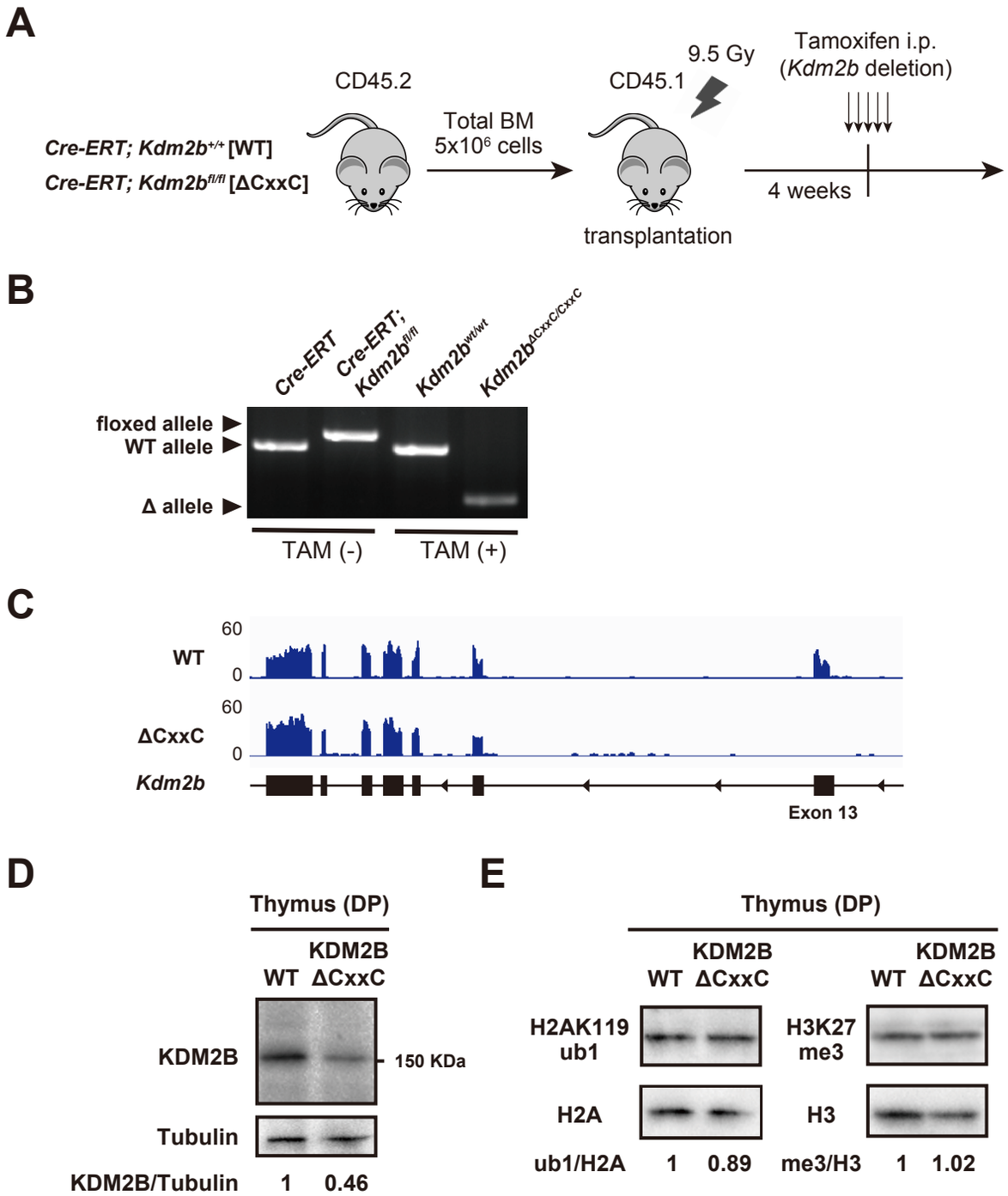

Figure S1. Generation of ΔCxxC mice lacking the ZF-CxxC domain.
(A) Experimental design of the hematopoietic-specific deletion of *Kdm2b* exon 13. (B) Complete deletion of *Kdm2b* exon 13 in hematopoietic cells detected by genomic PCR. Deletion of *Kdm2b* exon 13 in PB CD45.2^{+} hematopoietic cells from recipient mice repopulated with *Kdm2b*$^{fl/fl}$*;Cre-ERT* BM cells before and 8 weeks after the tamoxifen treatment. Floxed, the floxed *Kdm2b* allele; Δ, the floxed *Kdm2b* allele after the removal of exon 13 by Cre recombinase. (C) Snapshots of RNA-seq signals at the *Kdm2b* gene locus in LSK cells isolated from recipient mice repopulated with WT and ΔCxxC hematopoietic cells. The structure of the *Kdm2b* gene locus including relevant exons is indicated at the bottom. (D) The KDM2BΔCxxC protein in the thymus of ΔCxxC mice detected by a Western blot analysis. Total thymic cells were collected 8 weeks after the tamoxifen treatment. (E) Global levels of H2AK119ub1 and H3K27me3 in WT and ΔCxxC DP thymocytes. Cells were collected by cell sorting 8 weeks after the tamoxifen treatment. H2AK119ub1 and H3K27me3 levels were normalized to the amount of H2A and H3, respectively, and are indicated at the bottom.

