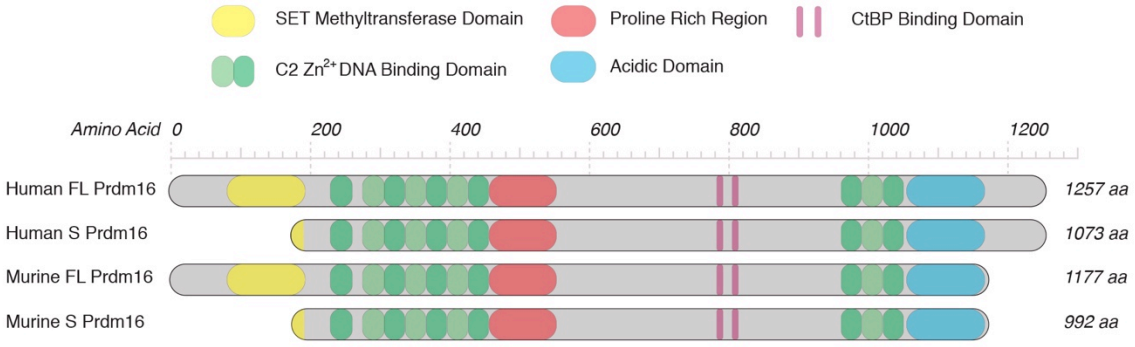


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HsSPRDM16       ------------------------------------------------------------------------------------------------------ 0
MsSPRDM16       ------------------------------------------------------------------------------------------------------ 0

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MsFPRDM16       KRKMEIGERFGPYVVTPRAALKEADFGWEQMLTDTEVSSQESCIKKQISEDLGSEKFCVDANQAGSGSWLKYIRVACSCDDQNLAMCQINEQIYYKVIKD 200
HsSPRDM16       ------------------------------------------------------------------------------MCQISEQIYYKVIKD 15
MsSPRDM16       ------------------------------------------------------------------------------MCQINEQIYYKVIKD 15
                                                                                                 ****.********** 

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MsFPRDM16       IEPGEELLVHVKEGAYSLGVMAPSLDEDPTFRCDECDELFQCRLDLRRHKKYACSSAGAQLYEGLGEELKPEGLGVGSDGQAHECKDCERMFPNKYSLEQ 300
HsSPRDM16       IEPGEELLVHVKEGVYPLGTVPPGLDEEPTFRCDECDELFQSKLDLRRHKKYTCGSVGAALYEGLAEELKPEGLGGG-SGQAHECKDCERMFPNKYSLEQ 114
MsSPRDM16       IEPGEELLVHVKEGAYSLGVMAPSLDEDPTFRCDECDELFQCRLDLRRHKKYACSSAGAQLYEGLGEELKPEGLGVGSDGQAHECKDCERMFPNKYSLEQ 115
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HsFPRDM16       HMVIHTEEREYKCDQCPKAFNWKSNLIRHQMSHDSGKRFECENCVKVFTDPSNLQRHIRSQHVGARAHACPDCGKTFATSSGLKQHKHIHSTVKPFICEV 398
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MsFPRDM16       IAEKYFGPGFMSMQEKKLGSLPYHSVFPFQFLPNFPHSLYPFTDRALAHNLLVKAEPKSPRDALKVGGPSAECPFDLTTKPKEAKPALLAPKVPLIPSSG 800
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MsSPRDM16       IAEKYFGPGFMSMQEKKLGSLPYHSVFPFQFLPNFPHSLYPFTDRALAHNLLVKAEPKSPRDALKVGGPSAECPFDLTTKPKEAKPALLAPKVPLIPSSG 615
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HsFPRDM16       EEQPLDLSIGSRARASQNGGGREPRKNHVYGERKLGAGEGLPQVCPARMPQQPPLHYAKPSPFFMDPIYSRVEKRKVTDPVGALKEKYLRPSPLLFHPQM 898
MsFPRDM16       EEQPLDLSIGSRARASQNGGGREPRKNHVYGERKPGVSEGLPKVCPAQLPQQPSLHYAKPSPFFMDPIY-RVEKRKVADPVGVLKEKYLRPSPLLFHPQM 899
HsSPRDM16       EEQPLDLSIGSRARASQNGGGREPRKNHVYGERKLGAGEGLPQVCPARMPQQPPLHYAKPSPFFMDPIYSRVEKRKVTDPVGALKEKYLRPSPLLFHPQM 714
MsSPRDM16       EEQPLDLSIGSRARASQNGGGREPRKNHVYGERKPGVSEGLPKVCPAQLPQQPSLHYAKPSPFFMDPIY-RVEKRKVADPVGVLKEKYLRPSPLLFHPQM 714
                ********************************** *..****:****::****.*************** *******:****.***************** 

HsFPRDM16       SAIETMTEKLESFAAMKADSGSSLQPLPHHPFNFRSPPPTLSDPILRKGKERYTCRYCGKIFPRSANLTRHLRTHTGEQPYRCKYCDRSFSISSNLQRHV 998
MsFPRDM16       SAIETMTEKLESFAAMKADSGSSLQPLPHHPFNFRSPPPTLSDPILRKGKERYTCRYCGKIFPRSANLTRHLRTHTGEQPYRCKYCDRSFSISSNLQRHV 999
HsSPRDM16       SAIETMTEKLESFAAMKADSGSSLQPLPHHPFNFRSPPPTLSDPILRKGKERYTCRYCGKIFPRSANLTRHLRTHTGEQPYRCKYCDRSFSISSNLQRHV 814
MsSPRDM16       SAIETMTEKLESFAAMKADSGSSLQPLPHHPFNFRSPPPTLSDPILRKGKERYTCRYCGKIFPRSANLTRHLRTHTGEQPYRCKYCDRSFSISSNLQRHV 814
                ********************************************************************************************** 

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MsFPRDM16       RNIHNKEKPFKCHLCNRCFGQQTNLDRHLKKHEHEGAPVSQHSGVLTNHLGTSASSPTSESDNHALLDEKEDSYFSEIRNFIANSEMNQASTRMDKRPEI 1099
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MsSPRDM16       RNIHNKEKPFKCHLCNRCFGQQTNLDRHLKKHEHEGAPVSQHSGVLTNHLGTSASSPTSESDNHALLDEKEDSYFSEIRNFIANSEMNQASTRMDKRPEI 914
                **********************************.******.************************************************** :**.::: 

HsFPRDM16       QIVDGSAQCPGLASEKQEDVEEEDDDDLEEDDEDSLAGKSQDDTVSPAPEPQAAYEDEEDEEPAASLAVGFDHTRRCAEDHEGGLLALEPMPTFGKGLDL 1198
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HsSPRDM16       QIVDGSAQCPGLASEKQEDVEEEDDDDLEEDDEDSLAGKSQDDTVSPAPEPQAAYEDEEDEEPAASLAVGFDHTRRCAEDHEGGLLALEPMPTFGKGLDL 1014
MsSPRDM16       QDLDSNPPCPGSASAKPEDVEEEEEEELEEEDDDSLAGKSQEDTVSPTPEPQGVYEDEEDEEPPS-LTMGFDHTRRHMQ---------------------- 992
                * :*... *** ** * ******::::***:*:********:*****:****..*********.: *::*******  : 

HsFPRDM16       RRAAEEAFEVKDVLNSTLDSEALKHTLCRQAKNQGSLDAWLKVTGATSESGAFHPINHL 1257
MsFPRDM16       ------------------------------------------------------------ 1177
HsSPRDM16       RRAAEEAFEVKDVLNSTLDSEALKHTLCRQAKNQGSLDAWLKVTGATSESGAFHPINHL 1073
MsSPRDM16       ------------------------------------------------------------ 992
```

Figure S1: Genomic structure and conservation of PRDM16. Alignment of primary amino acid sequences of short and full PRDM16 isoforms from human and mouse. Alignment performed using ClustalW2 multiple sequence alignment. Asterisks (*****) indicate fully-conserved residues, colons (**:**) indicate strong conservation with scoring of >0.5 using the PAM250 matrix. Periods (**.**) represent weak conservation with scoring of =<0.5. Conserved domains are colored as follows – SET methyltransferase (PR) domain (yellow), Zn-finger DNA binding domains (green, two hues to indicate distinct adjacent domains), proline-rich domain (red), CtBP biding domain motifs (fully-conserved PFDLT and PLDLS sequences) (violet), acidic domain (blue). Of note is the absence of nearly all of the PR domain in the short mouse and human isoforms and the strong conservation overall between human and mouse.

