## S-Table 1. qPCR primers

| Gene  | Forward | Reverse | Forward | Reverse |
|-------|---------|---------|---------|---------|
| Gata-2 | 5'-CGTCTCTGTGATGTAAGGC-3'; | 5'-GTCTGACCTTCCAGCTGATGTAAGGC-3'; | 5'-CTCCCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Smad4  | 5'-ACAAGTCAATGCTGCAAGTAGTAATG-3'; | 5'-GGTCTGACCTTCCAGCTGATGTAAGGC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| p16   | 5'-GAAGTCAATGCTGCAAGTAGTAATG-3'; | 5'-GCTCTGACCTTCCAGCTGATGTAAGGC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Ezh2  | 5'-TTGTTGGCAGAAAGCTGTAATGTAAGGC-3'; | 5'-TCCCTGACCTTCCAGCTGATGTAAGGC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| cd34  | 5'-AAACTAACAACACAACTTACCTCTGGGAA-3'; | 5'-AGTCAATGCTGCAAGTAGTAATG-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| c-Kit | 5'-ATTTCTTGTCTTCTCGTACT-3'; | 5'-GCCCAAGCCTATTAATGCTG-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Foxo3a| 5'-TGCTTGCCAGGGGTAATGCTG-3'; | 5'-CTGCTTCGCTGAGGCTG-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Angpt1| 5'-CTCCCCTCAGGAAATAGCTG-3'; | 5'-AGCCCGACATGCTG-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| p53   | 5'-CTGCTTGCCAGGGGTAATGCTG-3'; | 5'-CTGCTTCGCTGAGGCTG-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Runx1 | 5'-CTGCTTGCCAGGGGTAATGCTG-3'; | 5'-CTGCTTCGCTGAGGCTG-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Notch | 5'-CTCCCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Bm1-1 | 5'-TAAGGATTTTGCTATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Tie2  | 5'-CTCGTGAAGGGCCAAGAGTCAA-3'; | 5'-TGGTAGGAAAGGACTGTTGAC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Oct4  | 5'-TGCCTGAGGAAAGGACTGTTGAC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Nanog | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Klf4  | 5'-CTCGGAAACCGAAGGACTGTTGAC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; |
| Rps18 | 5'-CTGCTTGCCAGGGGTAATGCTG-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; |
| c-Myc | 5'-GAGGCTTTTGAAGGACTGTTGAC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; |
| Hoxb4 | 5'-CCGCTTTTGAAGGACTGTTGAC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; |
| Cxcr4 | 5'-CGACGACGGCGAAGGACTGTTGAC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| Gapdh | 5'-CTGCTTGCCAGGGGTAATGCTG-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
| CD166 | 5'-TCTCGGAGGTTAAGGACTGTTGAC-3'; | 5'-CTTCTCTCAGGAAATATGTAATG-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; | 5'-ATTCTGTGAGGCTCTGAGT-3'; |
S-Figure 2

A. Bone Marrow Cells
   Mx1 Neg × Mx1 Pos
   All Cells, Ungated
   YFP Selection
   FSC
   YFP
   FSC
   100% × 100%
   0.06% × 99.9%
   75.0% × 41.9%
   0.09% × 97.2%
   61.1% × 17.1%

B. Spleen Cells
   Mx1 Neg × Mx1 Pos
   All Cells
   YFP Selection
   FSC
   YFP
   FSC
   100% × 100%
   1.70% × 88.1%
   8.1% × 11.7%
   1.97% × 82.3%
   98.2% × 17.6%

C. Bone Marrow Cells
   Vav Neg × Vav Pos
   All Cells, Ungated
   YFP Selection
   FSC
   YFP
   FSC
   100% × 100%
   1.67% × 88.1%
   8.1% × 11.7%
   1.97% × 82.3%
   98.2% × 17.6%

D. Spleen Cells
   Vav Neg × Vav Pos
   All Cells
   YFP Selection
   FSC
   YFP
   FSC
   100% × 100%
   1.67% × 88.1%
   8.1% × 11.7%
   1.97% × 82.3%
   98.2% × 17.6%

