

Supplementary Tables

Supplementary Table S1: Oligos for targeted mutagenesis of *EVI2B* Peak 1

	Oligos 5' – 3'
Peak 1 mut1 (5'-3')	gtaatatatttcttagtcactttgtggtttagggcacttccggaaccag
Peak 1 mut2 (5'-3')	attgcacttccggaaccagctagggataaacttctctttttctac
Peak 1 mut3 (5'-3')	tgctagaggaacattttaaactctgaagggaaccaccattttcctttctta

Supplementary Table S2: sequences of primers used for Q-PCR in this study

	Gene name	Species	Primer 5' – 3'
Forward	<i>GAPDH</i>	Human	ccacatcgctcagacacat
Reverse	<i>GAPDH</i>	Human	ccaggcgccaatacg
Forward	<i>Gapdh</i>	Mouse	ccagcctcgtcccgtagac
Reverse	<i>Gapdh</i>	Mouse	cccttgactgtgccgttg
Forward	<i>Evi2b</i>	Mouse	cgtaatccgcaactgaaacac
Reverse	<i>Evi2b</i>	Mouse	gacaaagcacaatgaagaccag
Forward	<i>EVI2B</i>	Human	cagtttgctagaggaacattttaaact
Reverse	<i>EVI2B</i>	Human	atccatttcagaatatttctctcgttacc
Forward	<i>Cebpa</i>	Mouse	gaccattagccttggtgtactgtatg
Reverse	<i>Cebpa</i>	Mouse	tggatcgattgtgcttcaagtt
Forward	<i>CEBPA</i>	Human	caagaagtcggtggacaaga
Reverse	<i>CEBPA</i>	Human	ggtcattgtcactggtcagc

Supplementary Table S3: sequences of primers used in ChIP-PCR

	Name	Species	Primer 5' – 3'
Forward	Peak 1	Human	CTTTGTGGTTTATTGCACTTC
Reverse	Peak 1	Human	GGGTGGTTCAATTCAGATTTA
Forward	Peak 2	Human	CGTGAATCTGGACTGGTT
Reverse	Peak 2	Human	AAAATGCTGGGATTACAGG
Forward	Control region	Human	CCACCAAGAAGTCACACAAATAGAA
Reverse	Control region	Human	TTGATATTCTGTCCCTCACCC
Forward	5'extreme	Human	CTTGGACATTGAAAGGCATCAG
Reverse	5'extreme	Human	TTCCAGCTAATTCCCACATAATTC
Forward	5'shoulder	Human	TCTTACCTCAGCTGTAACTTCTTT
Reverse	5'shoulder	Human	TCCTTTCTTAGCCAAATCACCA
Forward	Peak 1.1	Human	CTGGTTCCGGAAGTGCAATA

Reverse	Peak 1.1	Human	ACTCAAATCATAGGGCTCCAAA
Forward	Mid	Human	TTGGAGCCCTATGATTTGAGTG
Reverse	Mid	Human	TCTTCGGGACTCCATCCTAAA
Forward	Peak 1.2	Human	GAAGTGAGTGATGTGTTCTGTTTAC
Reverse	Peak 1.2	Human	CTTTAGAGGAGGAAACGGTGAC
Forward	3'shoulder	Human	CCAAGTGCAGGTAACCAATAAA
Reverse	3'shoulder	Human	AAGAATGAGATCCTGTCATTTGC
Forward	3'extreme	Human	TTGGATAGTACCATTCTGAGAGTATATG
Reverse	3'extreme	Human	GAGAGCTTCTACCACAAGGATATT

Supplementary Table S4: murine *Evi2b*-targeting shRNA sequences:

	shRNA name	shRNA sequence 5' – 3' (target sites in bold)
Forward	NSC#1	aaccccc atctc gcttggg cgagag taatcaagag ttactctc gccaag cgagat ttttggaa
Reverse	NSC#1	tcgattccaaaa atctc gcttggg cgagag taactcttgatt actctc gccaag cgagat gggggtt
Forward	NSC#2	aaccccc catcac ctt tacctata attcaagag attatag gtaga aggtgat ggttttggaa
Reverse	NSC#2	tcgattccaaaa accatcac ctt tacctata atctctga attatag gtaga aggtgat gggggggtt
Forward	shRNA#3	aaccccc gcatatta tcggt actattt caagag aatag taccg attaata tggtttttggaa
Reverse	shRNA#3	tcgattccaaaa accatcac ctt tacctata atctctga attatag gtaga aggtgat gggggggtt
Forward	shRNA#4	aaccccc gtgcat cagtt gtctcact tatcaagag taagt gagaca actgat gcactttttggaa
Reverse	shRNA#4	tcgattccaaaa agtgc atcagtt gtctcact tactcttgata agt gagaca actgat gcacgggggtt

Supplementary Table S5: human *EVI2B*-targeting shRNA sequences:

	shRNA name	shRNA sequence 5' – 3' (target sites in bold)
Forward	NSC	aaccccc atctc gcttggg cgagag taatcaagag ttactctc gccaag cgagat ttttggaa
Reverse	NSC	tcgattccaaaa atctc gcttggg cgagag taactcttgatt actctc gccaag cgagat gggggtt
Forward	shRNA#5	aaccccc acaggtatt ggcta attctc atcaagag tgagaatt agcca atacct gtttttggaa
Reverse	shRNA#5	tcgattccaaaa acaggtatt ggcta attctc actcttgatgag aattag cca atacct gtgggggtt
Forward	shRNA#6	aaccccc gatca agat cttaat gaat cc tcaagag ggattc atta agatctt gatctttttggaa
Reverse	shRNA#6	tcgattccaaaa gatca agat cttaat gaat cc tcttgagg attc atta agatctt gatcgggggtt

Supplementary material and methods

Cell lines and treatments

K562 cells stably expressing human p42 C/EBP α -ER, p30 C/EBP α -ER, or with control ER (estrogen receptor) alone were cultured in phenol red-free RPMI medium supplemented with 10% charcoal-stripped FBS.¹ K562 cells expressing fusion proteins or ER alone were treated with 1 μ M β -estradiol (E₂) (Sigma-Aldrich, St. Louis, MO, USA) or ethanol vehicle control as described before.² 32D/G-CSF-R cells were kept in RPMI medium supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA), 1% penicillin/streptomycin and supernatant from HEK293 cells transduced with constructs coding for IL-3 (dilution 1:500). Differentiation was induced by 100 ng/ml G-CSF (Amgen, Thousand Oaks, CA, USA). Differentiation was assessed by staining of cytopun cells with May-Grünwald Giemsa (DiaPath, Martinengo, BG, Italy). NB4 cells were kept in RPMI medium supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA), 1% penicillin/streptomycin. Differentiation was induced by 1 μ M ATRA (Sigma-Aldrich, St. Louis, MO, USA).

