**Supplementary Materials and Methods**

**RNA extraction**

Total RNA was extracted from FACS-sorted lineage-negative (Lineage- negative for the expression of CD3, CD8a, CD19, B220, GR1, CD11b, CD11c, Ter119) fetal liver cells using the RNeasy Micro Kit (Qiagen), according to the manufacturer's instructions. RNA from whole fetal livers was isolated using the RNeasy Mini columns (Qiagen). All RNAs were quantified with a Nanodrop 2000 spectrophotometer (Thermo Scientific) and assessed with an Agilent 2100 Bioanalyzer.

**Microarray analysis**

For whole transcriptome analyses, FACS-sorted, lineage-negative fetal liver cells were isolated from WT and Fl/ko Vav1:iCre littermates at E15.5 and total RNA was extracted, as described earlier. Five sample pairs were used in total for probe synthesis and hybridization to Affymetrix Mouse Gene 1.0 ST chips for whole-genome, gene-level microarrays (EMBL Genomics Core Facility, Heidelberg). Raw .CEL files were used for analysis of the microarray data with GeneSpring 12 GX software (Agilent Technologies). Background adjustment, quantile normalization and summarization of probes was performed on all chips using the Robust Multichip Analysis-16 (RMA 16) algorithm. Statistical analysis was carried out using a t-test unpaired test, while p-value computation was done asymptotically, without multiple testing correction. Gene ontology analyses were carried out using DAVID and Gene Set Enrichment Analysis (GSEA). Microarray data are deposited in the Gene Expression Omnibus (GSE53056).

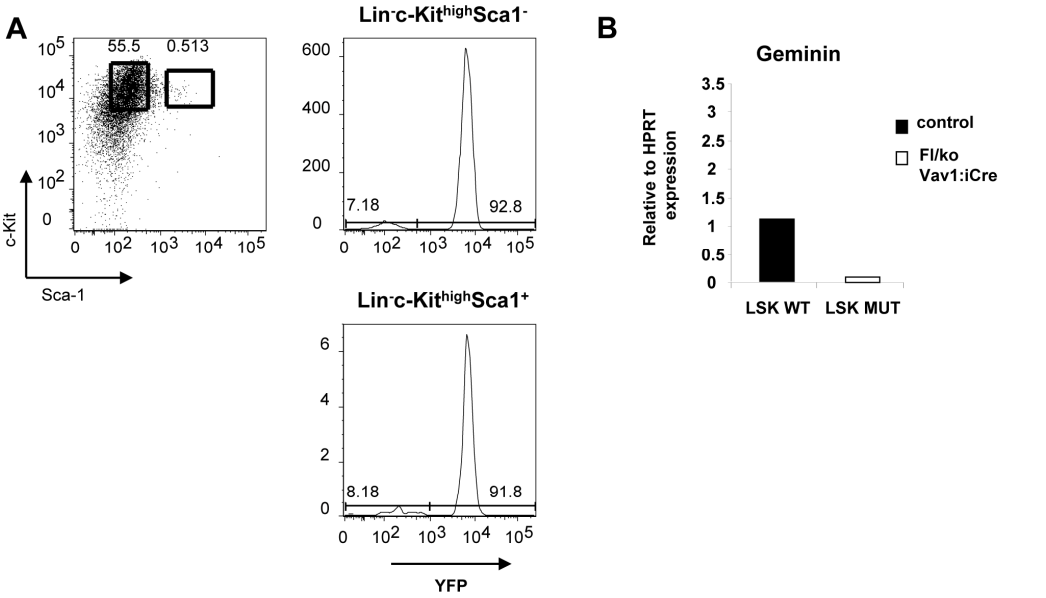
**Quantitative Real-Time PCR**

Expression of selected transcripts was validated using quantitative Real-Time PCR. RNA isolated from whole fetal liver or lineage-negative cells was reverse transcribed using the Primescript First Strand cDNA synthesis kit (Takara). Real-time PCR assays were performed using a Kapa SYBR FAST qPCR master mix with an Applied Biosystems Step One instrument. The primers used in the assays are listed in Table S5. To compare gene expression between WT and Fl/ko Vav1:iCre samples, the $\Delta\Delta C_T$ method was used. Data were normalized against endogenous HPRT and beta Actin (ACTB) levels. All reactions were performed in triplicates using RNA purified from three biological replicates.