E. MX1 CRE
   MX1 Cre
   Controls
   Samples

F. VAV CRE
   VAV Cre
   Controls
   Samples
| Tissue | Population                                      | Population Abbr | Marker                                      |
|--------|------------------------------------------------|-----------------|---------------------------------------------|
| Bone marrow | Hematopoietic stem cell | HSC             | LinSc1:\textsuperscript{1} ckit:\textsuperscript{1} Flt3\textsuperscript{1} |
|        | Multipotent progenitor                     | MPP             | LinSc1:\textsuperscript{1} ckit:\textsuperscript{1} Flt3\textsuperscript{1} |
|        | Lymphoid primed multipotent progenitor      | LMPP            | LinSc1:\textsuperscript{1} ckit:\textsuperscript{1} Flt3\textsuperscript{1} |
|        | Common lymphoid progenitor                  | CLP             | LinFlt3:IL-7:Sc1:\textsuperscript{1+}+\textsuperscript{1+} |
|        | Common myeloid progenitor                   | CMP             | LinSc1:\textsuperscript{1} ckit:\textsuperscript{1} FcyR\textsuperscript{1+}CD34\textsuperscript{1+} |
|        | Granulocyte/macrophage progenitor           | GMP             | LinSc1+ ckit:\textsuperscript{1} FcyR\textsuperscript{1+}CD34\textsuperscript{1+} |
|        | Megakaryocyte/erythroid progenitor          | MEP             | LinSc1+ ckit:\textsuperscript{1} FcyR\textsuperscript{1+}CD34\textsuperscript{1+} |
|        | Progenitor B cell                           | Pro-B           | B220:\textsuperscript{CD19}CD43:\textsuperscript{1} ckit:\textsuperscript{1} IgM\textsuperscript{1} |
|        | Pre B cell                                  | Pre-B           | B220:\textsuperscript{CD19}CD43:\textsuperscript{1}IgM\textsuperscript{1} |
|        | Large Pre-B                                 | Large Pre-B     | B220:\textsuperscript{CD19}CD43:\textsuperscript{1}IgM\textsuperscript{1}BP1\textsuperscript{1+} |
|        | Small Pre-B                                 | Small Pre-B     | B220:\textsuperscript{CD19}CD43:\textsuperscript{1}IgM\textsuperscript{1}CD2\textsuperscript{1} |
|        | Immature B cell                             | Imm B           | B220:\textsuperscript{CD19}CD43:\textsuperscript{1}IgM\textsuperscript{1}IgD\textsuperscript{1} |
|        | Mature B cell or Recirculating B cell        | Mat B or Recirc| B220:\textsuperscript{CD19}IgM\textsuperscript{1+}IgD\textsuperscript{1+} |
|        | Macrophage                                  | Macro           | Mac1:Gr1:\textsuperscript{1+}CD11\textsuperscript{1} |
|        | Granulocytes                                | Gran            | Mac1:Gr1:\textsuperscript{1+}CD11\textsuperscript{1} |
|        | Plasmacytoid dendritic cell                 | pDC             | CD11b:CD11c:\textsuperscript{1+}B220:PDCA1\textsuperscript{1} |
| Thymus | Double negative                             | DN              | CD4:\textsuperscript{CD8}                  |
|        | Double positive                             | DP              | CD4:\textsuperscript{CD8}                  |
|        | Helper T cells                              | CD4+            | CD4:\textsuperscript{CD8}                  |
|        | Cytotoxic T cells                           | CD8+            | CD4:\textsuperscript{CD8}                  |
| Spleen | Immature B cell                             | Imm B           | B220:\textsuperscript{CD19}IgM\textsuperscript{1+}IgD\textsuperscript{1+} |
|        | Transitional B cell 1                       | Trans B 1       | B220:\textsuperscript{CD19}IgM\textsuperscript{1+}IgD\textsuperscript{1+}CD21\textsuperscript{1+}CD23\textsuperscript{1+} |
|        | Transitional B cell 2                       | Trans B 2       | B220:\textsuperscript{CD19}IgM\textsuperscript{1+}IgD\textsuperscript{1+}CD21\textsuperscript{1+}CD23\textsuperscript{1+} |
|        | Mature B cell or Recirculating B cell        | Mat B or Recirc| B220:\textsuperscript{CD19}IgM\textsuperscript{1+}IgD\textsuperscript{1+} |
|        | Follicular B cell                           | FO B            | B220:\textsuperscript{CD19}CD21:\textsuperscript{1+}CD23\textsuperscript{1+} |
|        | Marginal zone B cell                        | MZ B            | B220:\textsuperscript{CD19}CD21:\textsuperscript{1+}CD23\textsuperscript{1+} |
S-Figure 7

% SMYD2 protein expression

K562  KAS-4  MOLT-4  CCRF-CEM  RS4;11  SEM  HL-60  THP-1  NALM-6  CCL-120
SUPPLEMENTAL TABLES AND FIGURE LEGENDS

S-Table 1. PCR primers employed for analysis of SMYD2 Hematopoietic Stem Cell (HSC) targets of Figure 4B. Primers used for Smyd2 genotyping are provided in S-Methods.

S-Figure 1. Expression of Smyd2 throughout hematopoiesis in the mouse. Smyd2 is most highly expressed in the HSC and in the early progenitor of both myeloid and lymphoid lineages. Smyd2 is expressed throughout T cell development, whereas only modest expression is observed beyond early B cell stages. The myeloid lineage has varied expression of Smyd2 in its committed progenitors and very low expression onward. Adapted from Seita et.al (40).