RNA isolation, cDNA preparation and quantitative RT-PCR

RNA from murine BM populations was isolated using RNeasy Micro or Mini Kit (Qiagen Sciences, Germantown, MD, USA) depending on number of sorted cells according to manufacturer's protocol, which included DNaseI treatment. RNA from cell lines was isolated with Tri Reagent RT (Molecular Research Center, Cincinnati, OH, USA) and treated with DNaseI (Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions. cDNA was prepared using SuperScript II reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA). Primer sequences used for Quantitative RT-PCR can be found in Supplementary Table S2.

ChIP-qPCR

U937 cell line was grown in RPMI supplemented with 10% FBS to obtain 20 million cells. The proteins were cross-linked with DNA by using 1% formaldehyde for 10 min at RT and quenched with 0.125 M glycine for 5 min at RT. Cells were washed two times with ice-cold PBS and harvested. The cells were lysed with 0.5% SDS lysis buffer in the presence of protease inhibitor cocktail (PIC, Nacalai Tesque). The nuclear pellet was collected, resuspended in IP buffer supplemented with PIC, and sonicated using Bioruptor (Diagenode) to shear DNA to lengths between 100 and 300 bp. 35 cycles of sonication was performed at high amplitude, 30 s on and 30 s off. 50 uL Dynabeads M-280 Sheep anti-Rabbit IgG (Thermo Fisher Scientific) were washed thrice with PBS/ 5 mg/mL BSA and PIC, then incubated with 5 μ g C/EBP α antibody (Genetex GTX100674) on tube rotator for 3 h at 4°C. The bead-antibody complex was washed thrice with PBS-BSA-PIC solution, incubated with sonicated chromatin from 18 million cells for 2 h at 4°C, washed one time with the following 3 buffers: low salt, high salt, and LiCl immune complex wash buffers, and twice with TE buffer. The beads-antibody-chromatin complex was then resuspended in elution buffer, reverse-crosslinked for 4 h or overnight at 65°C, proteinase K treated for 1 h at 45°C, DNA-purified using QIAquick PCR Purification Kit (Qiagen), and eluted in 60 μ L nuclease-free water. 1 μ L of the ChIP DNA was used for qPCR. ChIP-qPCR was performed on three biological replicates with three technical replicates for each primer set. We compared the enrichment of peak 1.1 and peak 1.2 to the average of enrichment on both distal regions and calculated the p-values using student's t-test to determine whether the differences are significant. Primer sequences used for ChIP-qPCR can be found in Supplementary Table S3.

ChIP-PCR using K562 cells was performed as previously described.²

Flow cytometric analysis and BM sorting

Cell suspensions were pre-incubated with anti-EVI2B PE monoclonal antibody (1:250) (MEM-216, recognizing an extracellular epitope, Exbio Praha, Vestec, Czech Republic) for 30 min. and then incubated 15 min. with an antibody cocktail containing CD19 PE-Cy7 (LT-19), CD3 APC (UCHT1), and CD45 APC-Cy7(MEM-28) (Exbio Praha, Vestec, Czech Republic) or a cocktail containing CD3 Pacific Blue (UCHT1), CD19 Pacific Blue (LT-19) (Exbio Praha, Vestec, Czech Republic), CD123 PE-Cy7 (6H6) (Affymetrix eBioscience, San Diego, CA, USA), CD16 BV510 (3G8) (BioLegend, San Diego, CA, USA), CD56 FITC (B159), CD14 PerCP-Cy5.5 (M5E2), CD11c APC (B-ly6), and HLA-DR APC-H7 (L243) (BD Biosciences, San Jose, CA, USA).

Mouse BM cells were isolated by crushing the long bones, hips and spine. Single cell suspensions from BM, spleen, and blood isolated from *Evi2b* KO mice were analyzed using the following antibodies: CD45/B220 APC, CD45/B220 PE (RA3-6B2), CD3 PE (17A2), Gr1 APC (RB6-8C5), and Mac1 APC (M1/70). B cells were detected as APC and PE double positive, T cells as PE positive, and granulocytes as APC positive. BM transplant recipients were analyzed using the following antibodies: CD45.2 PE/Cy7 (104) and CD45.1 PB (A20). Cell sorting of HSC and progenitor populations was performed in 2 steps. First, Lin⁺ cells were labeled with biotinylated lineage markers: CD45/B220 (RA3-6B2), CD3 (145-2c11), Ter119 (TER-119), Gr1 (RB6-8C5), and CD11b (M1/70) and depleted using anti-biotin magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) on MACS separator (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's protocol. Second, Lin⁻ cells were labeled with different combinations of the following antibodies: c-kit-PE or FITC (2B8), Sca1 APC (E13-161.7) or Pe-Cy7 (D7), CD150 Pe-Cy7 (TC15-12F12.2), CD48 FITC (HM48-1), FcγRII/III Pe-Cy7 (93), CD34 FITC (RAM34), Flt3 APC (A2F10), IL7R PE (A7R34), streptavidin-eFluor450

(Affymetrix eBioscience, San Diego, CA, USA). To isolate mature cell populations lineage depletion step was not performed. Nucleated marrow cells were labeled with the following antibodies: Gr1 FITC (RB6-8C5), Mac1 APC (M1/70), B220 APC (RA3-6B2), CD4 PE (GK1.5), and CD8 PE (53-6.7). To sort different hematopoietic populations the following sorting strategies were used: LKS cells – Lin⁻, c-kit⁺, Sca1⁺; long-term hematopoietic stem cells (LT-HSC) – Lin⁻, c-kit⁺, Sca1⁺; CD150⁺, CD48⁻; short-term hematopoietic stem cells (ST-HSC) – Lin⁻, c-kit⁺, Sca1⁺, CD150⁻, CD48⁺; common myeloid progenitors (CMP) – Lin⁻, c-kit⁺, Sca1⁻, FcγRII/III^{low}, CD34⁺; granulocyte monocyte progenitors (GMP) – Lin⁻, c-kit⁺, Sca1⁻, FcγRII/III^{high}, CD34⁺; megakaryocyte erythroid progenitors (MEP) – Lin⁻, c-kit⁺, Sca1⁻, FcγRII/III⁻, CD34⁻; common lymphoid progenitor (CLP) – Lin⁻, Flk⁺, Il7R⁺, cKit⁺, Sca1⁺; granulocytes – Gr1⁺, CD11b⁺; B cells – B220⁺; T cells – CD4⁺ and CD8⁺ (single positives). Differentiation of human cord blood CD34⁺ cells was assessed using CD11b APC, and CD15 Pe-Cy7 antibodies (eBioscience). NB4 cells differentiation was assessed using CD11b APC antibodies (eBioscience).