**K562 Differentiation assays**

*In vitro* differentiation assays were carried out using K562 cells stably expressing control shRNA (shControl), or shRNA against human Geminin (shGeminin). K562 cells were treated with Hemin, according to the protocol described by Lam et al. (2010) with minor adjustments. Briefly, $5x10^5$ cells/ml were treated with 40μM hemin (Sigma-Aldrich) for 4 days. Benzidine (Sigma-Aldrich) staining solution (0.2% w/v in 0.5M acetic acid) supplemented with hydrogen peroxide (final concentration 0.0012%) was used to detect hemoglobin (Hb) expressing cells. Untreated cells stained with benzidine served as a control for spontaneous Hb production and the pigmentation served as threshold above which Hb expression was recorded.

For megakaryocyte (MK) hematopoiesis, K562 cells were induced with the phorbol ester, TPA (*12-O-tetradecanoylphorbol-13-acetate*, Sigma-Aldrich), in a variation of the method described by Hsu and Yung (2003) whereby $5x10^5$ cells/ml were treated with 40nM TPA for 3 days. MK differentiation was assessed by FACS using CD41a and CD61 antibodies (Mattia et al., 2002). Cell viability was assessed using the Live/Dead stain (Invitrogen).




**E**

| Genotype | Number of newborn pups |
|---|---|
| Fl/WTVav1:iCre or WT/WT Vav1:iCre | 4 |
| FL/WT | 8 |
| WT/ko | 7 |
| WT/koVav1:iCre | 4 |
| FL/ko | 6 |
| FL/koVav1:iCre | 0 |
| **Total** | 29 |

**Fig. S1. Deletion of the Geminin gene in fetal hematopoietic system leads to embryonic lethality.** (A) Male mice of the reporter line R26EYFP were crossed to female Vav1:iCre mice to examine the Cre recombinase activity in E15.5dpc fetal liver. Cre

recombinase excises a flanked stop sequence allowing expression of YFP protein under the control of the promoter of R26 locus. $EYFP^{+}$ cells in immunophenotypically defined HSCs ($Lin^{-}$c-$Kit^{high}$Sca-$1^{+}$) and HPCs ($Lin^{-}$c-$Kit^{high}$Sca-$1^{-}$) fetal liver of E15.5 R26EYFP Vav1:iCre embryos are shown. Representative FACS analysis using antibodies against lineage, c-Kit, Sca-1 is presented. (B) Total RNA was isolated from LSK (Lin-c-KithighSca1+) cells and HPC (hematopoietic progenitor cells Lin-c-KithighSca1-) and analyzed by real time PCR using specific primers for the detection of the mouse Geminin mRNA and HPRT as a loading control. The values represent mRNA expression for Geminin gene normalised for HPRT expression. (C) Embryos at E15.5dpc (Fl/koVav1:iCre) that lack Geminin expression are pale, anaemic and show reduced size of fetal liver compared to the wild type mice. (D) Total fetal liver cellularity of control (N=16) and Fl/koVav1:iCre (N=11) embryos are depicted in the bar graph. (E) Genotyping of offspring was performed by PCR analysis using DNA from newborn mice and primers detecting the wild type (WT), floxed (Fl) or deleted (KO) alleles of the Geminin gene. No pups of Fl/koVav1:iCre genotype were identified.