S-Figure 2. Representative Cre deletion efficiencies monitored in bone marrow and spleen via FACS analyses of YFPLSL. 100% of the cell sample population from either murine bone marrow (A, C) or spleen (B, D) are viewed as side scatter (SSC) versus forward scatter (FSC). Left columns of each panel (Mx1-Neg or Vav-Neg) are Smyd2^floxflox controls, whereas the right columns of each panel show percentages of Smyd2^floxfloxMx-1Cre or Smyd2^floxfloxVav-Cre deletion. The internal YFP stop allele allows production of fluorescence following SMYD2 deletion prior to extracellular staining. BM deletion frequencies ranged from 75-88%, whereas splenic deletion frequencies ranged from 61-82%. PCR data demonstrating the genotype of sample mice either for Mx-1-Cre (E) or Vav-Cre (F) deletions. Cre negative mice were used as controls against Cre positives mice. Lanes 1: Standard 1kb ladder followed by a non-template and additional controls. Upper panels: Cre recombination alleles. Middle panels: Smyd2. Lower panels: YFP (selectable marker). A-D confirm that Cre-mediated deletion (upper panels E-F) produce equivalent YFP signals when assayed in Bone Marrow (BM) and in spleen (SP) cells, whereas Cre-negative mice do not. Primers and methods provided in S-Methods.

S-Figure 3. Smyd2^floxfloxMx-1Cre or Smyd2^floxfloxVav-Cre deletion efficiencies in bone marrow and spleen. A. RT-qPCR analysis demonstrating >90% reduction of SMYD2 RNA. Fold change calculated as RQ (relative quantification, 2△△Ct) compared to untreated control (CTRL). B. Western blotting confirms ~90% reduction of SMYD2 detected by anti-SMYD2 serum relative to CTRL detected by pre-immune sera.

S-Figure 4. Hematopoietic population definitions and antibody conjugates employed for their detection by flow cytometry. Sources of antibodies noted in Materials and Methods.

S-Figure 5. Representative FACS plots documenting conditional Smyd2 loss in Smyd2^floxfloxMx1Cre mice. Smyd2^f/f mice were crossed with Mx-1cre, in which targeting is
initiated within the HSC lineage following induction of its recombination activity by IFN-α. The flow cytometric data shown here are representative of the results displayed in tabular format in Fig. 1. Stain combinations are displayed in S-Fig.4. Cells were sorted based on expression of YFP generated by crosses of Rosa26-Lox-Stop-Lox-YFP (YFP<sup>LSL</sup>) reporter mice with Smyd2<sup>flox/flox</sup>Mx1Cre. Representative deletion efficiencies are shown in S-Fig.2. Shown here are percentages; total cell numbers and statistics are provided in Fig. 1.

**S-Figure 6. Representative FACS plots documenting Smyd2 loss of Polycytoid Dendritic Cells (pDC) in Smyd2<sup>flox/flox</sup>Vav-Cre.** Smyd2<sup>f/f</sup> mice were crossed with Vav-Cre, which initiates recombination within fetal liver HSCs and within HSC-proximal endothelial cells (51). The flow cytometric data shown here are representative of the results displayed in tabular format in Fig. 1. Stain combinations are displayed in S-Fig.4. Cells were sorted based on expression of YFP generated by crosses of Rosa26-Lox-Stop-Lox-YFP (YFP<sup>LSL</sup>) reporter mice crossed with Smyd2<sup>flox/flox</sup>Vav-Cre mice. Representative deletion efficiencies are shown in S-Figs.2 and 3. Shown here are percentages; total cell numbers and statistics are provided in Fig. 1.

**S-Figure 7. SMYD2 si- and sh-RNA transduction efficiencies in leukemias.** Cultured cells of 10 leukemias were either transiently transfected with SMYD2 si-RNA (black dots) or stably transduced with retrovirus SMYD2-pRSMX-PG (grey dots) as described in S-Methods and previously (30). KD levels for each leukemia were quantified relative to parallel controls. SMYD2 protein was fractionated on Western blots (77), botted with anti-SMYD2 Ab and KD levels quantified with a Typhoon PhosphorImager and ImageQuant software (Amersham Pharmacia Biotech) as detailed in S-Methods. Control levels were set at 100%. The data shown represent 2 independent experiments.

**SUPPLEMENTAL METHODS**

**Mice and Mx-1-Cre activation.** Smyd2-floxed mice<sup>32</sup> were crossed with Mx1-Cre (48) or Vav-Cre (51) mice for deletion of SMYD2 at various stages of B cell development. Mice were bred and housed in the pathogen free animal facility of the University of Texas. All experiments received approval from the Institutional Animal Care and Use Committees (Protocol ID AUP-2012-00169). Mice of 8–10 weeks of age were used for peripheral B cell analysis and those 4–6 weeks of age were used for bone marrow cultures. PCR genotyping from tail DNA was used to confirm genetic profiles.