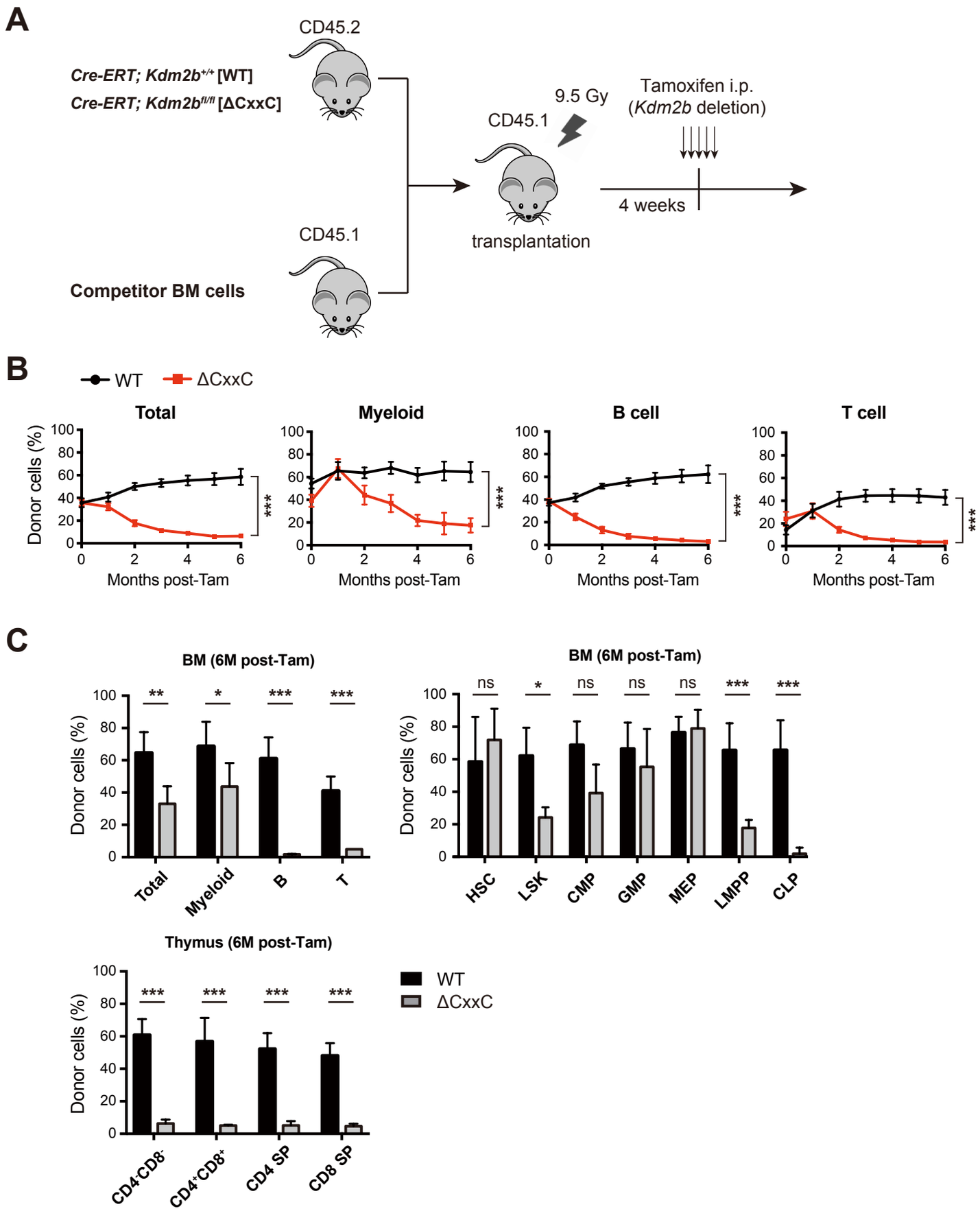

Figure S2. Deletion of KDM2B ZF-CxxC impaired the repopulating capacity of HSCs.
(A) Experimental scheme of competitive repopulation assays. (B) Contribution of donor cells to PB hematopoiesis. The chimerism levels of total CD45.2 hematopoietic cells and myeloid (Gr-1$^+$ and/or Mac-1$^+$) and lymphoid (B220$^+$; CD4$^+$ or CD8$^+$) cells in PB are shown as means ±SD (n=5). (C) BM and thymus analysis 4 months after the injection of tamoxifen. The chimerism levels of CD45.2 donor cells in total BM hematopoietic cells, myeloid cells, lymphoid cells, HSCs, LSK cells, and myeloid and lymphoid progenitors in BM and in thymocytes are shown as means ± SD (WT, n=5; ΔCxxC, n=5). *, P < 0.05; **, P < 0.01; ***, P < 0.001 by the Student' s t-test; n.s., not significant.