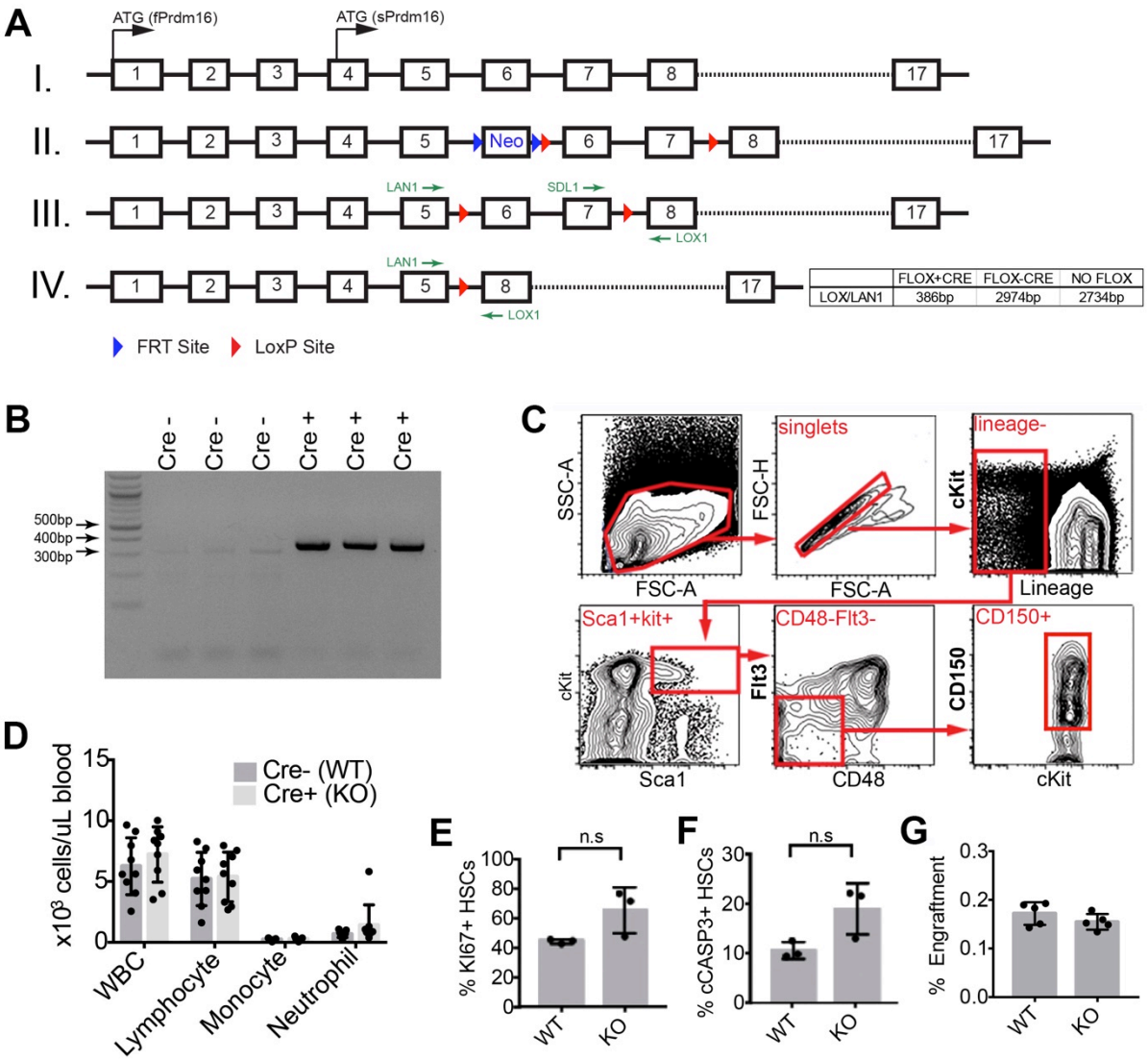
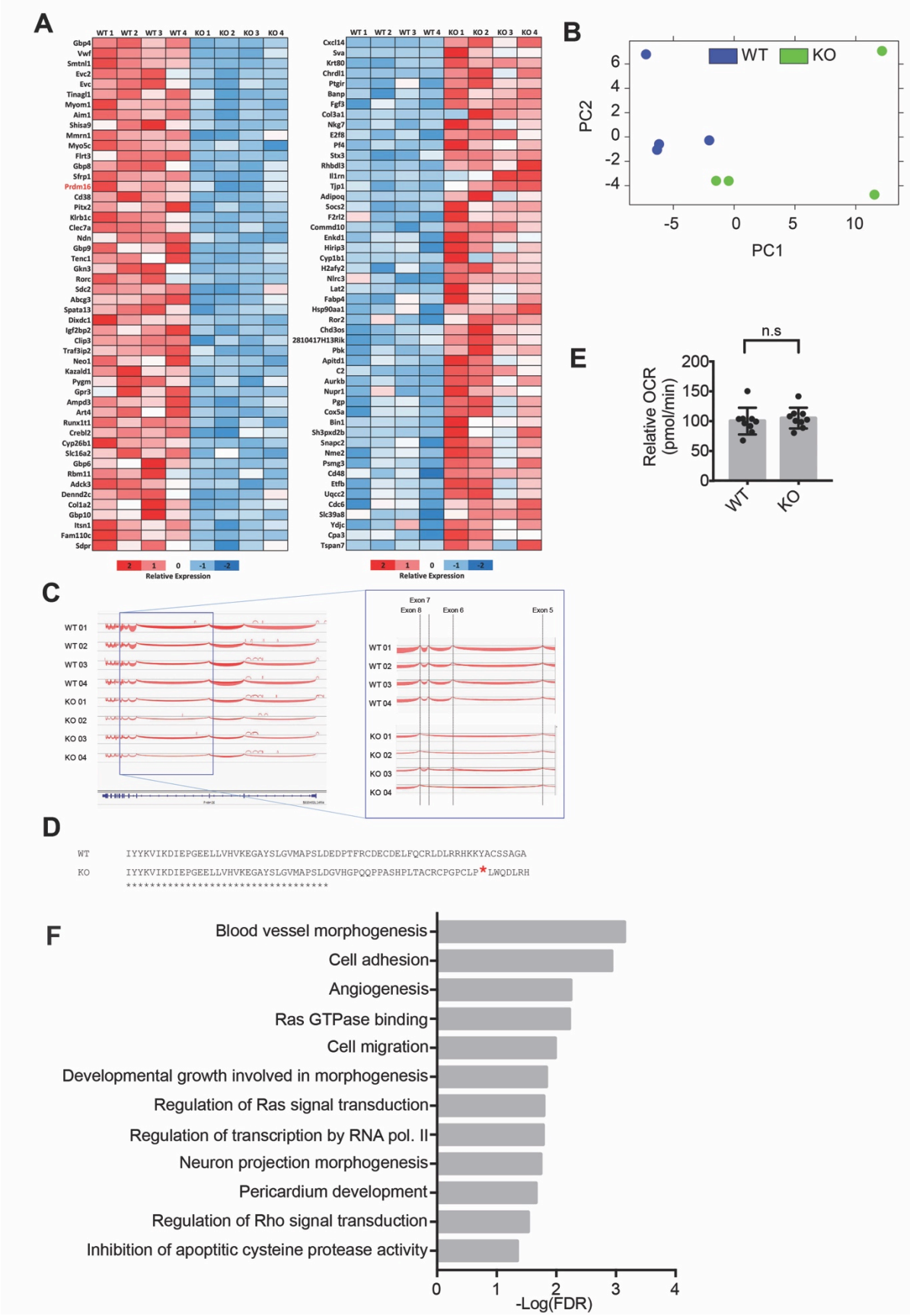


Figure S2: Generation and phenotype of *Prdm16*$^{fl/fl}$*.Vav-Cre* mice. (**A**) Schematic representation of the generation of *Prdm16*$^{fl/fl}$ mice – I. Map of *Prdm16* exons and introns, including the two start codons for *fPrdm16* and *sPrdm16*, respectively. II. Insertion of a Neomycin-resistance cassette flanked by FRT sites, and of LoxP sites around exons 6 and 7. III. Map of the *Prdm16*$^{fl/fl}$ genomic structure after removal of the Neo cassette by crossing to a FLP-recombinase$^{+/-}$ mouse, with the highlighting (in green) of LAN1, SDL1, and LOX1 primers for use in genotyping. IV. Deletion of *Prdm16* exon 6 and 7 in the presence of Cre recombinase, with LAN1/LOX1 locations and a table illustrating differences in LOX1/LAN1 amplicon length with or without genomic excision. **(B)** LOX1/LAN1 PCR of hematopoietic PB cells from three Cre- and three Cre+ mice showing the presence of a 380bp amplicon in the excised mice. (**C**) Gating scheme of adult BM HSCs (Lin$^-$cKit$^+$Sca$^+$Flt3$^-$CD48$^-$CD150$^+$) (**D**) Blood counts for white blood cells (WBC), lymphocytes, monocytes and neutrophils in peripheral blood of *Vav-Cre*$^{-/-}$ *Prdm16*$^{fl/fl}$ (WT) and *Vav-Cre*$^{+/-}$ *Prdm16*$^{fl/fl}$ (KO) mice. (*n* = 9). (**E**) Percentage of KI67$^+$ and (**F**) Cleaved caspase3$^+$ BM HSCs from WT and KO mice (*n* = 3). (**G**) Percent of WT or KO donor CD45.2 cells in BM of recipient mice 24-hours post-transplant. (*n* = 5 recipient mice). (n.s. = *P* > 0.05).


A
WT 1 WT 2 WT 3 WT 4 KO 1 KO 2 KO 3 KO 4
Gbp4
Vwf
Smtnl1
Evc2
Evc
Tinagl1
Myom1
Aim1
Shisa9
Mmrn1
Myo5c
Flrt3
Gbp8
Sfrp1
Prdm16
Cd38
Pitx2
Klrb1c
Clec7a
Ndn
Gbp9
Tenc1
Gkn3
Rorc
Sdc2
Abcg3
Spata13
Dixdc1
Igf2bp2
Clip3
Traf3ip2
Neo1
Kazald1
Pygm
Gpr3
Ampd3
Art4
Runx1t1
Crebl2
Cyp26b1
Slc16a2
Gbp6
Rbm11
Adck3
Dennd2c
Col1a2
Gbp10
Itsn1
Fam110c
Sdpr
Cxcl14
Sva
Krt80
Chrdl1
Ptgir
Banp
Fgf3
Col3a1
Nkg7
E2f8
Pf4
Stx3
Rhbdl3
Il1rn
Tjp1
Adipoq
Socs2
F2rl2
Commd10
Enkd1
Hirip3
Cyp1b1
H2afy2
Nlrc3
Lat2
Fabp4
Hsp90aa1
Ror2
Chd3os