Plasmids, targeted mutagenesis and luciferase assays

C/EBP binding sites were mutated using QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA) using manufacturer's instructions. For luciferase assays K562 cells were transiently transfected by electroporation with 4 μg luciferase reporter vector carrying human *EVI2B* fragments, 0.5 μg pNull-Renilla, and increasing amounts of pCDNA3-*CEBPA* or its mutant forms. pCDNA3 was used to maintain a constant amount of DNA during transfection. 24 hours later luciferase and Renilla reporter activities in cell lysates were assessed using Dual-Luciferase Reporter Assay System, following manufacturer's

instructions (Promega, Madison, WI, USA). Luciferase signal was normalized to Renilla reporter activity.

Lentiviral vectors and transductions

For production of lentiviral particles HEK293T cells were co-transfected with shRNA carrying lentiviral vector pGhU6 and packaging plasmids pMD2.VSVG and pCMVdR8.74. For *Evi2b* knockdown in murine HSPC, sorted LKS cells were plated into StemSpan SFEM medium (Stemcell Technologies, Vancouver, BC, Canada) supplemented with the following cytokines: 10 ng/ml mIL-3, 20 ng/ml hIL-6, 100 ng/ml mSCF, 50 ng/ml mTPO, 100 ng/ml mFlt-3 ligand (all from PeproTech, Rocky Hill, NJ, USA). Virus was added to MOI of 40 for 6 hours. For transduction of 32D/G-CSF-R and NB4 cells lentivirus was added to MOI of 20 overnight. For *EVI2B* downregulation in primary human cells, CD34⁺ cord blood cells were kept in RPMI medium supplemented with 20% FBS (Thermo Fisher Scientific, Waltham, MA, USA), 1% penicillin/streptomycin, 10 ng/ml hIL-3, 20 ng/ml hIL-6, 50 ng/ml hSCF (all from PeproTech, Rocky Hill, NJ, USA). Virus was added to MOI of 20 for 6 hours. shRNA target sites in murine and human *EVI2B* mRNA can be found in Supplementary Table S4 and S5, respectively. All transductions were performed in the presence of polybrene (8 µg/ml) (Sigma-Aldrich, St. Louis, MO, USA).

Western blot analysis and antibodies

Single-cell suspensions (5×10^6) were solubilized (30 min. on ice) in lysis buffer containing 1% mild detergent laurylmaltoside (Calbiochem; Merck Millipore, Darmstadt, Germany), 20mM Tris (pH 8.2), 100mM NaCl, mixture of protease inhibitors (Protease Inhibitor Cocktail Set III, Calbiochem; Merck Millipore, Darmstadt, Germany), 10mM EDTA, 50mM NaF, 10mM pyrophosphate, 5mM iodoacetamide and 5mM diisopropyl fluorophosphate (Sigma-Aldrich, St.

Louis, MO, USA), nuclei were removed by centrifugation, the supernatant mixed 1:1 with reducing 2x SDS sample buffer and separated on 7% SDS-PAGE gels. Immunoblots were stained with mouse monoclonal antibody mEVI2B-06 (10 µg/ml). The hybridoma producing the mAb was generated by a standard protocol from splenocytes of F1(Balb/c x B10A) mice immunized by recombinant protein corresponding to the predicted intracellular domain of the protein (aa 402-421). Myc-tag was detected by monoclonal antibody 9E10 (Exbio Praha, Vestec, Czech Republic). Actin was detected by rabbit polyclonal antibody (1:1000 dilution; Sigma-Aldrich, St. Louis, MO, USA). α -tubulin was detected by monoclonal antibody TU-01 (P. Draber, Institute of Molecular Genetics, Prague, Czech Republic). Mouse neurofibromin (NF1) was detected by rabbit polyclonal antibody (dilution 1:500; Santa Cruz Biotechnology, Dallas, Texas, USA). Secondary antibodies were goat anti-mouse Ig-HRP and anti-rabbit-HRP (1:5000, both from Bio-Rad, Hercules, California, USA).

Cell cycle and apoptosis analysis

For cell cycle analysis cells were resuspended in phosphate-citrate buffer solution (pH 4.8) and kept at RT for 20 min. Afterwards, cells were stained with 1.5 µg/ml pyronin Y (Sigma-Aldrich, St. Louis, MO, USA) and 2 µg/ml Hoechst 33342 (Sigma-Aldrich, St. Louis, MO, USA). For apoptosis analysis were used Annexin V–Dy647 (Apronex, Vestec, Czech Republic) and Cleaved Caspase-3 Alexa Fluor 647 (D3E9) (Cell Signaling Technology, Danvers, MA, USA). Cells were stained with 1 µl of Annexin V–Dy647 per sample (dilution 1:100) for 15 minutes at RT protected from light. For Cleaved Caspase-3 staining cells were fixed with 4% paraformaldehyde and permeabilized with methanol according to manufacturer's protocol. Cells were stained with 1 µl of Cleaved Caspase-3 (dilution 1:50). Percentage of apoptotic cells was determined by flow cytometry.

Generation of *Evi2b* KO mice

Targeting vector for Evi2b⁻ allele. The targeting construct used for the disruption of the *Evi2b* gene consists of a self-excising ACN cassette³ that was introduced in place of exon 2 of the *Evi2b* gene and of two homology arms of 2.5 kb that bracket the deleted sequence. The targeting construct was abutted to a cassette coding for the diphtheria toxin fragment A.⁴