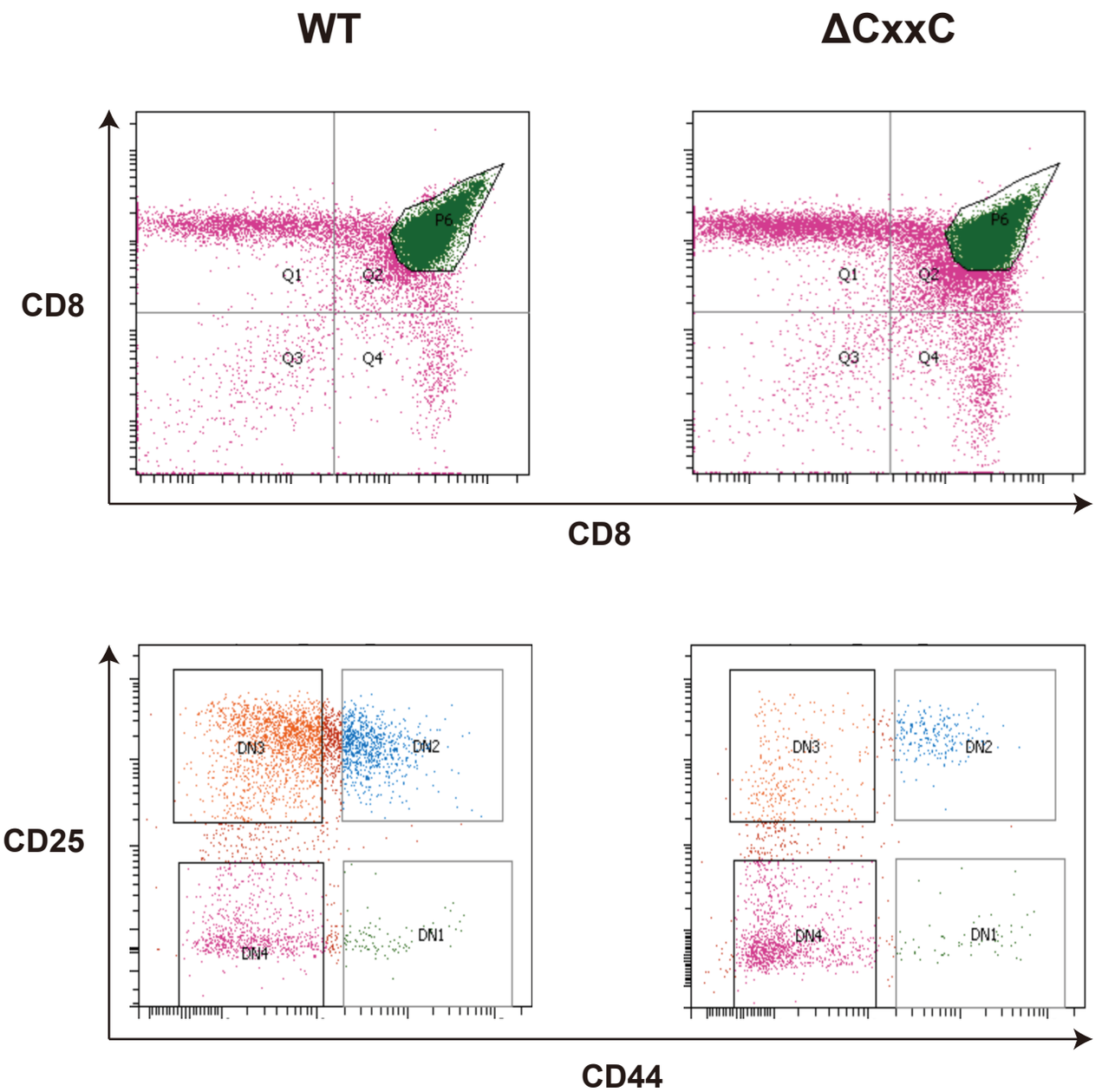

Figure S3. Representative flow cytometric profiles of WT and ΔCxxC DN and DP thymocytes. Sorting gates for RNS-sequencing are depicted.

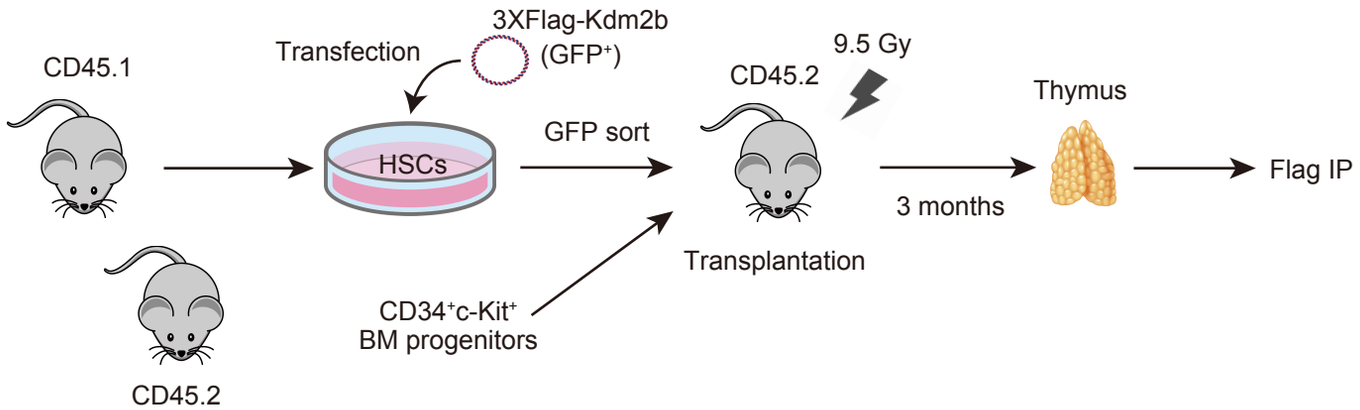

Figure S4. Strategy for generating hematopoietic chimera expressing 3xFlag-KDM2B.
$CD150^{+}CD34^{-}$LSK HSCs (CD45.1) were transduced with a 3xFlag-KDM2B virus. Transduced cells were sorted using GFP as a marker antigen, then transplanted into lethally irradiated mice (CD45.2) with $CD34^{+}$c-Kit^{+} rescue cells (CD45.2). Total thymocytes were harvested 3 months after transplantation and were subjected to a ChIP analysis using an anti-Flag antibody.

