*veterinary sciences*

MDPI

*Article*

# The lysine methyltransferase SMYD2 is required for definite Hematopoietic Stem Cell production in the mouse embryo

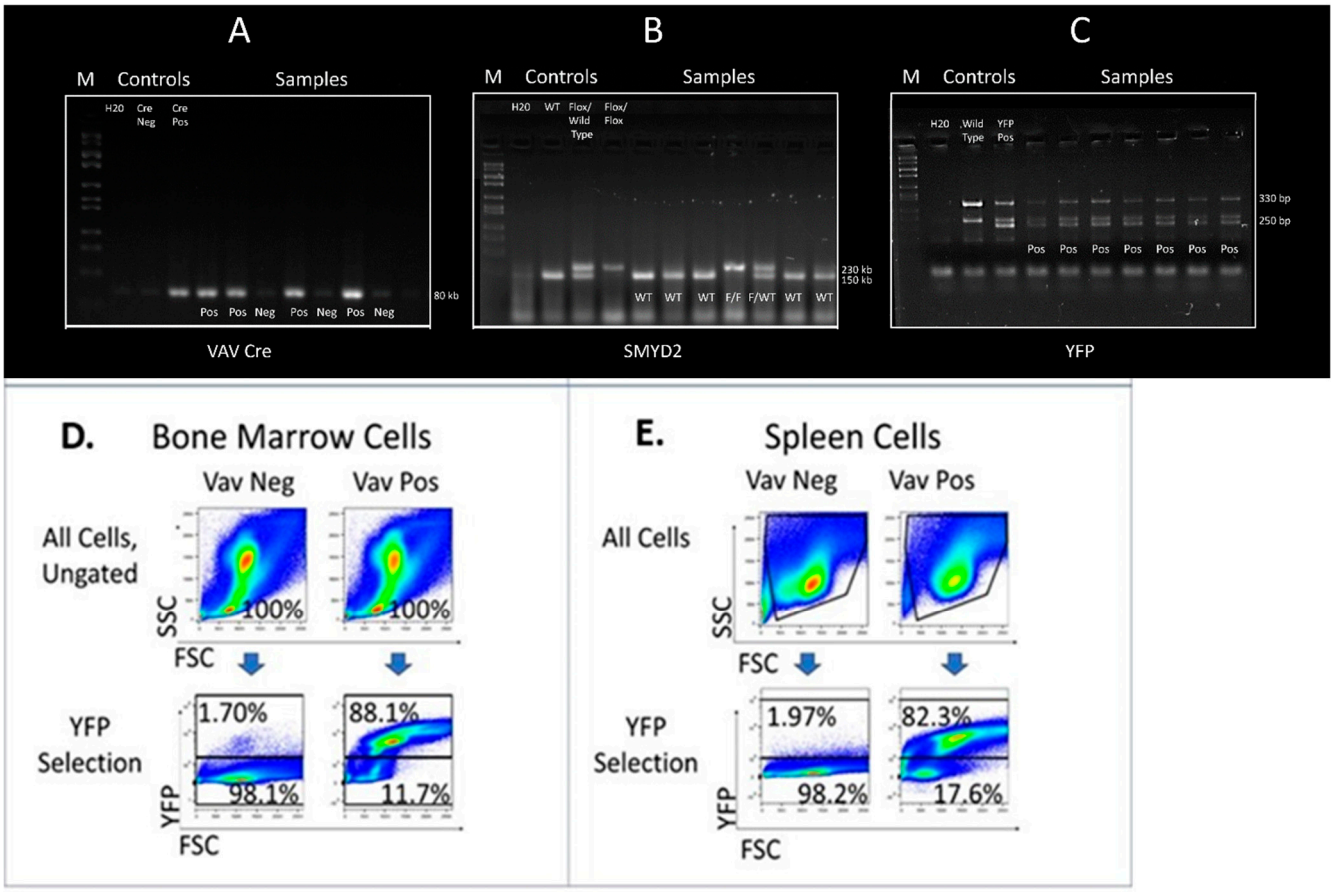


**Figure S1. Assessment of *vav*-Cre conditional knockout efficiency of *Smyd2*. A.** The presence of the flanked lox P sites on exon 2 of *Smyd2* were confirmed via PCR. Lanes 1: Standard 1kb ladder followed by a non-template and additional controls. Upper panels: *Cre* recombination alleles. Middle panels: *Smyd2*. Lower panels: *YFP* (selectable marker). Primers:5′GGTCTGGCTTTGGAGTTGAGCC3′; 5′GAGCTTCGTGGAGTGCA GGAC3′. Conditions: 94°C x 5′ (94°C x 30 min., 62°C x 30 min., 72°C x 30″) x 35 min; cycles, 72°C x 7 min. **B, C**. The YFP marker utilized to measure the deletion efficiency of *Smyd2* via PCR. Primers: 5′GGAGCGGGAGAAATGGATATG3′; 5′AAAGTCGCTCTGAGTTGTTAT3′;5′AAGACCGCGAAGAGTTTGTC3′ Conditions: 94°C x 5 min (94° C x 30 min, 58° C x 1min, 72° C x 1min) x 35 cycles, 72° C x 7 min. 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: 100v for ~30 min; visualization, UV. **D, E.** Representative Cre deletion efficiencies monitored in bone marrow and spleen via FACS analyses of $YFP^{LSL}$. 