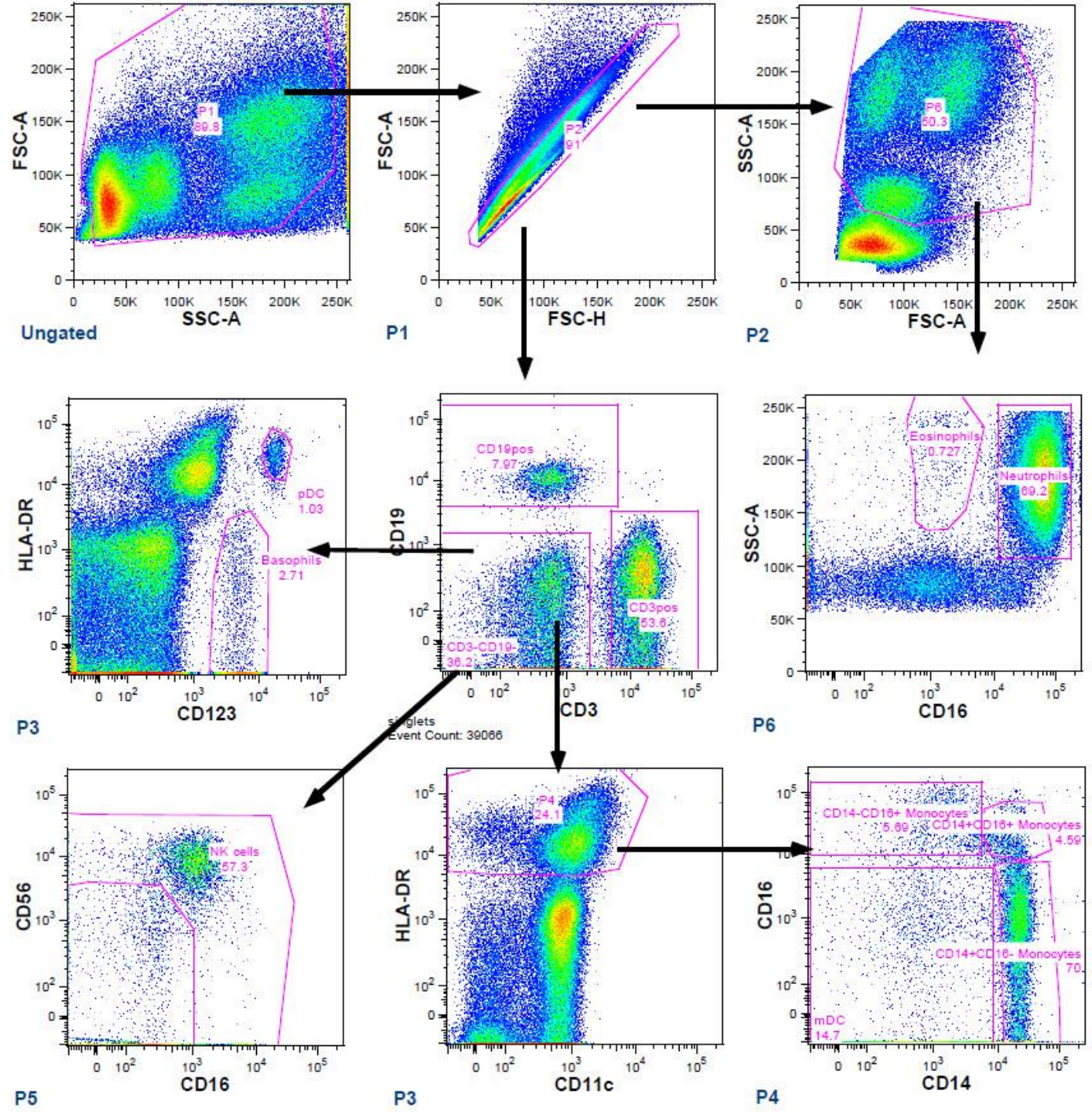
Isolation of recombinant embryonic stem (ES) cell clones. JM8.F6 C57BL/6N ES cells⁵ were electroporated with the targeting vector. After selection in G418, ES cell clones were screened for proper homologous recombination by Southern blot analysis. A *neo^r* specific probe was used to ensure that adventitious non-homologous recombination events have not occurred in the selected ES clones.

Production of knock-in mice. Mutant ES cells were injected into FVB blastocysts. The resulting chimeric males were bred with C57BL6/N females to obtain heterozygous F1 animals that were intercrossed to obtain homozygous mutant mice. Screening for the proper deletion of the ACN cassette was performed by PCR using the following pair of primers: sense 5'-agaagctgaggaacctagagggg-3' and antisense 5'-tgccataccacgaagacagga-3'. For genotyping the WT allele the following pair of primers was used: forward 5'-agaagctgaggaacctagagggg-3' and reverse 5'-gtgatggetgattggtcaaagtg-3', for genotyping the KO allele the following pair of primers was used: forward 5'-agaagctgaggaacctagagggg-3' and reverse 5'-tgccataccacgaagacagga-3'.

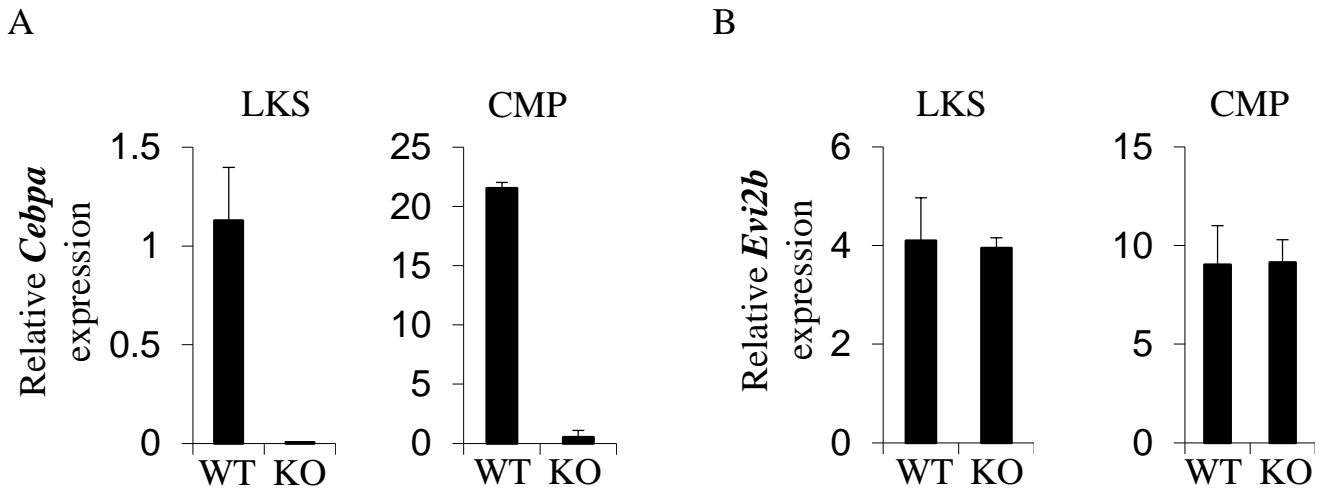
Supplementary References

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2. Liss A, Ooi CH, Zjablovskaja P, Benoukraf T, Radomska HS, Ju C, *et al.* The gene signature in CCAAT-enhancer-binding protein alpha dysfunctional acute myeloid leukemia predicts responsiveness to histone deacetylase inhibitors. *Haematologica* 2014; **99**: 697-705.
3. Bunting M, Bernstein KE, Greer JM, Capecchi MR, Thomas KR. Targeting genes for self-excision in the germ line. *Genes & development* 1999; **13**: 1524-8.
4. Soriano P. The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. *Development* 1997; **124**: 2691-700.
5. Pettitt SJ, Liang Q, Rairdan XY, Moran JL, Prosser HM, Beier DR, *et al.* Agouti C57BL/6N embryonic stem cells for mouse genetic resources. *Nature methods* 2009; **6**: 493-5.