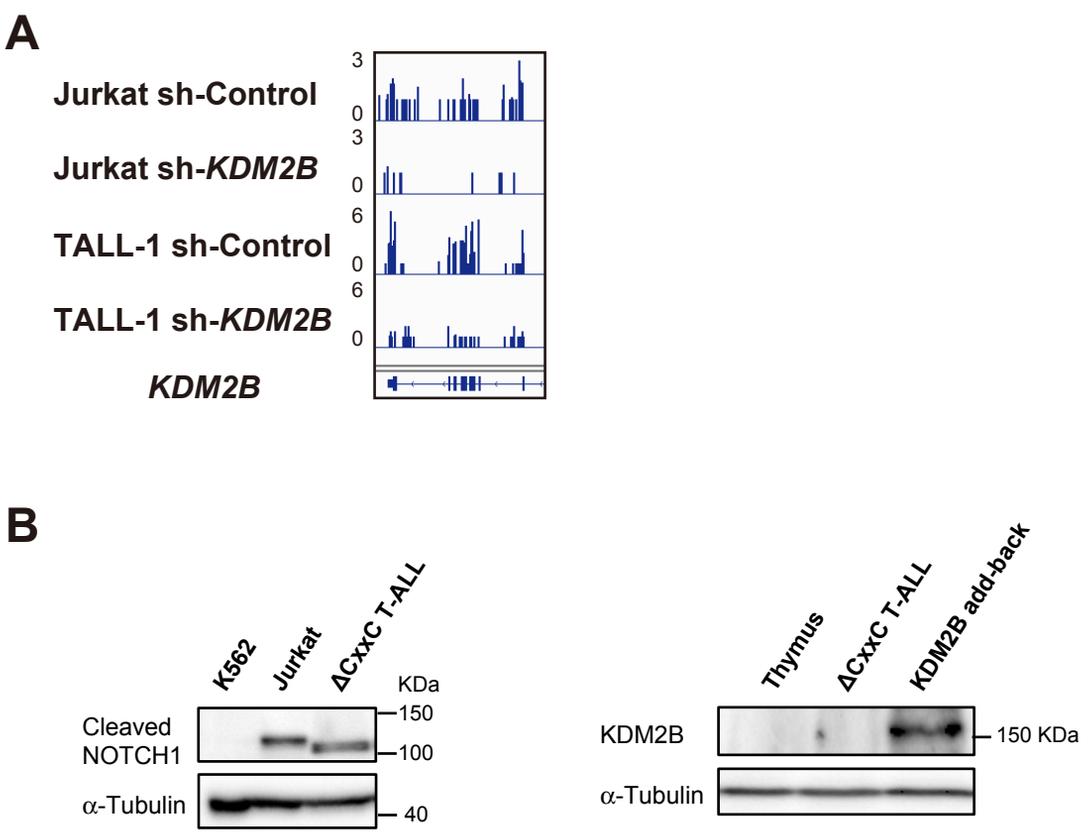

Figure S5. Validation of the role of KDM2B in the maintenance of T-ALL.
(A) Snapshots of RNA-seq signals at the *KDM2B* gene locus in human T-ALL cell lines stably transduced with control and *KDM2B* shRNA. The structure of the *KDM2B* gene locus including relevant exons is indicated at the bottom.
(B) Wstern blot analyses detecting active NOTCH1 (left panel) and KDM2B (right panel) in mouse ΔCxxC T-ALL cells.

Supplemental materials and methods

Flow cytometry and antibodies

We used monoclonal antibodies recognizing the following antigens in flow cytometric analyses and cell sorting: CD3e (145-2C11), CD4 (L3T4), CD8 (53-6.7), CD11b/Mac-1 (M1/70), CD11c (N418), CD16/32/FcγRII-III (93), CD25 (3C7), CD34 (RAM34), CD44 (IM7), CD45.1 (A20), CD45.2 (104), CD45R/B220 (RA3-6B2), CD48 (HM48-1), CD117/c-Kit (2B8), CD127/IL-7R (A7R34), CD135/FLT3 (A2F10), CD150 (TC15-12F12.2), Gr-1 (RB6-8C5), NK1.1(PK136), Sca-1 (D7), TCR γ/δ (GL3) and Ter-119. These antibodies were purchased from BD, BioLegend, and TOMBO. Dead cells were removed by staining with 0.5 μg/ml propidium iodide (Sigma-Aldrich). All flow cytometric analyses and cell sorting were performed on FACS Aria III or FACS Canto II (BD).

Quantitative RT-PCR

Total RNA was extracted using an RNeasy Mini kit (Qiagen) and reverse-transcribed by the ThermoScript RT-PCR system (Invitrogen) with an oligo-dT primer. Real-time quantitative PCR was performed with a StepOnePlus Real-Time PCR System (Life Technologies) using FastStart Universal Probe Master (Roche Applied Science) and the indicated combinations of the Universal Probe Library (Roche Applied Science). All data are presented as relative expression levels normalized to *Gapdh* expression. The primer sequences used were as follows: *Myc* forward 5′-CCTAGTGCTGCATGAGGAGA-3′, reverse 5′-TCCACAGACACCACATCAATTT-3′, *Gapdh* forward 5′-CTGACTTCAACAGCGACACC-3′, reverse 5′-TAGCCAAATTCGTTGTCATACC-3′.