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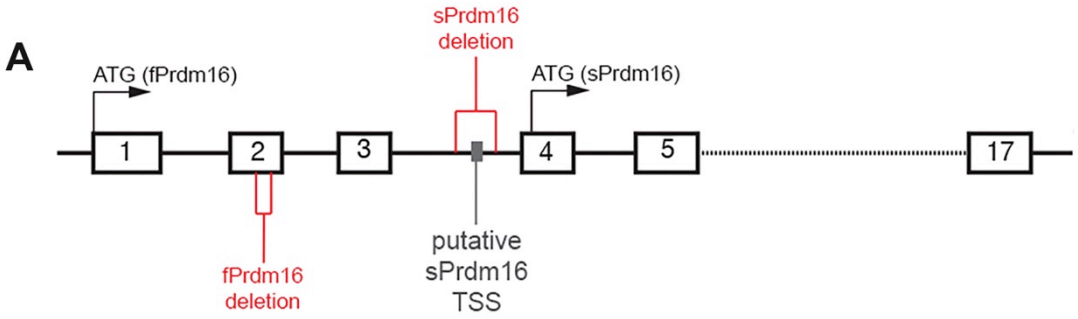
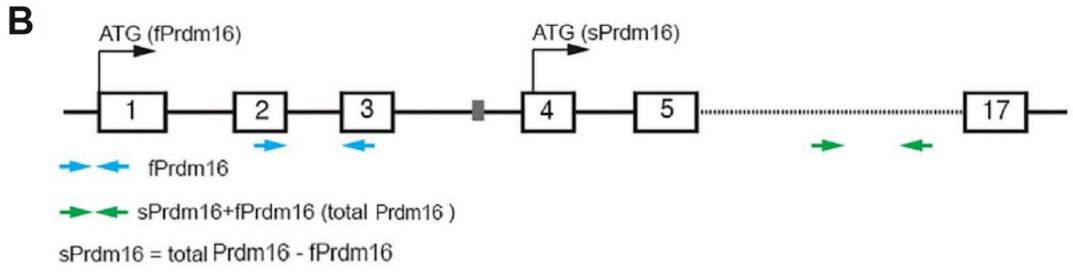
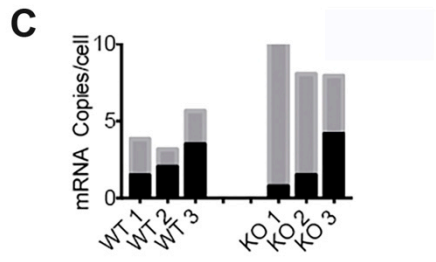
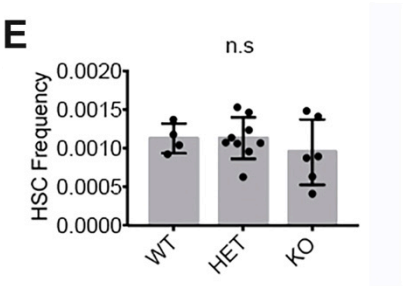
Pbk
Apitd1
C2
Aurkb
Nupr1
Pgp
Cox5a
Bin1
Sh3pxd2b
Snapc2
Nme2
Psmg3
Cd48
Etfb
Uqcc2
Cdc6
Slc39a8
Ydjc
Cpa3
Tspan7
2 1 0 -1 -2
Relative Expression
B
WT
KO
PC2
PC1
E
n.s
Relative OCR (pmol/min)
WT
KO
C
WT 01
WT 02
WT 03
WT 04
KO 01
KO 02
KO 03
KO 04
Exon 7
Exon 8
Exon 6
Exon 5
D
WT IYYKVIKDIEPGEELLVHVKEGAYSLGVMAPSLDEDPTFRCDECDELFQCRLDLRRHKKYACSSAGA
KO IYYKVIKDIEPGEELLVHVKEGAYSLGVMAPSLDGVHGPQQPPASHPLTACRCPGPCLP*LWQDLRH
F
Blood vessel morphogenesis
Cell adhesion
Angiogenesis
Ras GTPase binding
Cell migration
Developmental growth involved in morphogenesis
Regulation of Ras signal transduction
Regulation of transcription by RNA pol. II
Neuron projection morphogenesis
Pericardium development
Regulation of Rho signal transduction
Inhibition of apoptitic cysteine protease activity
-Log(FDR)

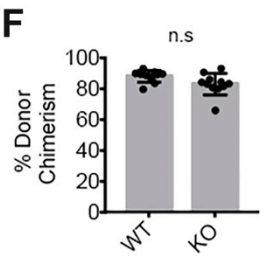
Figure S3: Genome-wide expression analysis of *Prdm16$^{fl/fl}$.Vav-Cre* mice (**A**) Heatmap of the top 50 genes (by *P*-value) up/downregulated in *Vav-Cre$^{+/-}$ Prdm16$^{fl/fl}$* (KO) BM HSCs and WT littermate BM HSC samples, as determined by RNAseq. (**B**) Principal component analysis (PCA) of individual WT and KO RNAseq samples (**C**) Quantification of *Prdm16* exons in RNAseq analysis of *Prdm16$^{fl/fl}$.Vav-Cre* and WT littermate HSCs using the Integrative Genomics Viewer (IGV) showing absence of reads at exons 6 and 7 in KO samples. (**D**) Analysis of *Prdm16$^{fl/fl}$.Vav-Cre* and WT littermate PRDM16 amino acid sequences showing a frameshift and early termination 25 amino acids downstream of the C-terminus of exon 5. (**E**) Basal oxygen consumption rate (OCR) of WT and KO mouse embryonic fibroblasts (MEFs) (n = 3 experiments with technical triplicates) (n.s. = P > 0.05) (**F**) GO pathways significantly downregulated in sorted Lin$^-$cKit$^+$Sca$^+$Flt3$^-$CD48$^-$ *Prdm16$^{fl/fl}$.Vav-Cre* FL HSCs compared to WT littermates. Values expressed as -Log$_{10}$ of the P-value, determined by PANTHER RNAseq analysis.