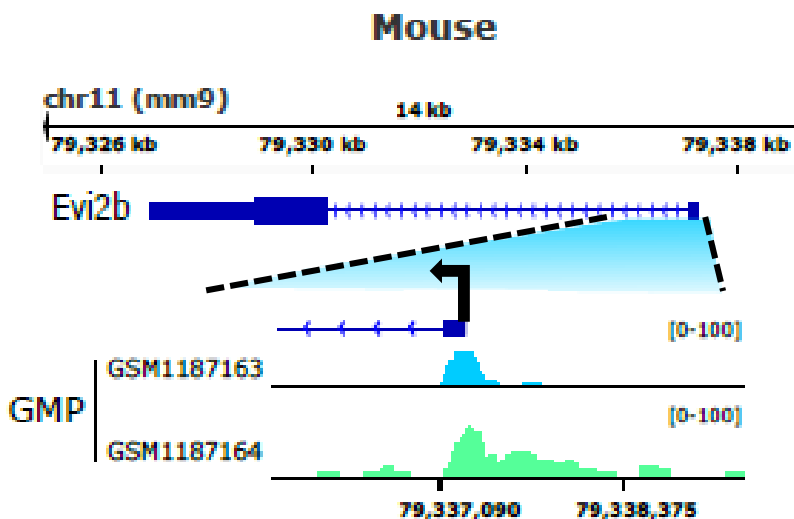
Supplemental Figures



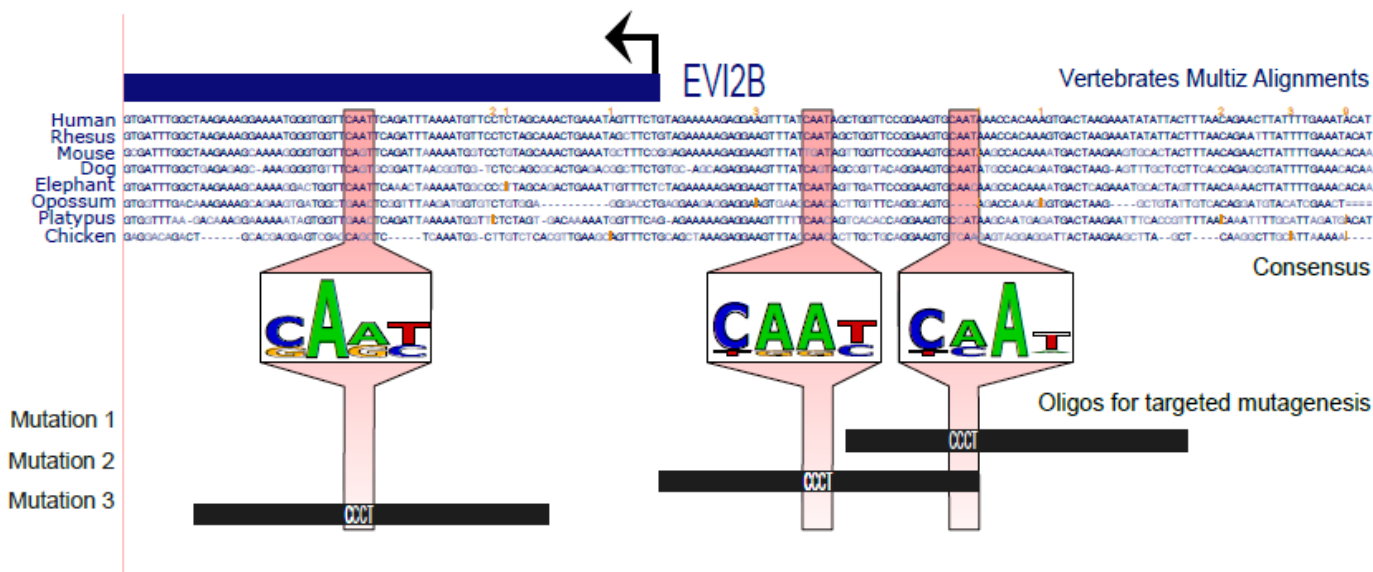
Supplementary Figure S1. Gating strategy defining distinct hematopoietic populations in human buffy coat. Basophils are defined as CD19⁻, CD3⁻, CD123⁺, HLA-DR⁻; eosinophils as CD16^{mid}, side scatter area ^{mid and high}; neutrophils as CD16⁺, side scatter area ^{mid and high}; monocytes as CD19⁻, CD3⁻, HLA-DR⁺, CD14⁺, CD16⁻; NK cells as CD19⁻, CD3⁻, CD56⁺, CD16⁺; B cells as CD19⁺, T cells as CD3⁺.



Supplementary Figure S2. *Evi2b* mRNA expression in *Cebpa* WT and KO mice. Quantitative RT-PCR in sorted murine LKS and CMP cells isolated from *Cebpa*^{loxP/loxP} MX1-CRE⁻ (WT) and *Cebpa*^{loxP/loxP} MX1-CRE⁺ (KO) mice treated with pI:pC. The y axis represents relative expression of (A) *Cebpa* and (B) *Evi2b* compared to *Gapdh*. Results represent the average and standard deviation of 3 WT and 3 KO mice.

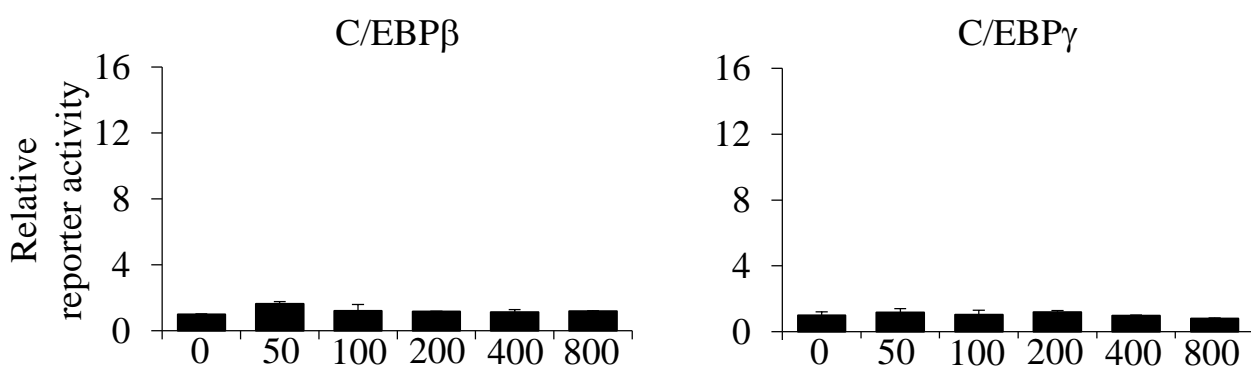


Supplementary Figure S3. Binding of endogenous C/EBP α to murine *Evi2b* promoter in GMP cells. Analysis of two ChIP-seq dataset from previous studies (GEO Ids: GSM1187163, GSM1187164) was performed. ChIP-seq data from mouse sorted GMP (granulocyte macrophage progenitor) cells showed C/EBP α binding sites within the *Evi2b* promoter (blue peak and green peak). Lower panel represents a zoom in the promoter region of *Evi2b*. Black arrow indicates transcriptional start site and direction of transcription.