**Mice sample size determination.** To ensure each of the animal experiments employed minimal sufficient numbers to insure reproducibility, the following criteria (6) were employed:
1. Sample size (n)—the number of mice in each experimental group; 2. Effect size—the magnitude of the statistical difference between groups (including the variance); 3. Type I error—the probability of incorrectly rejecting the null hypothesis; set to 0.06 in our study); 4. Type II error—probability of false negatives (inappropriately supporting the null hypothesis; set at 0.5 in our study); Power (1-\(\beta\))—the probability of detecting a bon fide positive (rejecting the null hypothesis; set at 0.8 in our study).

**Inclusion and exclusion criteria.** Factors employed (7) in this study included: Age—equivalent ages of both experimental and control mice of 8-10 weeks; 2. Sex—exclusively females in this study; 3. Health—mice showing signs of infection or disease (following consultation with our veterinarian) were excluded from the experimental protocol. All mice were not subjected to previous treatment history, were housed in a germ-free facility and were confirmed as “healthy” (eg. free of psychosocial, or emotional conditions) by attending veterinarians.

**Randomized controlled trials (RCT) and Blinding.** We acknowledge that RCT (8) is the best method used to establish causality (albeit there are various limitations). Random allocation is a technique that chooses individual subjects for treatment groups and control groups by chance with no regard to experimental protocol, condition of the subjects or other preferences. RCT, nor experimenter blinding was inappropriate for our studies and thus, were not employed.

**Mx1-Cre induction.** To activate Mx1-cre in vivo, 100 μg of pIpC (Sigma-Aldrich) was injected every other day for 5 days and samples taken at least two days after the final injection. Deletion efficiencies were monitored in both BM and spleen via FACS analyses of YFP\(^{\text{LSL}}\) as described by Srinivas et al. (47) (S-Fig 2).

**Flow Cytometry Antibodies.** Analytical cytometry was performed on a FACS Fortessa and followed by analysis using FlowJo (Tree Star) software. Single-cell suspensions were stained with antigen-specific monoclonal antibodies. Anti-mPDCA1-PE, Anti-Cd45R/B220-V605, Anti-Cd19-Alexa Flour 700, Anti-Cd86-APC-Cy7, Anti-I-A/I-E-Pacific Blue, Anti-Cd179a-PE, Anti-IL7Ra-V421, Anti-cKit-PE-Cy7, Anti-Cd11b-PerCP-Cy5.5, Anti-Cd3e-PerCP-Cy5.5, Anti-Gr-1-PerCP-Cy5.5, Anti-Cd45R/B220-PerCP-Cy5.5, Anti-Cd4-PerCP-Cy5.5, Anti-Cd8-PerCP CpCy5, Anti-NK1.1-PerCP-Cy5.5, Anti-Sca1-BV711, Anti-Cd135-APC and Anti-Cd150-BV605 were purchased from (Miltenyi Biotech). Others include Anti-Siglec1-PE-Cy7 (Biolegend), Anti-Cd34-Alexa Flour 700, Anti-Cd11c-PerCP-Cy5.5 (eBioscience), Anti-Cd179b-Biotin-V450 Streptavidin, Anti-AA4.1-PE (BD Biosciences) and Anti-Axl-APC. (R&D Systems). Antibodies were prepared in D-PBS/2% (vol/vol) FBS FACS buffer.
Flow cytometric analyses of hematopoietic progenitors. BM cells were isolated, RBC were ACK lysed, and cells were surface stained immediately thereafter. Gating was performed upon forward and side scatter profiles, discrimination of live vs. dead cells with 7-Amino-Actinomycin D (7-AAD). Cells were stained with pools of Lineage (Lin) specific antibodies: HSCs, (Lin)−Sca-1+c-Kit+(LSK)CD48−CD150+; Multipotent progenitors (MPPs), LSKCD48−CD150−; Common Lymphoid Progenitors (CLPs), Lin−Sca-1−c-KitIL-7Rα+Flk-2/Flt-3−; Common Myeloid Progenitors (CMP), Lin−Sca-1−c-Kit+IL-7ra−FcγRloCD34+; Granulocyte/Monocyte progenitors (GMP), Lin−Sca-1−c-Kit+IL-7Ra−FcγRhiCD34+; Megakaryocyte/Erythroid Progenitors (MEP), Lin−Sca-1−c-Kit+IL-7ra−FcγRloCD 34−. HSCs, CLPs, and CMP lineage mixes contained antibodies to B220, CD3e, CD4, CD5, CD8, CD11b, CD19, Gr-1, NK1.1, and TER-119 (purchased as noted in the previous section).