D

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WT          AGATTAGAGCTTACCATAGGCACTGAGCAAAGCAGGCAAGAGCTGGAAAGGTCTATTGCACCTAAAGCTGCCAGCCCGTGGTCCAGGTCTTCTTACAAGC    -656
SLTSS KO    AGATTAGAGCTTACCATAGGCACTGAGCAAAGCAGGCAAGAGCTGGAAAGGTCTATTGCACCTAAAGCTGCCAGCCCGTGGTCCAGGTCTTCTTACAAGC    -656

Intron3     AACCTCGGGGTGGGGGTGGGGAGACAGAGATCAGCAAACCAGTCTCTGCAGCTAAGTTCAGAAGATAGCATCAACAACAAAGAGAATGAGCAGAACACGG    -556
WT          AACCTCGGGGTGGGGGTGGGGAGACAGAGATCAGCAAACCAGTCTCTGCAGCTAAGTTCAGAAGATAGCATCAACAACAAAGAGAATGAGCAGAACACGG    -556
SLTSS KO    AACCTCGGGGTGGGGG----------------------------------------------------------------------------------    -556

                                                                                    Putative S-PRDM16 TSS
Intron3     TGAGGAAGCTGAGCATCTGTGTGCTGGCCAGGGCCAAACTGAGACTTTCCAGTTGAAAGGAAACAATGTTTTCTGGCATTCAGGTGGTGTGATGTCCGAC    -456
WT          TGAGGAAGCTGAGCATCTGTGTGCTGGCCAGGGCCAAACTGAGACTTTCCAGTTGAAAGGAAACAATGTTTTCTGGCATTCAGGTGGTGTGATGTCCGAC    -456
SLTSS KO    ----------------------------------------------------------------------------------------------------    -456

Intron3     AGGAGCAGGTACACTCAGGCAAAAGTGTTGTGCACGCACAGGCTCACACACCAGGACTTGGGTGCAGCCACACACCCTCATAACACCTTTAGCTTCAGAC    -356
WT          AGGAGCAGGTACACTCAGGCAAAAGTGTTGTGCACGCACAGGCTCACACACCAGGACTTGGGTGCAGCCACACACCCTCATAACACCTTTAGCTTCAGAC    -356
SLTSS KO    ----------------------------------------------------------------------------------------------------    -356

Intron3     ATGCTCTTTCTAGGTCAACACCCCACTGTCTTTGGGGGGTCATGTCAGACACAAGAAGGCTCTGCTTTCTCACACCTGGACAGCCTACTGGAAGGTTTTA    -256
WT          ATGCTCTTTCTAGGTCAACACCCCACTGTCTTTGGGGGGTCATGTCAGACACAAGAAGGCTCTGCTTTCTCACACCTGGACAGCCTACTGGAAGGTTTTA    -256
SLTSS KO    ----------------------------------------------------------------------------------------------------    -256

Intron3     CTGTGCTAGAGACTCGTCATTGCCTAACCTGACTGAAAGCTATTCTCTACGAACTAGAAAGGCCACCTGCTTCTCTCCTCCAATCTCCTGGAGGTTCCTA    -156
WT          CTGTGCTAGAGACTCGTCATTGCCTAACCTGACTGAAAGCTATTCTCTACGAACTAGAAAGGCCACCTGCTTCTCTCCTCCAATCTCCTGGAGGTTCCTA    -156