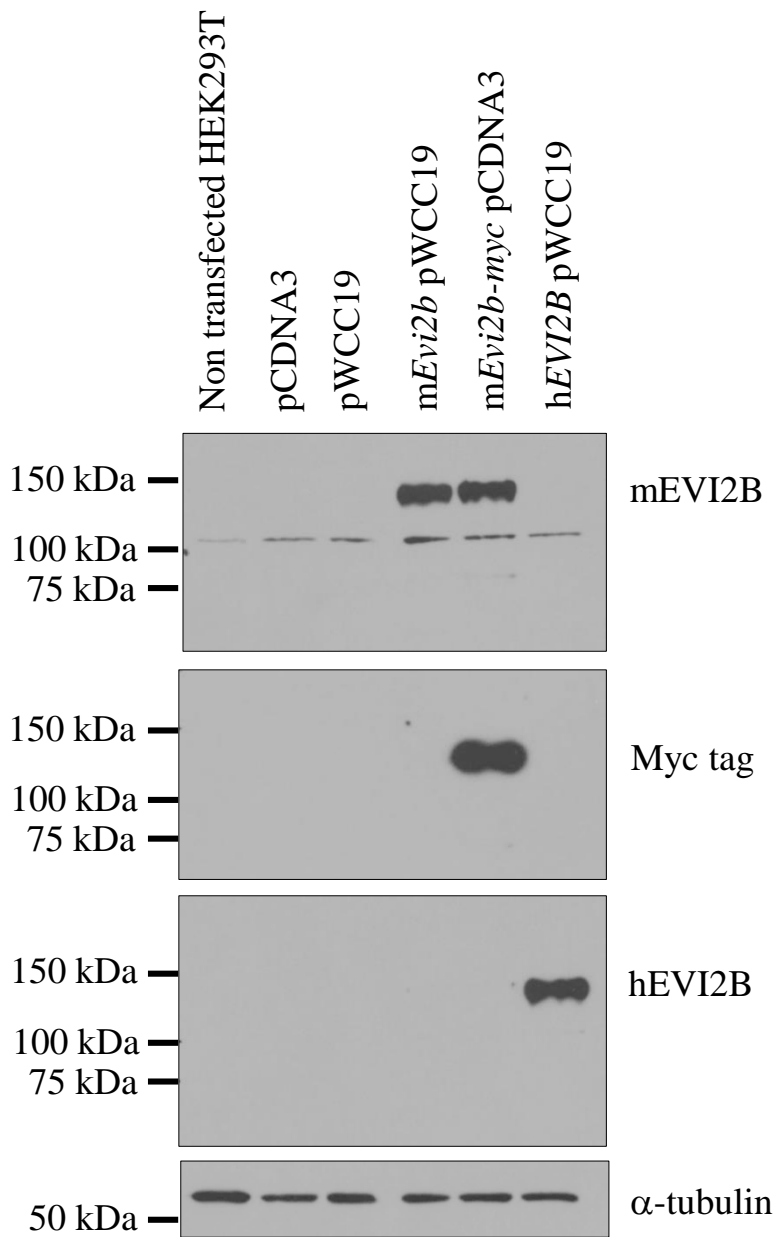


Supplementary Figure S4. Conservation of C/EBP consensus sites through 8 vertebrate genomes.

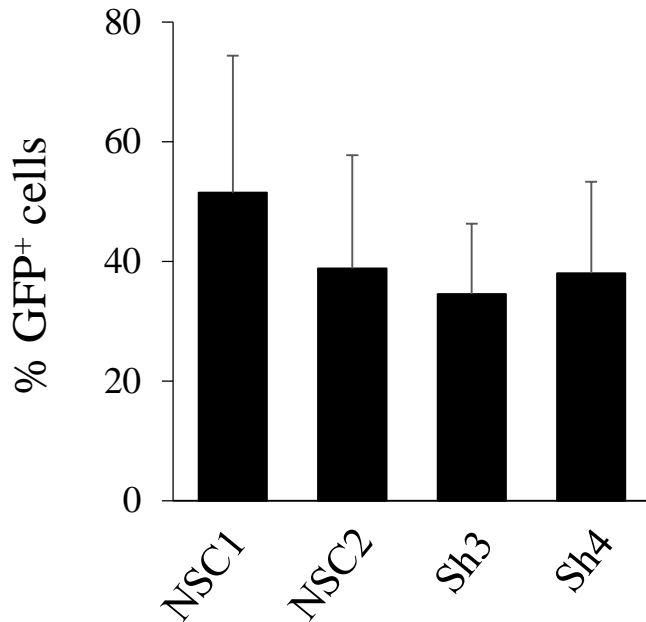
Conservation of C/EBP consensus (CAAT) in the promoter region of *EVI2B* through genomes of human, rhesus, mouse, dog, elephant, opossum, platypus and chicken. Arrow indicates *EVI2B* transcriptional start site and direction of transcription. The lower part of the Figure demonstrates the C/EBP consensus sequence conservation at the particular position, which is indicated by the overall height of each stack. Black rectangles with white sequence inside represent oligos used for mutagenesis of consensus site from CAAT to CCCT.



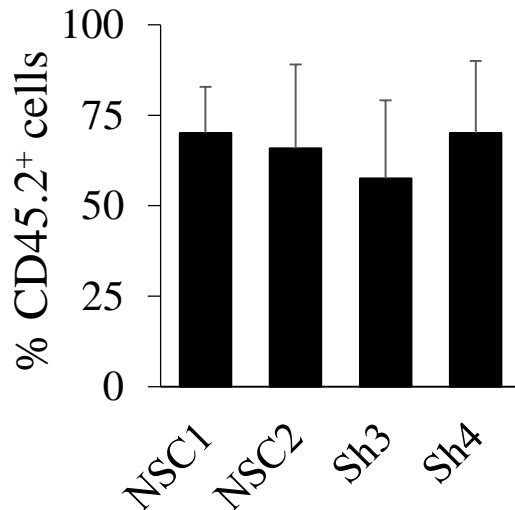
Supplementary Figure S5. C/EBP β and C/EBP γ do not transactivate *EVI2B* promoter. Luciferase reporter assay in K562 cells electroporated with human *EVI2B* peak1 construct and increasing amounts of C/EBP β (left panel) or C/EBP γ (right panel) expression plasmid. The y axis represents relative reporter activity compared to empty vector control.