RNA sequencing

An RNeasy Plus Micro Kit (Qiagen) was used to extract total RNA from samples and cDNA was synthesized using a SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech). S220 Focused-ultrasonicator 386 (Covaris) was used for the fragmentation of cDNA and cDNA libraries were then generated using a NEBNext Ultra DNA Library Prep Kit (New England BioLabs). Sequencing was performed using HiSeq1500 (Illumina) with a single-read sequencing length of 60 bp. TopHat (version 2.0.13; with default parameters) was used to map reads to the reference genome (UCSC/mm10) with annotation data from iGenomes (Illumina). Gene expression levels were quantified using Cuffdiff (Cufflinks version 2.2.1 with default parameters).

ChIP assays and ChIP-seq

In ChIP assays of FLAG-tagged KDM2B and EZH2, total thymocytes were fixed with 1% PFA at 25°C for 10 min, lysed in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, pH 8.0, 1% NP-40 substitute, 0.5% sodium deoxycholate, and 0.1% SDS) and sonicated by a homogenizer (NR-50M; Micro-tec Co.). After centrifugation, supernatants were subjected to immunoprecipitation using an anti-FLAG antibody (F1084; Sigma) and anti-EZH2 antibody (C15410039; Diagenode). Sheep anti-mouse IgG Dynabeads and sheep anti-rabbit IgG Dynabeads were used to capture the antibodies. In ChIP assays of human KDM2B, human T-ALL cell lines were fixed, lysed and sonicated with the same procedure. Immunoprecipitation was performed using an anti-KDM2B (09-864; Millipore). Sheep anti-rabbit IgG Dynabeads were used to capture the antibody. In ChIP assays of H2AK119ub1 and H3K27me3, DP thymocytes were fixed

with 0.5% PFA at 37°C for 2 min, lysed in ChIP buffer (10 mM Tris-HCl, pH 8.0, 200 mM NaCl, 1 mM CaCl2 and 0.5% NP-40 substitute), sonicated by a bioruptor (UCD-300; Cosmo Bio), and digested with micrococcal nuclease (M0247S; New England Biolabs). After centrifugation, supernatants were subjected to immunoprecipitation using an anti-H2AK119ub1 antibody (8240S; Cell Signaling Technology) or anti-H3K27me3 antibody (07-449; Millipore). Sheep anti-rabbit IgG Dynabeads were used to capture the antibodies. ChIP-seq libraries were prepared using a ThruPLEX DNA-seq kit (Rubicon Genomics). Sequencing was performed using HiSeq1500 (Illumina) with a single-read sequencing length of 60 bp. Bowtie2 (version 2.2.6; default parameters) was used to map the reads to the reference genome (UCSC/mm10). Peaks were called using MACS2 v2.1.1 with a q-value of <0.05 for KDM2B and EZH2, <0.2 for BCOR and $<10^{-10}$ for NOTCH1. ChIP peaks that overlapped with those of a corresponding input (distance between centers <10 kb) were removed. Reads per million mapped reads (RPM) values of the sequenced reads were calculated every 1,000 bp bin, with a shifting size of 100 bp using Bedtools. To visualize with Integrative GenomicsViewer (Broad Institute), the RPM values of the immunoprecipitated samples were normalized by subtracting the RPM values of the input samples in each bin and converted to a Bigwig file using the Wigtobigwig tool. In order to evaluate the mark of each gene, the RPM values of the region from 2 kb upstream to 2 kb downstream of the TSS of immunoprecipitated samples were divided by the RPM of the corresponding input.

Quantitative PCR was performed with an ABI StepOnePlus thermal cycler, SYBR Premix Ex Taq II (Takara Bio) and the following primers: -3033 from *Myc* TSS forward, 5′-TCTCCCTCCCCT TTTTCAGT-3′, reverse 5′-TGGCGTGTCATGAAACAGAT-3′; -296 from *Myc* TSS forward, 5′-CAGGGCAAGAACACA GTTCA-3′, reverse 5′-

GCTCCGGGGTGTAAACAGTA- 3′; and +558 from *Myc* TSS forward, 5′-GAGCTCCTCGAGCTG TTTG-3′, reverse 5′-ACACAGGGAAAGACCACCAG-3′.