SLTSS KO    ----------------------------------------------------------------------------------------------------    -156

Intron3     GGACCAGGTGGCCTGGGTGGACAGAGTCTCTCCTTGCCTAGAACTGGGTGAGGTCTTAGACAACCTGCCATCCTGCCTGACCCCCGAGGCCAGTACAGCG    -56
WT          GGACCAGGTGGCCTGGGTGGACAGAGTCTCTCCTTGCCTAGAACTGGGTGAGGTCTTAGACAACCTGCCATCCTGCCTGACCCCCGAGGCCAGTACAGCG    -56
SLTSS KO    ------------------------------------------------------------CCATCCTGCCTGACCCCCGAGGCCAGTACAGCG    -56

Intron3     TGATATAACACACACAGGACAGGCACCTCTGACCTCAGGTGTGTTTCTTCCCCAG    0
WT          TGATATAACACACACAGGACAGGCACCTCTGACCTCAGGTGTGTTTCTTCCCCAG    0
SLTSS KO    TGATATAACACACACAGGACAGGCACCTCTGACCTCAGGTGTGTTTCTTCCCCAG    0
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Figure S4: Targeting of *sPrdm16* TSS. (**A**) Schematic representation of regions targeted in CRISPR/Cas9 *Prdm16* isoform-deletion experiment, highlighting exon structure, *fPrdm16* and *sPrdm16* start codons, and putative *sPrdm16* TSS in intron 3. (**B**) Schematic of subtractive qPCR used to determine copy number of *sPrdm16* mRNA. Probes in exon 2/3 are specific for *fPrdm16* and probes in exon14/15 are used to calculate total *Prdm16*. *sPrdm16* is calculated by subtracting *fPrdm16* from *tPrdm16*. (**C**) Quantification based on subtractive qPCR of *Prdm16* isoform copy number from mice deleted for the *sPrdm16* putative TSS (KO) compared to WT littermates. (**D**) FL HSC frequency in WT, KO, or TSS-heterozygous (HET) mice. (**E**) Peripheral blood (PB) donor chimerism of transplanted WT or KO BM HSCs in competitive transplants with CD45.1 BM, measured 16-weeks post-transplant (*n* = 10-11 recipients from 3 independent experiments). (**F**) Alignment of sequencing results from intron 3 of a WT mouse and a putative *sPrdm16-TSS KO* (SLTSS KO). Sequencing results are aligned to the final 755 nucleotides of *Prdm16* intron 3 and illustrate loss of the putative *sPrdm16* TSS from the SLTSS KO mice, highlighted in yellow.

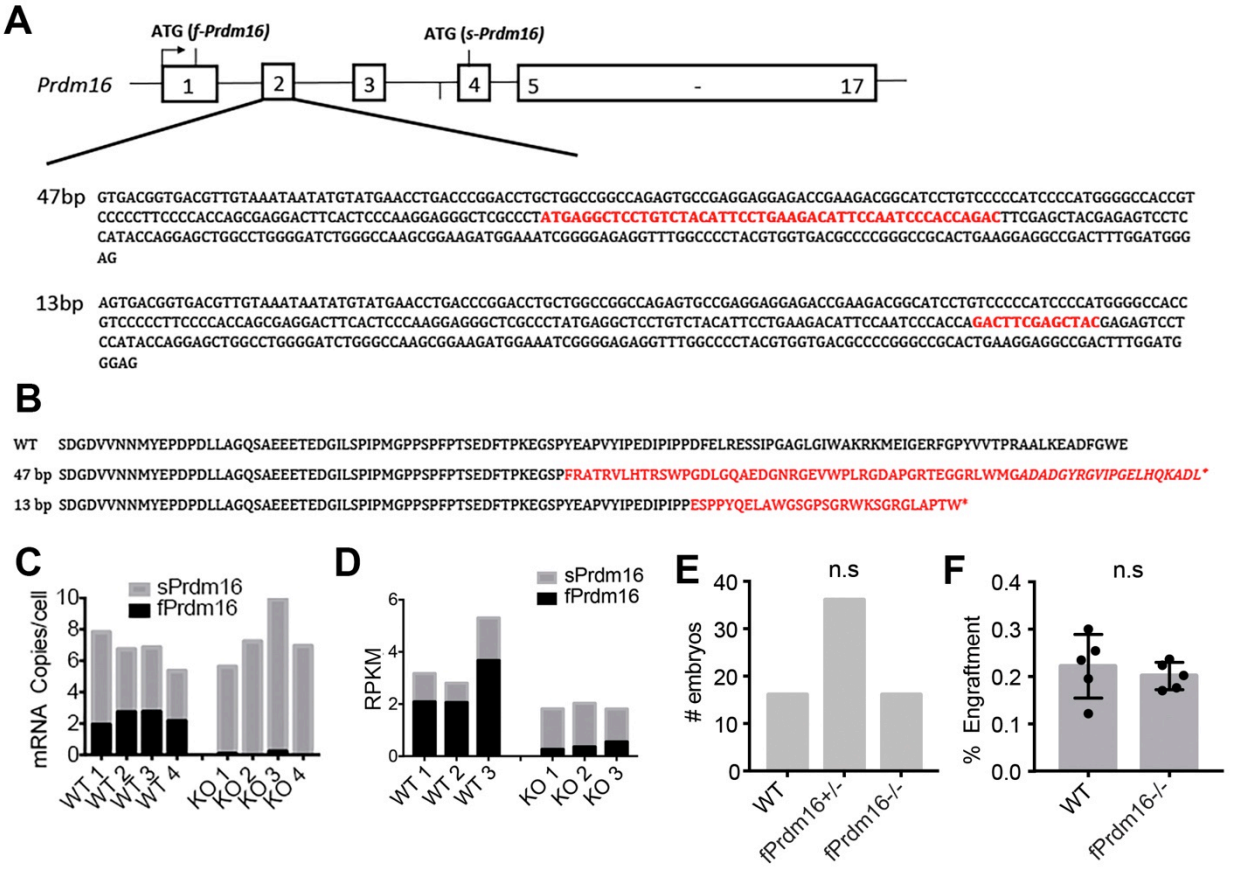