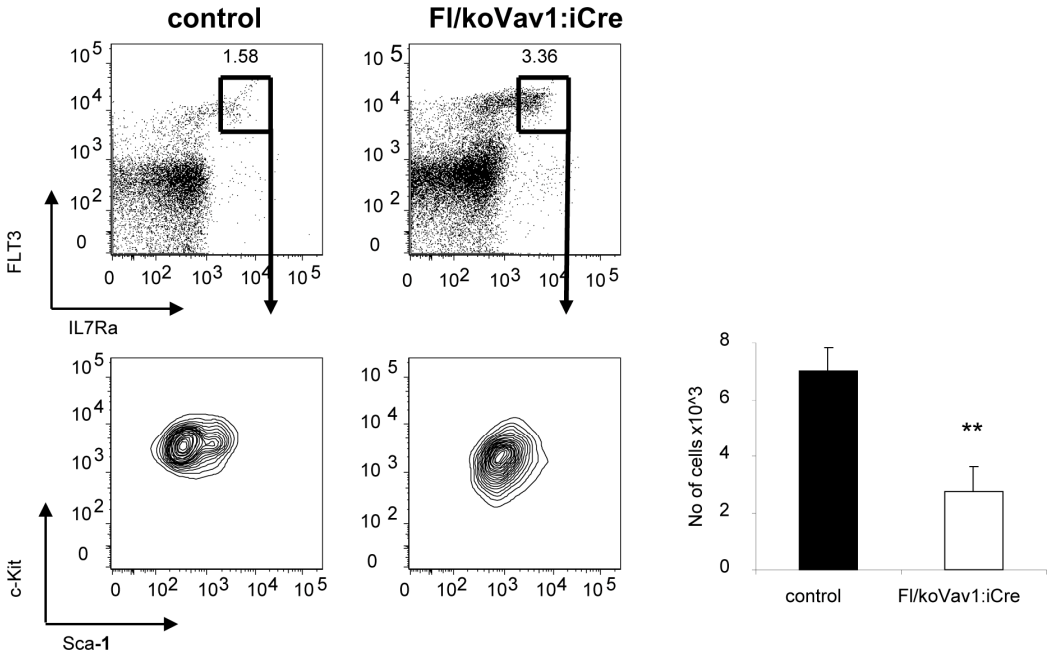



**Fig. S2. Reduced number of common lymphoid progenitors (CLPs) in the absence of Geminin.** (a) Representative FACS analysis using antibodies recognizing Lineage, c-Kit, Sca-1, Flt3 and IL7Rα antibodies. c-Kit and Sca-1 expression on $Lin^{-}Flt3^{+}IL7Ra^{+}$ cells is shown (b) Average absolute cell numbers of $Lin^{-}Flt3^{+}IL7R\alpha^{+}$ cells from fetal liver of E15.dpc control (N=15) and Fl/koFl/kokoVav1:iCre (N=8) embryos is shown in the bar graph (± SE), $^{**}p < 0.01$.