Sorting was performed on a FACS Aria (BD Biosciences). Lineage gates and compensation settings were determined by single color controls. Analysis was performed with FlowJo (Tree Star, Inc.). Definitions of cell populations also are defined in S-Fig.1. Antibody conjugates are listed in S-Fig.4.

RT-qPCR and endpoint PCR. Total cellular RNA was isolated using an RNaseasy Mini Kit (QIAGEN, Santa Clara, CA). Synthesis of cDNA was performed with qScript cDNA supermix (Quanta). RT-qPCRs were performed using PerfeCTa SYBR Green FastMix (Quanta) with 1 µL of 20X-diluted cDNA generated from 500 ng of total RNA. RT-qPCR primers were designed to amplify the junction between two exons. Primer sequences for qPCR are listed in Supplemental Table 1. CT values were normalized against Gapdh. qPCR was performed with an initial denaturation step of 10 min at 95°C, followed by 15s at 95°C and 60s at 60°C for 30 cycles using an Mx3000P™ QPCR system (Stratagene, La Jolla, CA). The normalized level of mRNA was determined as 2−Ct(GOI)/2−Ct(CTL), where Ct is the threshold cycle, GOI is the transcript of interest, and CTL is the housekeeping control (assuming that Ct is inversely proportional to the initial concentration of mRNA and that the amount of product doubles with every cycle). All PCR products were analyzed via gel electrophoresis. 15ul of each sample and loading dye was run on a 2.0% agarose gel with the addition of ethidium bromide. Electrophoresis was ran at 100v for approximately 30 minutes before visualization in a UV light box.

Primers employed for SMYD2 target genes are listed in S-Table I. Genotyping primers employed for S-Figure are: Primers: MX-1-Cre: 5’GCGGTCTGGCGAGTAAAAACTATC3’; 5’GTGAAACA GCATTGCTGTCACCTT3’, Ran at: 94° C x 2’ (94° C x 20”, 60° C x 20”, 72° C x 20”) x 35 cycles, 72° C x 2’. Vav-Cre: 5’AGATGCCAGGACATCAGGAACCTG3’;
5'ATCAGCCACACACCAGACAGATC3', Ran at: 94° C x 5' (94° C x 30", 64° C x 45", 72° C x 45") x 35 cycles, 72° C x 7'. Smyd2: 5'GGTCTGGCT TTGGAGTTGAGCC3'; 5'GAGCTTCGTGGAGTGCAGGAC3', Ran at: 94°C x 5' (94°C x 30", 62°C x 30", 72°C x 30") x 35 cycles, 72°C x 7'; YFP: 5'GGAGCGGGAGAAATGGATATG3'; 5'AAAGTCGCTCTGAGTTGTTAT3'; 5'AAGACCGCGAAGAGTTTGTC3', Ran at: 94° C x 5' (94° C x 30", 58° C x 1', 72° C x 1') x 35 cycles, 72° C x 7'.

**Extraction of bone marrow cells from mice.** Bone marrow (BM) cells from 6-10 weeks old mice were harvested by flushing cavities of femur and tibia with chilled PBS followed by filtering through 40 μm strainer to yield a single-cell suspension. Filtered cells were further incubated with lysis buffer (RBC Lysis Buffer, BioLegend) to lyse erythrocytes. After washing with PBS, cells were subjected to further experiments. All mouse experiments were subject to institutional approval by the University of Texas Animal Research Center.

**Mouse immunization and ELISA.** Mice were immunized with 4-Hydroxy-3-nitrophenylacetyl hapten conjugated to Keyhole Limpet Hemocyanin lysine (NP-KLH) at a molar ratio ~17:1 (NP/KLH) as we previously described (77). Antigen was precipitated on alum at a concentration of 1 mg/ml and delivered by intraperitoneal (100μg) or subcutaneous (50 μg) injection. Blood serum was collected at 0, 14, and 28 days after inoculation and analyzed via ELISA.

ELISA plates were coated with goat–anti-mouse Ig (M+G+A), incubated with serially diluted sera (1:50 to 1:36X10^4) and developed with horseradish peroxidase (HRP)-conjugated goat Ab specific for each mouse IgG isotype (Southern Biotechnology Associates). Plates were developed with HRP-conjugated goat Abs specific for mouse IgM and IgG isotypes and Dako TMB One-Step substrate. Antibody concentrations were calculated within linear dilution range, using standard curves generated with purified mouse mlgG antibodies.