Figure S5: Generation of *fPrdm16*$^{-/-}$ mice. (**A**) Map of the 47bp and 13bp deletions (in red) obtained from mice using CRISPR/Cas9 pronuclear injection. (**B**) Comparison of the frameshifted amino acid sequence (red) leading to premature stop codons in both mice. (**C**) Subtractive qPCR quantification of *Prdm16* isoform mRNA copy number from *Δ47-fPrdm16*$^{-/-}$ mice (KO) and WT littermates shows selective loss of *fPrdm16* in the mutants. (**D**) Calculation of *fPrdm16* and *sPrdm16* RPKM obtained by subtracting Exon 1-3 RPKM (specific *fPrdm16* RPKM) from Exon 1-17 RPKM (total *Prdm16*) to calculate *sPrdm16* RPKM. (C). (**E**) Mendelian distribution of E13-15 embryos of *Δ47-fPrdm16* mice (n = 62). (**F**) Percent donor CD45.2 cells in BM of recipient mice 24 hours after transplantation of FL cells from either *Δ47-fPrdm16* (fPrdm16$^{-/-}$) or WT littermates. (n = 5 recipient mice). (n.s. = $P > 0.05$)

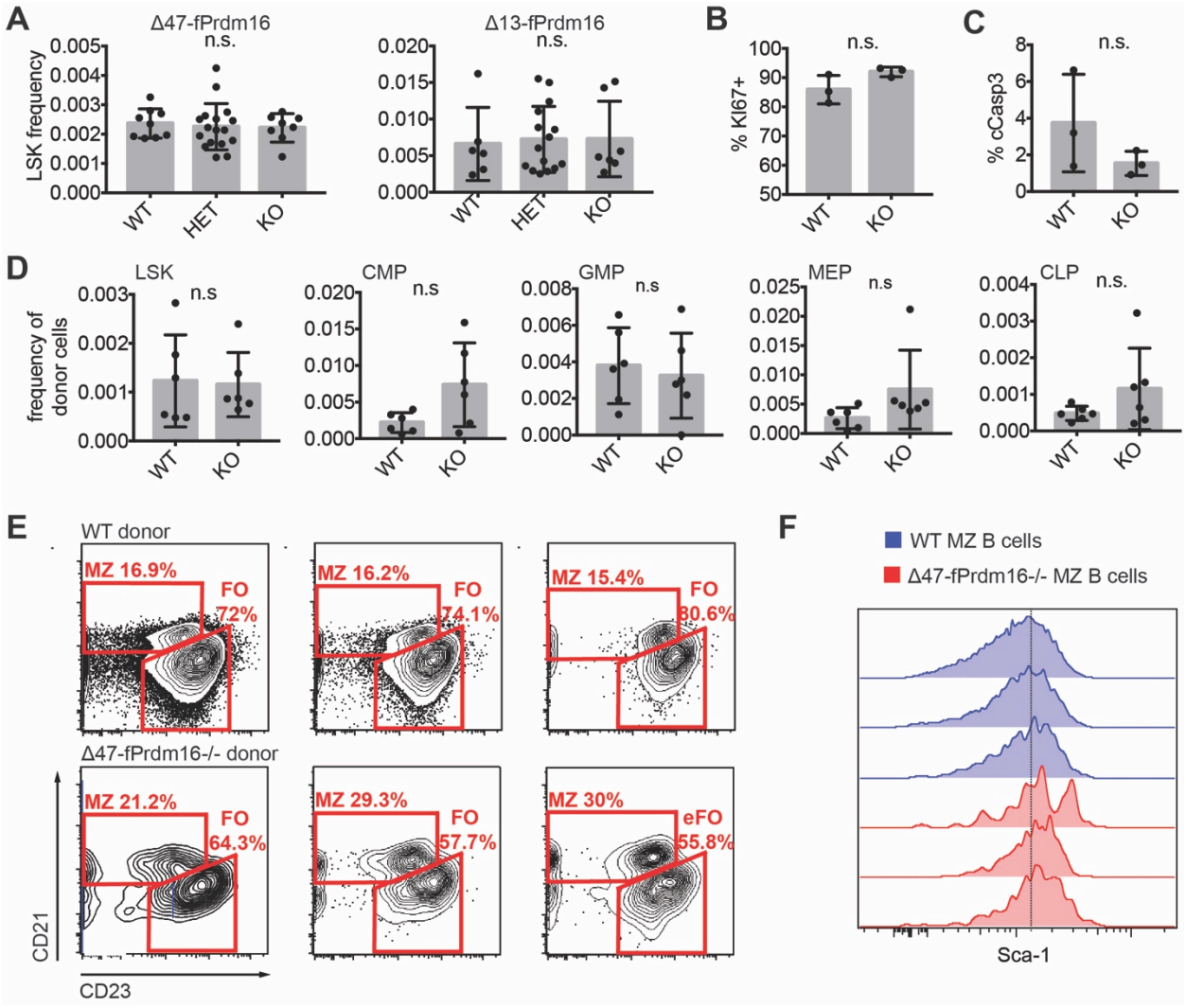

Figure S6: Function of *fPrdm16*$^{-/-}$ HSCs. (**A**) LSK frequency in FL of *fPrdm16*$^{+/+}$ (WT), *fPrdm16*$^{+/-}$ (HET), and *fPrdm16*$^{-/-}$ (KO) mice from *Δ47-fPrdm16*$^{-/-}$ or *Δ13-fPrdm16*$^{-/-}$ mice. (**B**) Percent KI67^{+} and (**C**) cleaved Caspase3^{+} FL HSCs in *Δ47-fPrdm16*$^{-/-}$ and WT littermate embryos (*n* = 3). (**D**) Donor repopulation in progenitor populations 16 weeks after competitive transplantation of *Δ47-fPrdm16*$^{-/-}$ or WT littermate FL cells, expressed as percent of CD45.2 donor cells within a progenitor population: LSK (Lin^{-}Sca1^{+}cKit^{+}), CMP (Lin^{-}Sca1^{-}cKit^{+}CD34^{+}CD16/32^{lo}), GMP (Lin^{-}Sca1^{-}cKit^{+}CD34^{+}CD16/32^{mid}), MEP (Lin^{-}Sca1^{-}cKit^{+}CD34^{-}CD16/32^{lo}), and CLP (Lin^{-}Sca1^{lo}cKit^{lo}IL7ra^{+}Flt3^{+}) (*n* = 6 recipients from 2 independent transplants). (**E**) Flow cytometry plots of donor-derived splenic B-cells 16 weeks after competitive transplantation of *Δ47-fPrdm16*$^{-/-}$ or WT littermate FL cells. (MZ: marginal zone B-cells; FO: follicular B-cells). (**F**) Histograms of SCA1 MFI in donor MZ B-cells illustrating higher Sca1 in the MZ B-cells from *Δ47-fPrdm16*$^{-/-}$ than from WT littermate FL cells.