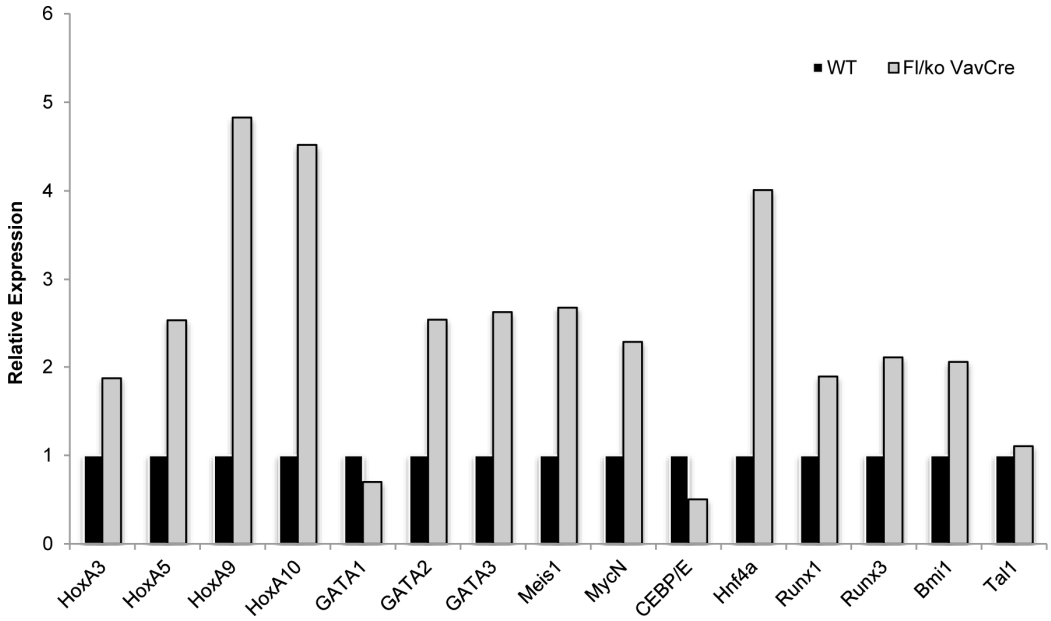



**Fig. S3. Quantitative PCR validation of selected differentially expressed genes.** The mRNA levels of selected genes that were shown to be differentially expressed in the cDNA microarrays were examined with qPCR. Also included were genes that did not appear in the dataset (eg. CEBP/E). Total fetal liver RNA from either WT or Fl/KO Vav1:iCre embryos was used in reverse transcription reactions followed by real-time qPCR. The values were normalized against endogenous HPRT or beta-Actin and expressed as fold-change over WT.

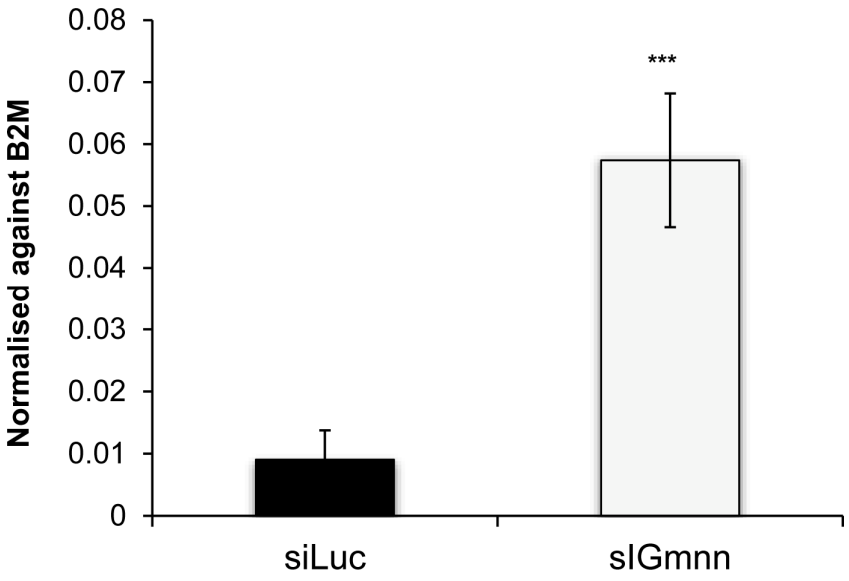



**Fig. S4. Geminin knockdown in K562 cells leads to de-repression of HoxA9.** Transcriptional activation of HoxA9 in siGmnn-treated cells was detected by quantitative real-time PCR. Normalisation of expression values was carried out using the endogenous levels of B2M. *** p-value<0.005. Results are representative of at least three independent experiments carried out in triplicates.