100% of the cell sample population from either murine bone marrow or spleen viewed as side scatter (SSC) versus forward scatter (FSC). Left columns of each panel (Vav-Neg) are $Smyd2^{flox/flox}$ controls, whereas the right columns of each panel show percentages of $Smyd2^{flox/flox}$*Vav-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%. .

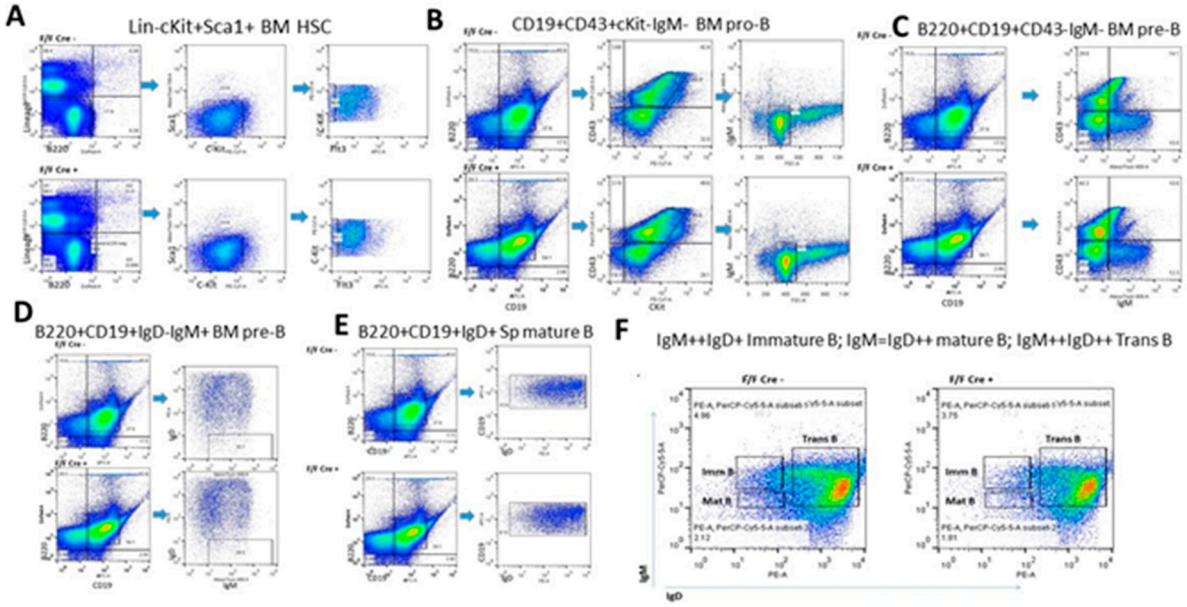


**Figure S2. Analysis of hematopoietic populations following SMYD2 deletion with *vav-Cre*.** We crossed *Smyd*$^{F/F}$ mice with *Vav-Cre* mice (JAX stock #008610), which initiates recombination within E12.5 embryonic epithelium determined FACS profiles. Cells were sorted based on expression of YFP generated by crosses of *Rosa26-Lox-Stop-Lox-YFP* (YFP$^{LSL}$) reporter mice (S-Fig.1). S-Figure 3 provides the stain combinations employed to identify population detection by flow cytometry**. A.** Lin-cKit+Sca1+ BM HSC adult BM HSC; **B.** CD19+CD43+cKit-IgM- BM pro-B cells; **C.** B220+CD19+CD43-IgM- BM large pre-B cells; **D.** B220+CD19+IgD-IgM+CD43- BM small pre-B cells; **E.** B220+CD19+IgD+ Splenic (Sp) mature B cells; **F.** IgM$^{hi}$IgD$^{low}$ immature Sp B cells; IgM$^{low}$IgD$^{hi}$ Sp mature B cells; IgM$^{hi}$IgD$^{hi}$ transitional (Trans) Sp B cells. Total cell numbers were determined as: Total # femur/spleen/thymus cells harvested/sample X number of cells/gate. Statistical analyses performed by student's t-test on 4-6 experimental replicates; *p≤0.05; **p≤0.005; ***p≤0.001.