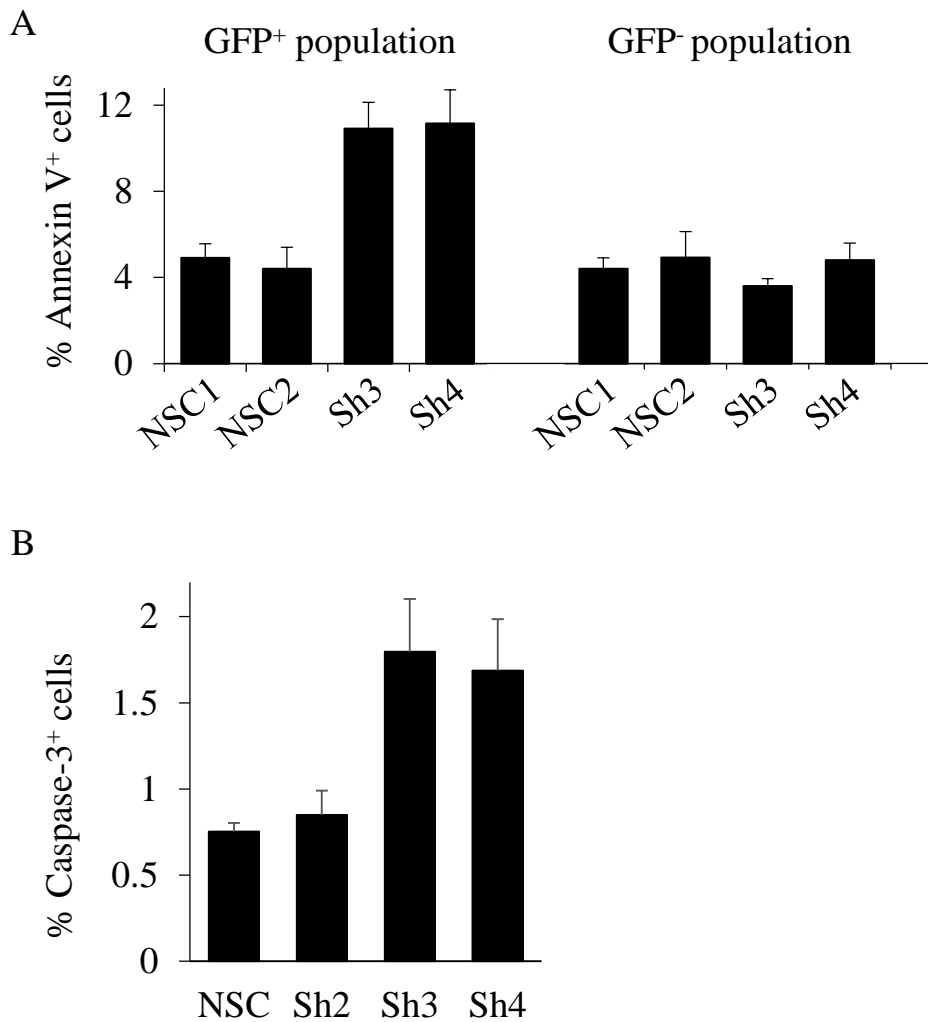
Supplementary Figure S6. Validation of murine and human EVI2B antibodies. Western blot analysis of HEK293T cells transfected with either empty vector pCDNA3, empty vector pWCC19, murine *Evi2b* pWCC19, murine *Evi2b-myc* pCDNA3, and human *EVI2B* pWCC19. Protein extracts were run in a SDS-PAGE and stained with antibodies against mouse EVI2B, human EVI2B, Myc tag, and α -tubulin. Membrane was stripped between staining with different antibodies. Positions of m.w. standards (kDa) are shown on the left.



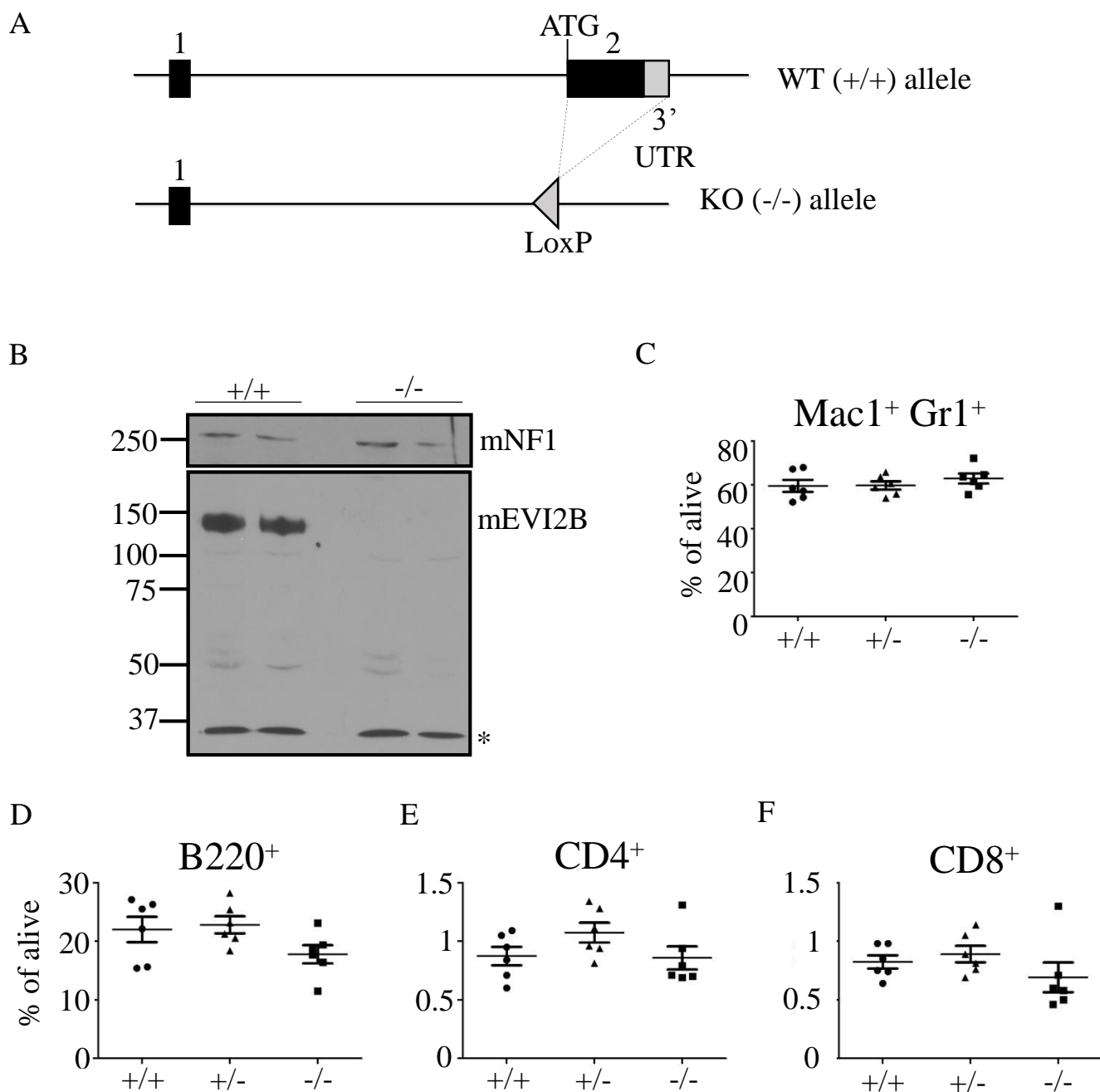
Supplementary Figure S7. Assessment of GFP positivity upon transduction of LKS cells. Flow cytometric analysis of LKS cells transduced with control (NSC1 and NSC2) or *Evi2b* shRNA constructs (Sh3 and Sh4). Analysis was done 2 days after transduction. Y axis indicates % of GFP⁺ cells. Each group represents the average of at least 4 independent transductions.