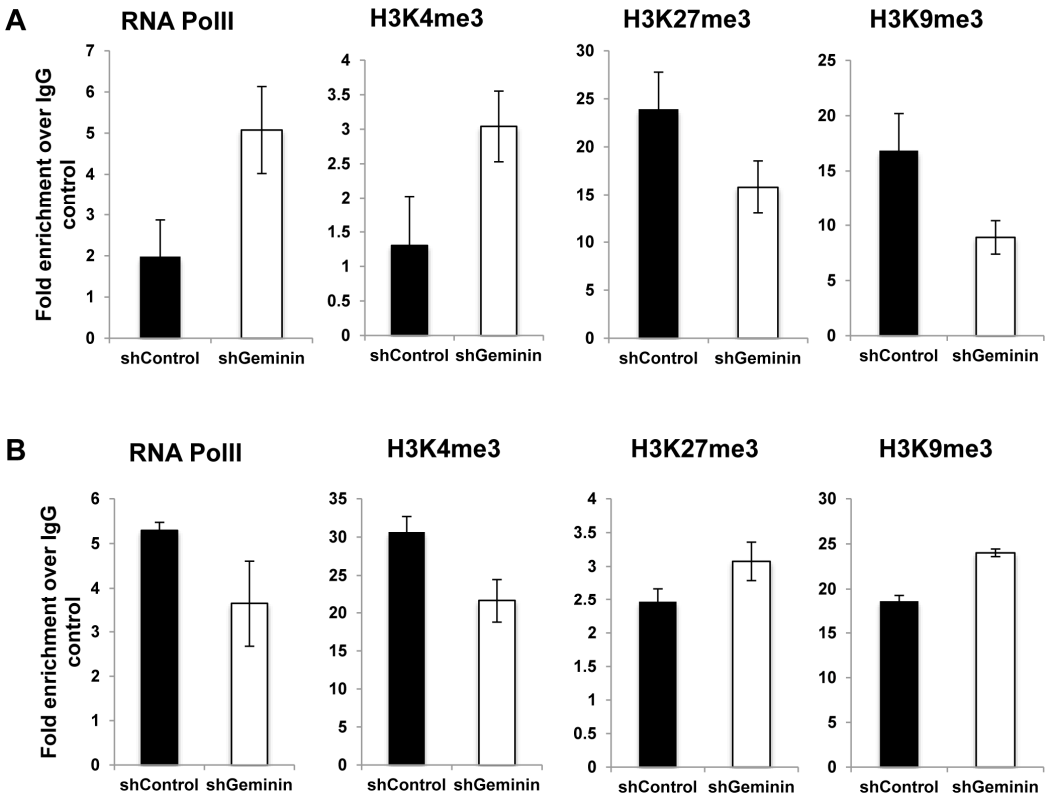



**Fig. S5. Silencing of Geminin in K562 cells modulates epigenetic marks on the HoxA10 and Gata1 genes.** Chromatin immunoprecipitation was performed in K562 cells expressing shControl or shGeminin using antibodies against RNA Polymerase II, H3K4me3, H3K9me3 and H3K27me3. A genomic region of 1.0 kb and 1.5 kb downstream of the transcription start site was examined in HOXA10 (A) and GATA1 (B) genes respectively. Enrichment was determined as fold change over non-specific IgG values. ChIP followed by real-time PCR were performed as described in materials and methods. Data shown are representative of three independent experiments performed in triplicates.