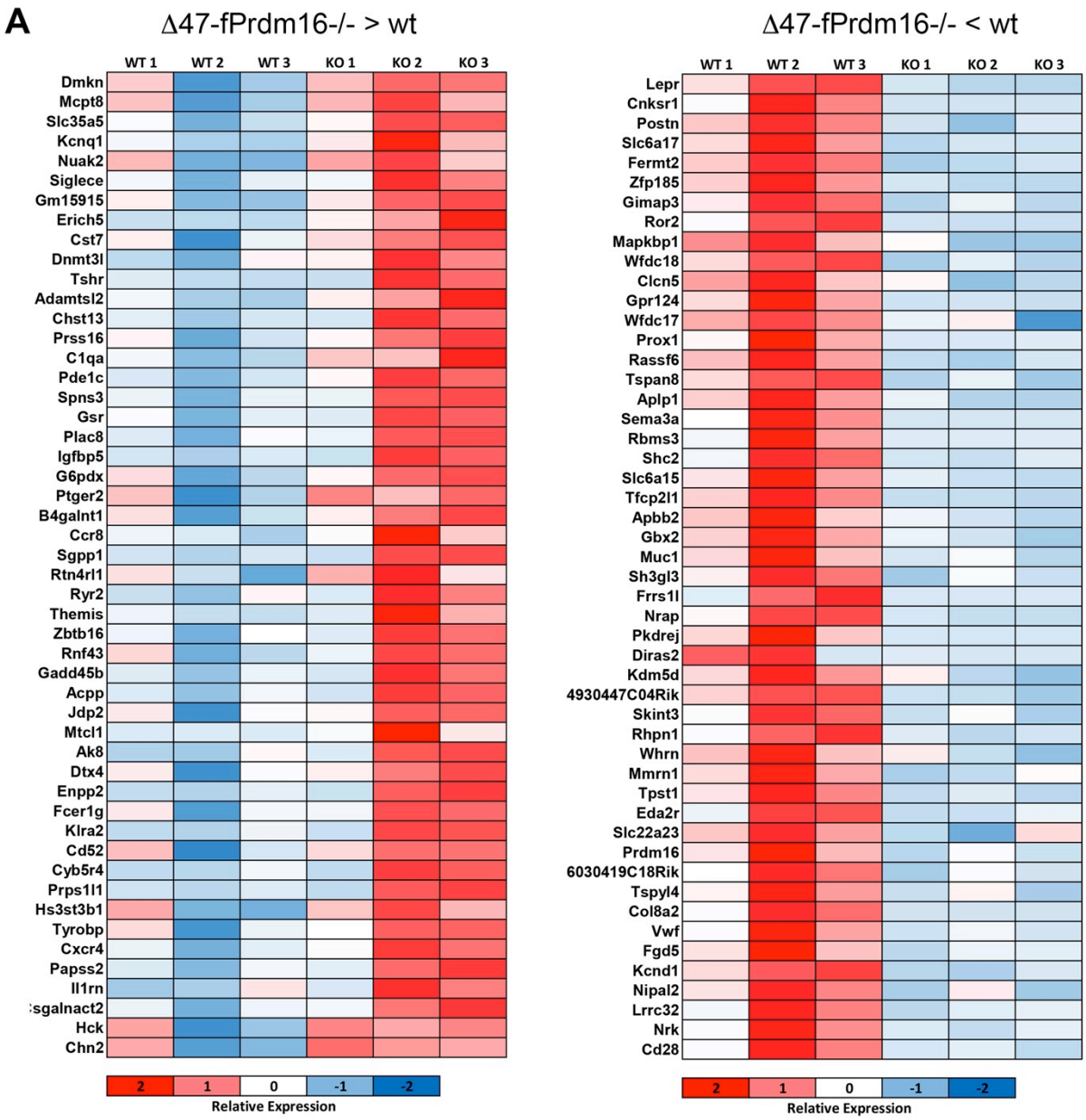
A
Δ47-fPrdm16-/- > wt
WT 1
WT 2
WT 3
KO 1
KO 2
KO 3
Dmkn
Mcpt8
Slc35a5
Kcnq1
Nuak2
Siglece
Gm15915
Erich5
Cst7
Dnmt3l
Tshr
Adamtsl2
Chst13
Prss16
C1qa
Pde1c
Spns3
Gsr
Plac8
Igfbp5
G6pdx
Ptger2
B4galnt1
Ccr8
Sgpp1
Rtn4rl1
Ryr2
Themis
Zbtb16
Rnf43
Gadd45b
Acpp
Jdp2
Mtcl1
Ak8
Dtx4
Enpp2
Fcer1g
Klra2
Cd52
Cyb5r4
Prps1l1
Hs3st3b1
Tyrobp
Cxcr4
Papss2
Il1rn
sgalnact2
Hck
Chn2
2
1
0
-1
-2
Relative Expression
Δ47-fPrdm16-/- < wt
WT 1
WT 2
WT 3
KO 1
KO 2
KO 3
Lepr
Cnksr1
Postn
Slc6a17
Fermt2
Zfp185
Gimap3
Ror2
Mapkbp1
Wfdc18
Clcn5
Gpr124
Wfdc17
Prox1
Rassf6
Tspan8
Aplp1
Sema3a
Rbms3
Shc2
Slc6a15
Tfcp2l1
Apbb2
Gbx2
Muc1
Sh3gl3
Frrs1l
Nrap
Pkdrej
Diras2
Kdm5d
4930447C04Rik
Skint3
Rhpn1
Whrn
Mmrn1
Tpst1
Eda2r
Slc22a23
Prdm16
6030419C18Rik
Tspyl4
Col8a2
Vwf
Fgd5
Kcnd1
Nipal2
Lrrc32
Nrk
Cd28
2
1
0
-1
-2
Relative Expression
B
PC2
10
0
-10
-20
-20
-10
0
10
20
PC1
KO
WT

Figure S7: Genome-wide expression analysis in *fPrdm16*$^{-/-}$ HSCs. (**A**) Heatmap of the top 50 genes (by *P*-value) up/downregulated in *Δ47-fPrdm16*$^{-/-}$ (KO) FL HSCs compared to WT littermates, as determined by RNAseq. (**B**) Principal component analysis (PCA) of *Δ47-fPrdm16*$^{-/-}$ (KO) and WT littermate FL HSC RNAseq samples.