**Microbead enrichment of Sca1+c-kit+ Hematopoietic Stem Cells (HSCs).** BM of Mx-1Cre/Smyd2flox/flox CKO and aged-matched controls was harvested from by crushing two tibias, two femurs, two pelvises, and one spine from each of 4 mice. BM cells were enriched for stem cells using anti–mouse CD117 MicroBeads and an autoMACS machine (both Miltenyi Biotec) per manufacturer’s instructions as described (54). Sca1+c-Kit–enriched HSC populations were confirmed by staining with antibodies against lineage markers [c-Kit, Sca-1, CD150, and CD34 (eBioscience)] as previously described (54). Stained validated samples were cultured for other analyses and/or processed for RNA and analyzed for gene expression.
Apoptosis quantification of HSCs in cultures. HSCs washed twice in serum free media DMEM for 1 hr, were cultured for defined periods of time in serum free DMEM containing 0.1% bovine serum albumin. Apoptosis was quantified at appropriate times by visualization in an inverted fluorescence microscopy following addition of acridine orange. Numbers of apoptotic and normal cells was determined under high power fields (×100) from wells centers in duplicate at various time points. Apoptosis for each individual experiment was identified following 6 independent observations (with a mean of 4–5 independent experiments) by their morphology of shrunken, absent, or budding cytoplasm, condensation and fragmented nuclei. Cells were counted both on monolayer as well as supernatant above as apoptotic cells were not adherent.

To further validate the acridine orange staining method, in addition, to confirm that these condensed cells had undergone apoptosis, cells were gently harvested, prepared as a cytopsin, and stained with Giemsa on formalin-fixed, paraffin-embedded sections as recommended by the manufacturer (Giemsa Stain Kit; Abgen150670).

HSC sections also were stained for apoptotic-mediated DNA fragmentation by the terminal UDP-nick end labelling (TUNEL) reaction described previously (2) with modifications to reduce false positives (3) Each slide was counted for numbers of non-parenchymal TUNEL+ figures/50 apoptotic figures per 50 high power fields at 40X (i.e., mean apoptotic non-parenchymal cell numbers/high power field from 200 observations).

Culture and maintenance of leukemias. Acute Myeloblastic Leukemias (AMLs): L-60 (ATCC CRL-2724): Base medium ATCC-formulated RPMI-1640 Medium (Catalog No. 30-2001) plus fetal bovine serum to a final concentration of 20%. Cell density maintained between 3 x 10^5 and 3 x 10^6 viable cells/mL. Fresh media renewed every 2-3 days.

THP-1 (ATCC TIB-202): Base medium, ATCC-formulated RPMI-1640, Catalog No. 30-2001. For complete medium, add 2-mercaptoethanol to a final concentration of 0.05 mM and fetal bovine serum to a final concentration of 10%.

Chronic Myelogenous Leukemias (CMLs):

K-562 (ATCC CCL-243): Base medium, ATCC-formulated Iscove's Modified Dulbecco's Medium (Catalog No. 30-2005). Complete growth medium, fetal bovine serum to a final concentration of 10%. Cultures maintained by the addition of fresh medium at 1 x 10^5 viable cells/mL. Subculture at 1 x 10^6 cells/mL.

KAS-4 (ATCC CRL-2726): Base medium, 1:1 mixture of ATCC-formulated Eagle's Minimum Essential Medium (Catalog No. 30-2003) and F12 Medium. Complete medium: add to base fetal
bovine serum to a final concentration of 10%. For propagation: Remove the medium with floating
cells, and recover them by centrifugation. Rinse adherent cells with fresh 0.25% trypsin, 0.53 mM
EDTA solution, and let the culture sit at room temperature until they detach.

**MLL-Rearranged Acute Lymphoblastic Leukemias (MLLr)-B-ALL):**

RS4;11 (ATCC CRL-1873): The base medium, DMEM: F12 Medium (ATCC 30-2006). For
complete growth medium add: Extra 10 mM HEPES (for a final conc. of 25 mM), 10 ng/ml cholera
toxin, 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 100 ng/ml hydrocortisone, 20 ng/mL human
recombinant EGF (Thermo Fisher PHG0311) and Fetal Bovine Serum, 10% final conc (ATCC 30-
2020). Subcultivation ratio of 1:2 to 1: every 10 to 15 days; medium renewal: every 3 to 4 days.

SEM (ATCC CCL-119): Base medium ATCC-formulated RPMI-1640 Medium, (ATCC 30-2001).
For complete growth medium, add fetal bovine serum (ATCC 30-2020) to a final concentration of
10%.