Supplementary Figure S8. Presence of CD45.2⁺ donor cells in peripheral blood 4 weeks after transplantation. Recipient mice transplanted with LKS cells transduced with control (NSC1 and NSC2) or *Evi2b* shRNA constructs (Sh3 and Sh4) were bled 4 weeks post transplantation. Flow cytometric analysis was performed using antibodies against CD45.2 (donor cells). Y axis indicates % CD45.2⁺ cells. Each group contains at least 10 animals.

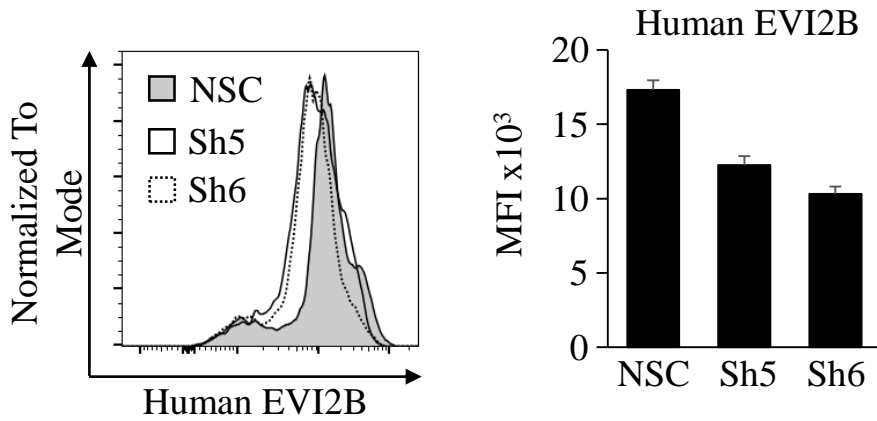


Supplementary Figure S9. *Evi2b* silencing increases apoptosis of HSPC. (A) Flow cytometric analysis of apoptosis in GFP⁺ and GFP⁻ populations in LKS cells transduced with the distinct shRNAs and plated into Methocult M3434 for 4 days. The y axis represents percentage of apoptotic Annexin V⁺ cells 6 days after transduction. Results represent the average of 3 independent experiments. (B) Flow cytometric analysis of apoptosis in LKS cells transduced with the distinct shRNA and plated into Methocult M3434 for 2 days. The y axis represents percentage of apoptotic Cleaved Caspase-3⁺ cells 4 days after transduction. Results represent the average of 3 independent experiments.

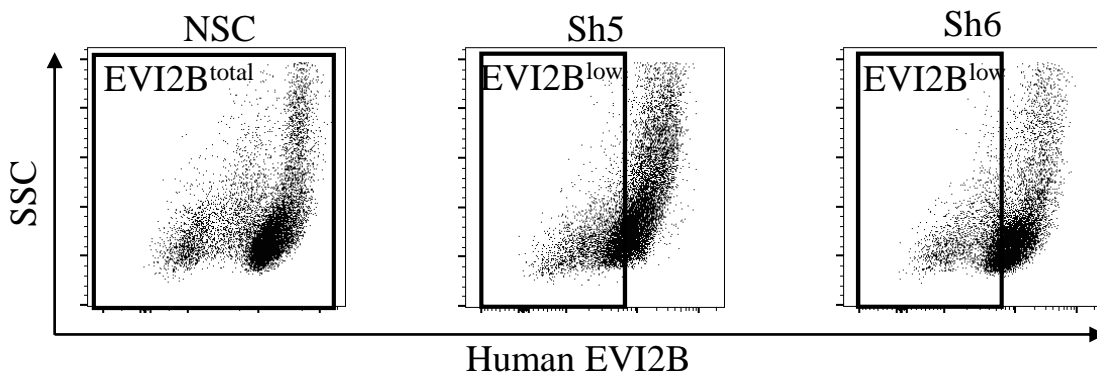


Supplementary Figure S10. Generation of *Evi2b* KO mice. (A) Schematic representation of the *Evi2b* WT (+/+; top) and *Evi2b* KO (-/-; bottom) alleles. Exons 1 and 2 are shown as boxes and the positions of the start codon (ATG) and 3' UTR are specified. The structure of the *Evi2b* KO allele is shown after CRE-mediated excision of the LoxP-flanked CAN cassette.³ (B) Western blot analysis of NF1 (upper panel) and EVI2B (lower panel) in *Evi2b* KO and WT murine BM. Non-specific band marked with star is used as a loading control. Positions of m.w. standards (kDa) are shown on the left. (C-F) Frequencies of (C) Mac1⁺/Gr1⁺ granulocytes, (D) B220⁺ B cells, (E) CD4⁺ T cells and (F) CD8⁺ T cells determined by flow cytometry in BM of *Evi2b* WT, heterozygous and KO mice. The y axis indicates percentage of cells. Each group contains at least 6 animals.

A



B



Supplementary Figure S11. Human *EVI2B* knockdown in CD34⁺ cord blood cells. (A) Flow cytometric analysis of human *EVI2B* expression in CD34⁺ cells after 10 days culture. Left panel shows a representative histogram demonstrating *EVI2B* levels in CD34⁺ cells transduced with control (NSC) or *EVI2B* downregulating constructs (Sh5, Sh6). The x axis represents fluorescence intensity. The y axis indicates number of cells normalized to mode. The right panel represents MFI (mean fluorescence intensity) of *EVI2B*. (B) Gating strategy used for experiments demonstrating impaired differentiation of CD34⁺ cells after *EVI2B* downregulation. Cells expressing low *EVI2B* levels in sh5 and sh6 transduced cells (*EVI2B*^{low}) were compared to *EVI2B*^{total} cells in NSC.