Retroviral vector and virus production

A retroviral vector and virus were made using the same method as described previously[14]. In ChIP assays, *Kdm2b* cDNA tagged with a 3xFlag in the retroviral vector pGCDNsam was used. A recombinant vesicular stomatitis virus glycoprotein-pseudotyped high titer retrovirus was generated using a 293 gpg packaging cell line. The virus in supernatants of 293 gpg cells was concentrated by centrifugation at 6,000×*g* for 16 hours. In *KDM2B* knockdown experiments, lentiviral vectors against human *KDM2B* (sh-*KDM2B*) and control sh-SCR were used as previously described.[33] Short hairpin sequences used in this study are sh-SCR, CAACAAGATGAAGAGCACCAA and sh-KDM2B(#1), GGAAGTTGAGAGTCTGCTTTG.

Transduction into HSCs and human T-ALL cell lines

$CD150^{+}CD34^{-}$LSK HSCs were sorted into 96-well microtiter plates coated with the recombinant human fibronectin fragment CH-296 (RetroNectin; Takara Shuzo) at 400 cells per well and incubated in S-Clone SF-O3 (Sanko Junyaku) supplemented with 1% fetal bovine serum, 1% L-glutamine, penicillin, streptomycin solution (GPS; Sigma), 50 mM 2-mercaptoethanol, 100 ng/mL mouse stem cell factor (SCF; PeproTech), and 100 ng/mL human thrombopoietin (TPO; PeproTech) for 24 hours. Cells were subsequently transduced with the indicated retrovirus at a multiplicity of infection of 750 in the presence of 1 μg/mL RetroNectin and 10 μg/mL protamine sulfate (Sigma) for 24 hours. After transduction, the cells were further incubated in the same medium and supplements

with 50 ng/mL SCF and 50 ng/mL TPO for 48 hours. Cells were then analyzed on FACS Aria III and GFP-positive cells were sorted for the transplantation assay. In human *KDM2B* knockdown experiments, human T-ALL cell lines (Jurkat and TALL-1) were incubated in RPMI 1640 (Gibco) supplemented with 15% fetal bovine serum and 1% L-glutamine, penicillin, streptomycin solution (GPS; Sigma). Cells were transduced with the indicated lentivirus in the presence of 10 ng/mL protamine sulfate (Sigma) for 48 hours. After transduction, the cells were further incubated in the same medium for 48 hours. The cells were then analyzed on FACS Aria III and mCherry-positive cells were sorted for growth assays and RNA extraction.

Generation of a *Kdm2b*-deficient T-ALL cell line and KDM2B add-back experiment

$CD4^+8^+$ T-ALL cells were isolated from the thymus of ΔCxxC T-ALL mice on FACS Aria III and cultured in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum and 1% L-glutamine, penicillin, streptomycin solution (GPS; Sigma). For the KDM2B add-back experiment, the cells were transduced with a 3xFLAG-tagged *Kdm2b* retrovirus in the presence of 10 ng/mL protamine sulfate (Sigma) for 24 hours. After transduction, the cells were further incubated in the same medium and supplements for 48 hours. Cells were then analyzed on FACS Aria III and GFP-positive cells were sorted for the growth and transplantation assays.

Western blot analysis

To detect non-histone proteins, lysates were prepared as follows to minimize the fragmentation of proteins by sonication. Cells were lysed in 0.1% NP-40 lysis buffer (300 mM NaCl) and centrifuged. The supernatants were kept on ice (solution A). Pellets were

resuspended in SDS sample buffer and sonicated using a Bioruptor (Cosmo Bio; solution B). Mixtures of solutions A and B were incubated at 95°C for 10 min. To detect histone proteins, cells were lysed in 2×SDS sample buffer, sonicated, and incubated at 95°C for 10 min. Proteins were separated by SDS-PAGE, transferred to a PVDF membrane, and detected by Western blotting using the following antibodies: anti-cleaved NOTCH1 (4147; Cell Signaling Technology), anti-KDM2B (ab137547; Abcam), anti-tubulin (MCA77G, Bio-Rad), anti-H2AK119ub (8240S; Cell Signaling Technology), anti-histone H2A (ab18255; Abcam), anti-H3K27me3 (07-449; Millipore), and anti-H3 (ab1791; Abcam). Protein bands were detected with enhanced chemiluminescence reagent (Immobilon Western; Millipore). The sequential reprobing of membranes with antibodies was performed after the removal of primary and secondary antibodies from membranes in 0.2 M glycine-HCl buffer (pH 2.5) and/or the inactivation of HRP by 0.1% NaN_3.