Cultures maintained by the addition of fresh medium or replacement of medium. Alternatively,
cultures can be established by centrifugation with subsequent resuspension at 2 to 3 X 10^5 viable
cells/mL. Maintain cell density between 2 to 3 X 10^5 and 1 to 2 X 10^6 viable cells/mL. Medium
renewal, add fresh medium (20% to 30% by volume) every 2 to 3 days

**T-Cell Acute Lymphoblastic Leukemias (T-ALLs):**

MOLT4 (ATCC CRL-1873): Base medium, ATCC-formulated RPMI-1640 (ATCC 30-2001. For
complete growth medium, add fetal bovine serum (ATCC 30-2020) to a final concentration of
10%. Cultures are maintained by addition or replacement of fresh medium at 5 X 10^5 viable
cells/mL with renewal every 3 to 4 days

CCRF-CEM (ATCC CCL-119): Base and complete medium same as for MOLT4. Cultures
maintenance same as with MOLT4. For renewal add fresh medium (20% to 30% by volume) every
2 to 3 days

**B-Cell Acute Lymphoblastic Leukemias (B-ALLs):**

NALM-6 (ATCC CRL-3273): Base medium, ATCC-formulated RPMI-1640 Medium, (ATCC 30-
2001. Complete growth medium, add to the base medium fetal bovine serum (final concentration
of 15%). Subculturing was maintained by addition of fresh medium with subsequent resuspension
at 1 x 10^5 viable cells/ml.

CCL-120/CCRF-SB (ATTC CCL-120): Base medium, ATCC-formulated Iscove’s Modified
Dulbecco’s Medium (ATTC Catalog No. 30-2005). For complete growth medium, add to the base
medium: fetal bovine serum to a final concentration of 10%. Cultures are maintained by centrifugation with subsequent resuspension in fresh medium at 3 to 5 x 10^5 viable cells/mL. Medium renewal: 2 to 3 days

**Small interfering (si)-RNA knockdown in leukemias.** Si-RNA-mediated gene knockdown of SMYD2 was utilized for inducing short-term gene silencing as previously described (30). Briefly, 50 nM of si-RNA (designed and synthesized by Qiagen specific for targeting SMYD2 mRNA: 5′-CAGGAACGACCGGTTAAGAGA-3) or a negative control (All Stars Negative Control si-RNA; Qiagen was transfected by electroporation into the following human leukemia: on the following leukemias: Acute Myeloblastic Leukemia (AML): HL-60 (ATCC CRL-2724) and THP-1 (ATCC TIB-202); Chronic Myelogenous Leukemia (CML): K-562 (ATCC CCL-243) and KAS-4 (ATCC CRL-2726); MLL-Rearranged Acute Lymphoblastic Leukemias (MLLr)-B-ALL): RS4;11 (ATCC CRL-1873) and SEM (ATCC CCL-119). T-Cell Acute Lymphoblastic Leukemia (T-ALL): MOLT4 (ATCC CRL-1873) and CCRF-CEM (ATCC CCL-119); B-Cell Acute Lymphoblastic Leukemia (B-ALL): NALM-6 (ATCC CRL-3273) and CCL-120/CCRF-SB (ATCC CCL-120). Cells were transiently transfected as described above.

**Western blotting.** Protein lysates were prepared and fractionated as previously described (77) on 12.5% SDS-PAGE. Following transfer to nylon, individual lanes were excised and blotted with the following commercial anti-human (h) antibodies: Anti-SMYD2 (ab108217; Abcam); anti-TP53 (DO-1 RUO; BD Pharmingen); anti-p19 (DCS100.1; Biorad); anti-p21 (14-6715-63; Thermo-Fischer); anti-p27 (C-19: sc-528; Santa Cruz); anti-HSP90 (AF3286; R&D Systems); anti-ERα (NR3A1; R&D Systems); anti-PARP1 (ab137653; Abcam); anti-β-catenin (ab16051; Abcam) and anti-GAPDH (ab181602; Abcam). After SDS-PAGE and transfer to nitrocellulose, blots were blocked with BSA (10% w/v), and then developed by chemiluminescence (Clarity Western ECL Substrate, Bio-Rad) with exposure time based on brightness of bands.

**Quantitative analysis of protein expression of leukemias following SMYD2 si-RNA knockdown.** Following SMYD2 KD as described above, lysates were subjected to Western blotting (77) with antibodies (detailed above) specific for p53, p21, ARF, p27, HSP90AB1, ERα, PARP1, PTEN and β-Catenin. Individual proteins bands were scanned and quantified using a Typhoon PhosphorImager and ImageQuant TL software. Three independent experiments were used to generate the quantitative analysis shown in Figure 7. Data is expressed as % KD relative to GADPH set at 100%.

**Short hairpin (sh)-RNA interference.** Leukemias were stably transduced with the inducible retrovirus pRSMX-PG (77) expressing the bacterial tetracycline repressor (Tet^r), the
blasticidin resistance gene and the following insert directed at exon 2 of Smyd2: 5'-CGATATTTCTGAATGGT GCAT-3'; 5'-GCTGTGAAAGTTTGAATCA-3'. Phoenix-A (ΦNX-A) amphototropic 293 cells were used to package \textit{pRSMX-PG-shRNA-SMYD2} using Fugene-6 reagent (Roche).