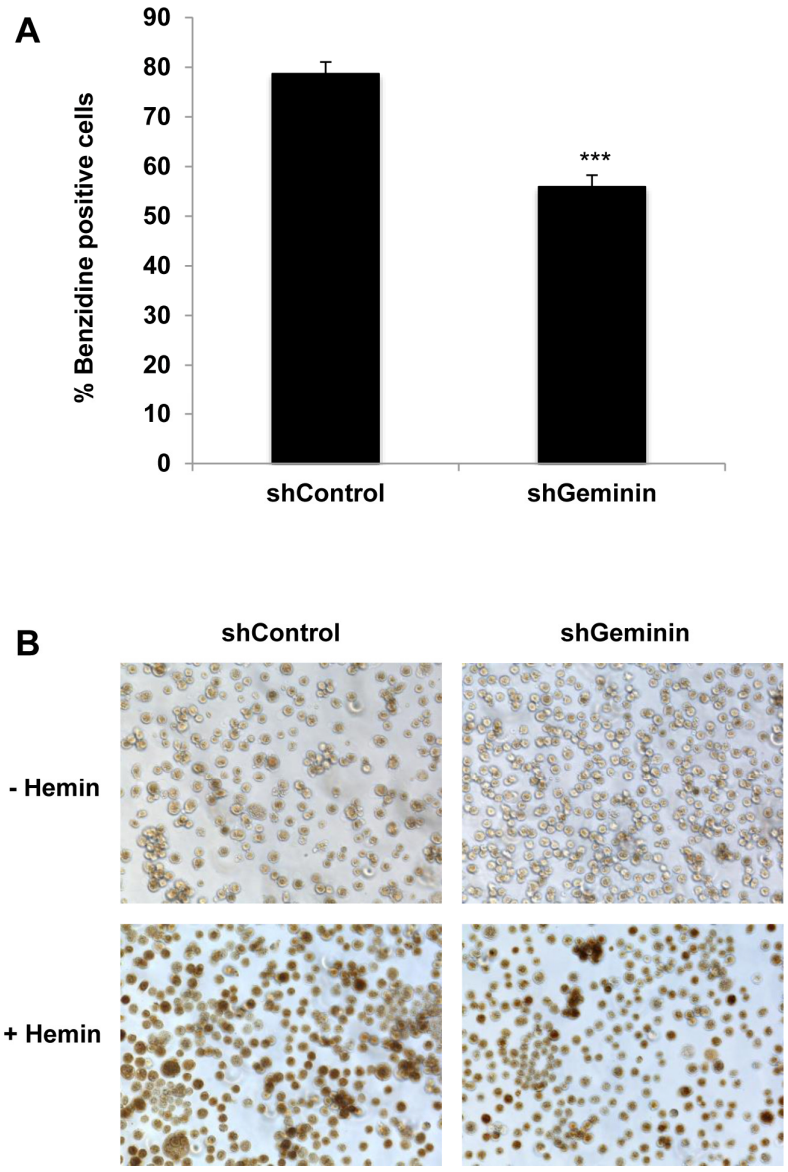



**Fig. S6. Hemin-induced differentiation of K562 cells is defective in the absence of Geminin.** K562 cells stably transfected with either shControl or shGeminin were treated with 40μM hemin for four days. Hemoglobin production was assayed using benzidine staining. (A) Graph depicts the percentage of Hemoglobin (Hb) expressing cells. Error bars represent the standard error of the mean. *** *p-value* <0.005 (B) Phase contrast micrographs (x200) of benzidine-stained cells upon treatment with hemin (bottom row). Cells were stained with benzidine in the absence of hemin to determine cells spontaneously expressing Hb (upper row).

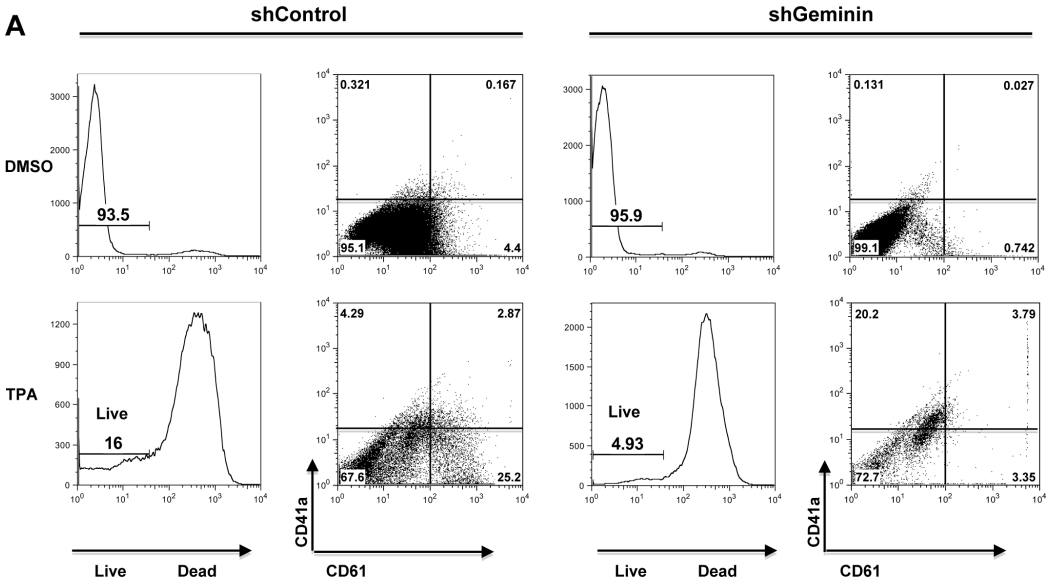



**Fig. S7. K562 cells do not differentiate towards mature megakaryocytes in the absence of Geminin.** K562 cells expressing control (shControl) or shRNA against Geminin (shGeminin) were treated with 40 nM TPA for three days to induce megakaryocytic differentiation. Cell viability and apoptosis were assessed using the Live/Dead stain (Invitrogen). Live cells were analysed for the expression of CD41a (an early marker of differentiation towards the megakaryocytic lineage) and CD61 (a marker of mature megakaryocytes). CD61 expression is markedly reduced in TPA treated cells depleted of Geminin.

**SUPPLEMENTARY TABLES**

**Table S1**

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**Table S2**

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**Table S3**

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**Table S4**

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**Table S5**

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