| Tissue | Population | Population Abbr | Marker | Lineage |
|---|---|---|---|---|
| Bone Marrow | Hematopoietic stem cell | HSC | $Lin^-Sca1^+ckit^+Flt3^-$ | B220, CD19, CD3e, CD4, CD8a, CD11b, Gr1, NK1.1, Ter119 |
| | Multipotent progenitor | MPP | $Lin^-Sca1^+ckit^+Flt3^{int}$ | |
| | Lymphoid primed multipotent progenitor | LMPP | $Lin^-Sca1^+ckit^+Flt3^{hi}$ | |
| | Common lymphoid progenitor | CLP | $Lin^-Flt3^+IL\text{-}7^+Sca1^{low}+ckit^{low}$ | |
| | Common myeloid progenitor | CMP | $Lin^-Sca1^-+ckit^+ FcyR^{int}CD34^{int}$ | |
| | Granulocyte/macrophage progenitor | GMP | $Lin^-Sca1^-+ckit^+ FcyR^{hi}CD34^{hi}$ | |
| | Megakaryocyte/erythroid progenitor | MEP | $Lin^-Sca1^-+ckit^+ FcyR^{low}CD34^{low}$ | |
| | Progenitor B cell | Pro-B | $B220^+CD19^-CD43^+ckit^+IgM^-$ | |
| | Pre B cell | Pre-B | $B220^+CD19^+CD43^-IgM^-$ | |
| | Large Pre-B | Large Pre-B | $B220^+CD19^+CD43^+IgM^-BP1^+$ | |
| | Small Pre-B | Small Pre-B | $B220^+CD19^+CD43^-IgM^-CD2^+$ | |
| | Immature B cell | Imm B | $B220^+CD19^+CD43^-IgM^{hi}IgD^-$ | |
| | Mature B cell or Recirculating B cell | Mat B or Recirc | $B220^+CD19^+IgM^{hi}IgD^+$ | |
| | Macrophage | Macro | $Mac1^+Gr1^{low}CD115^+$ | |
| | Granulocytes | Gran | $Mac1^+Gr1^{hi}CD115^-$ | |
| | Plasmacytoid dendritic cell | pDC | $CD11b^-CD11c^{low}B220^+PDCA1^+$ | |
| | | | | |
| Thymus | Double negative | DN | $CD4^-CD8^-$ | B220, CD19, CD3e, CD8a TCRB, TCRyΔ, CD11b, NK1.1, Ly-6G, CD11b, CD11c,Ter119 |
| | Double positive | DP | $CD4^+CD8^+$ | |
| | Helper T cells | CD4+ | $CD4^+CD8^-$ | |
| | Cytotoxic T cells | CD8+ | $CD4^-CD8^+$ | |
| | | | | |
| Spleen | Immature B cell | Imm B | $B220^+CD19^+IgM^{hi}IgD^-$ | |
| | Transitional B cell 1 | Trans B 1 | $B220^+CD19^+IgM^{hi}IgD^-CD21^-CD23^-$ | |
| | Transitional B cell 2 | Trans B 2 | $B220^+CD19^+IgM^{hi}IgD^+CD21^+CD23^+$ | |
| | Mature B cell or Recirculating B cell | Mat B or Recirc | $B220^+CD19^+IgM^{hi}IgD^{hi}$ | |
| | Follicular B cell | FO B | $B220^+CD19^+CD21^{int}CD23^{hi}$ | |
| | Marginal zone B cell | MZ B | $B220^+CD19^+CD21^{hi}CD23^{low}$ | |

**Figure S3.** Hematopoietic population definitions and antibody conjugates employed for their detection by flow cytometry. Sources of antibodies noted in S-Methods.

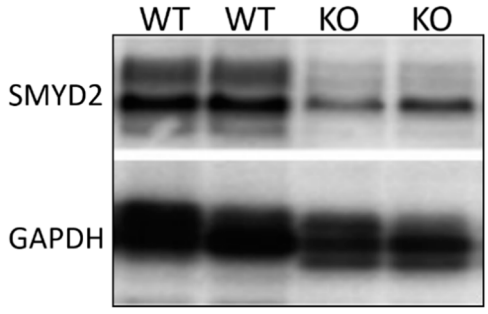


**Figure S4.** Protein deletion efficacy. Western blotting confirms reduction of SMYD2 in CKO detected by anti-SMYD2 serum relative to WT. Protein lysates were prepared and fractionated as previously described [11] on 12.5% SDS-PAGE. Following transfer to nylon, individual lanes were excised and blotted with the following commercial anti-human (h) antibodies: Anti-SMYD2 (ab108217; Abcam); 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.