Leukemias (∼100,000 /well in triplicate) were seeded in 6-well tissue culture plates and incubated for 24 hr with RPMI medium (supplemented with 2% FBS, 1% penicillin–streptomycin) at 37 °C. Leukemias were infected with \textit{pRSMX-PG-shRNA-SMYD2} at various titers, incubated with 2% RPMI medium for 5 days and then tested for expression by the presence of GFP under an inverted fluorescent microscope. Doxycycline (1-10 μg/mL) was applied for induction of shRNA expression. Leukemias were harvested at various time points and analyzed for SMYD2 knockdown. Knockdowns (KD) were considered sufficient for analysis when each reached 70 % or lower relative expressions compared to controls, as judged by RT-qPCR and/or Western blot analysis.

\textbf{Colony formation unit (CFU) assay.} Leukemias were infected with an SMYD2 sh-RNA (\textit{pRSMX-PG-shRNA-SMYD2} as detailed above. For CFU assays, ∼10\textsuperscript{6} cell cultures of each lymphoma were applied to methylcellulose medium (Methocult, STEMCELL Technologies). Colonies were visually counted after 14 days as detailed by DiLoreto et al. (4).

\textbf{Apoptosis.} CML, MLLr-B-ALL, B-ALL and AML leukemias were transfected with SMYD2 si-RNA as described above, seeded in 6-well plates and cultured for 3 days. To detect apoptosis, we utilized the caspase-3 ApoAlert Caspase-3 Colorimetric Assay Kit (Clontech, Palo Alto, USA). Cytosolic lysates were incubated with 50 μM p-nitroanilide (pNA) conjugated to the caspase cleavage site Asp-Glu-Val-Asp (DEVD) at 37°C for 1 hr. Hydrolyzed pNA was detected using a Multiscan MS colorimeter (ThermoLabsystems, Vantaa, Finland) at 405 nm. Measurements were performed by analytical cytometry on an ACS Aria (BD Biosciences) followed by analysis using FlowJo (Tree Star) software.

Identical apoptosis analyses were conducted on FACS-sorted cells of hematopoietic developmental stages of \textit{Mx-1Cre/Smyd2\textsuperscript{flox/flox}} CKO and control mice (Fig. 3).

\textbf{Yeast two-hybrid screening.} To isolate SMYD2-interacting proteins we employed the Matchmaker Gold Yeast Two-Hybrid system according to the protocol described in the Yeast-Maker Transformation System 2 User Manual (PT1172-1, Cat No. 630439) using a cDNA library (Clontech) to isolate SMYD2-interacting proteins as previously described. Briefly SMYD2 was cloned into pGBK7 and then was transformed and integrated into the yeast strain Y2H. The ensuing bait-integrated yeast strain was subjected to expression testing and checked for auto-
activation and toxicity (as determined by color and growth status of the diluent yeast) following spreading transformants on a series of nutrient-deficient, selective agar plates. The “prey”, a mouse embryonic cDNA library (Clontech), and the purified pGBK7-SMYD2 “bait” were transformed into yeast strain Y187 to allow interaction between prey and bait. Yeast colonies grown on selective agar medium (Double dropout media containing 40 µg/ml X-a-Gal and 200 ng/ml Aureobasidin A) were then harvested and subjected to further analysis.

Statistics. Groups of three to eight mice were used for statistical analysis. P values were calculated with a Student's t-test.

Power and sample size distribution. To determine the sample size needed to ensure that the margin of error (confidence interval was sufficiently small to be informative, we applied a formula generated a sample size (N), that was sufficient to obtain a margin of error (E) that did not exceed a specified value. Using the equation:

\[ n = \left( \frac{Z \sigma}{E} \right)^2 \]

in which Z=standard normal distribution for the desired confidence level (minimum of 95% confidence);

E=the margin of error; \( \sigma \) = standard deviation; Z=the value from the probabilities of a standard normal distribution; and \( \sigma \) = the standard deviation of the outcome of interest (5).

Accession numbers. Accession numbers included in the analysis of Figure 4B are: GSM365137, GSM365154, GSM365150, GSM365156, GSM365149, GSM176000, GSM175999, GSM176005, GSM272521, GSM272546, GSM272565, GSM272525, GSM272552, GSM476259, GSM312870, GSM176001, GSM272556, GSM272529, GSM272505, GSM272500, GSM272578, GSM33139, GSM219394, GSM219393, GSM219392, GSM331381, GSM176009, GSM628423, GSM256320, GSM256283, GSM256328, GSM399726, GSM175974, GSM175951, GSM176300, GSM100672, GSM100579, GSM100466, GSM100589, GSM100580, GSM306883, GSM312887, GSM312938. Further details are provided in the original source of these data (67-69). Individual data sets were obtained at: http://www.ncbi.nlm.nih.gov/projects/geo/info/qqtutorial.html.

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