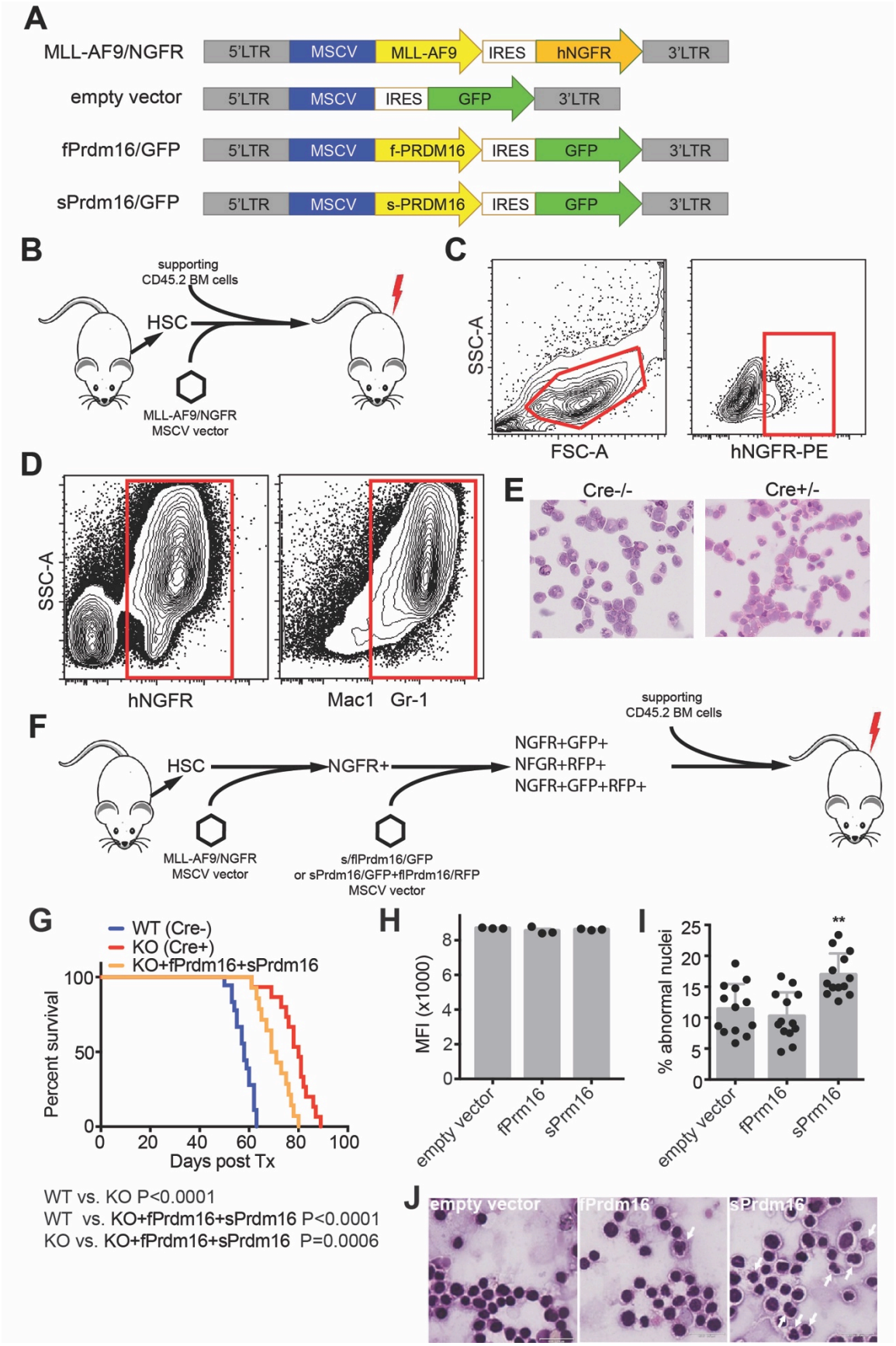
A
MLL-AF9/NGFR
5'LTR
MSCV
MLL-AF9
IRES
hNGFR
3'LTR
empty vector
GFP
fPrdm16/GFP
f-PRDM16
sPrdm16/GFP
s-PRDM16
B
supporting
CD45.2 BM cells
HSC
MLL-AF9/NGFR
MSCV vector
C
SSC-A
FSC-A
hNGFR-PE
D
hNGFR
Mac1
Gr-1
E
Cre-/-
Cre+/-
F
HSC
NGFR+
NGFR+GFP+
NFGR+RFP+
NGFR+GFP+RFP+
s/flPrdm16/GFP
or sPrdm16/GFP+flPrdm16/RFP
G
WT (Cre-)
KO (Cre+)
KO+fPrdm16+sPrdm16
Percent survival
Days post Tx
WT vs. KO P<0.0001
WT vs. KO+fPrdm16+sPrdm16 P<0.0001
KO vs. KO+fPrdm16+sPrdm16 P=0.0006
H
MFI (x1000)
empty vector
fPrm16
sPrm16
I
% abnormal nuclei
**
J
empty vector
fPrdm16
sPrdm16

Figure S8: Retroviral expression of *sPrdm16* and *fPrdm16* in MLL-AF9 leukemia. (**A**) Maps of retroviral vectors used in MLL-AF9 leukemia studies. (**B**) Experimental design of MLL-AF9 leukemia studies. (**C**) Representative flow cytometry plot showing hNGFR expression after transformation of HSCs with hNGFR-MLL-AF9 retroviral vector. (**D**) Representative flow cytometry plot showing hNGFR and myeloid marker expression in PB of moribund mice at the endpoint of survival experiments. (**E**) Representative hematoxylin and eosin (H&E) staining of purified hNGFR^{+} cells from moribund *Prdm16$^{fl/fl}$.Vav-Cre* (Cre+/-) and WT littermate (Cre-/-) mice. (**F**) Schematic of MLL-AF9 transformation of HSCs and forced expression of *fPrdm16*-GFP, *sPrdm16*-GFP, or *s-Prdm16*-GFP/*f-Prdm16*-RFP. **(G)** Survival curves of lethally irradiated mice transplanted with either *Prdm16$^{fl/fl}$.Vav-Cre*, WT littermate, or *Prdm16$^{fl/fl}$.Vav-Cre* transduced with *s-Prdm16*-GFP/*f-Prdm16*-RFP hNGFR+ MLL-AF9 cells. (*n* = 15 recipient mice in three independent experiments) (**H**) Quantification of GFP mean fluorescence intensity (MFI) of *Prdm16$^{fl/fl}$.Vav-Cre* MLL-AF9 cells expressing either empty vector, *fPrdm16*-GFP or *sPrdm16*-GFP (*n* = 3 MLL-AF9 lines). (**I**) Percent of cells with abnormal (elongated or multi-lobed) nuclei (by hematolylin/eosin stain) in BM of leukemic mice transplanted with *Prdm16$^{fl/fl}$.Vav-Cre* MLL-AF9 cells expressing either empty vector, *fPrdm16*-GFP or *sPrdm16*-GFP (*n* = 4 fields from 3 independent mice, each field containing at least 50 BM cells, ** = $P < 0.01$, One-way ANOVA for multiple comparisons;). (**J**) Representative images of the data presented in (I). Arrows indicate abnormal nuclei.

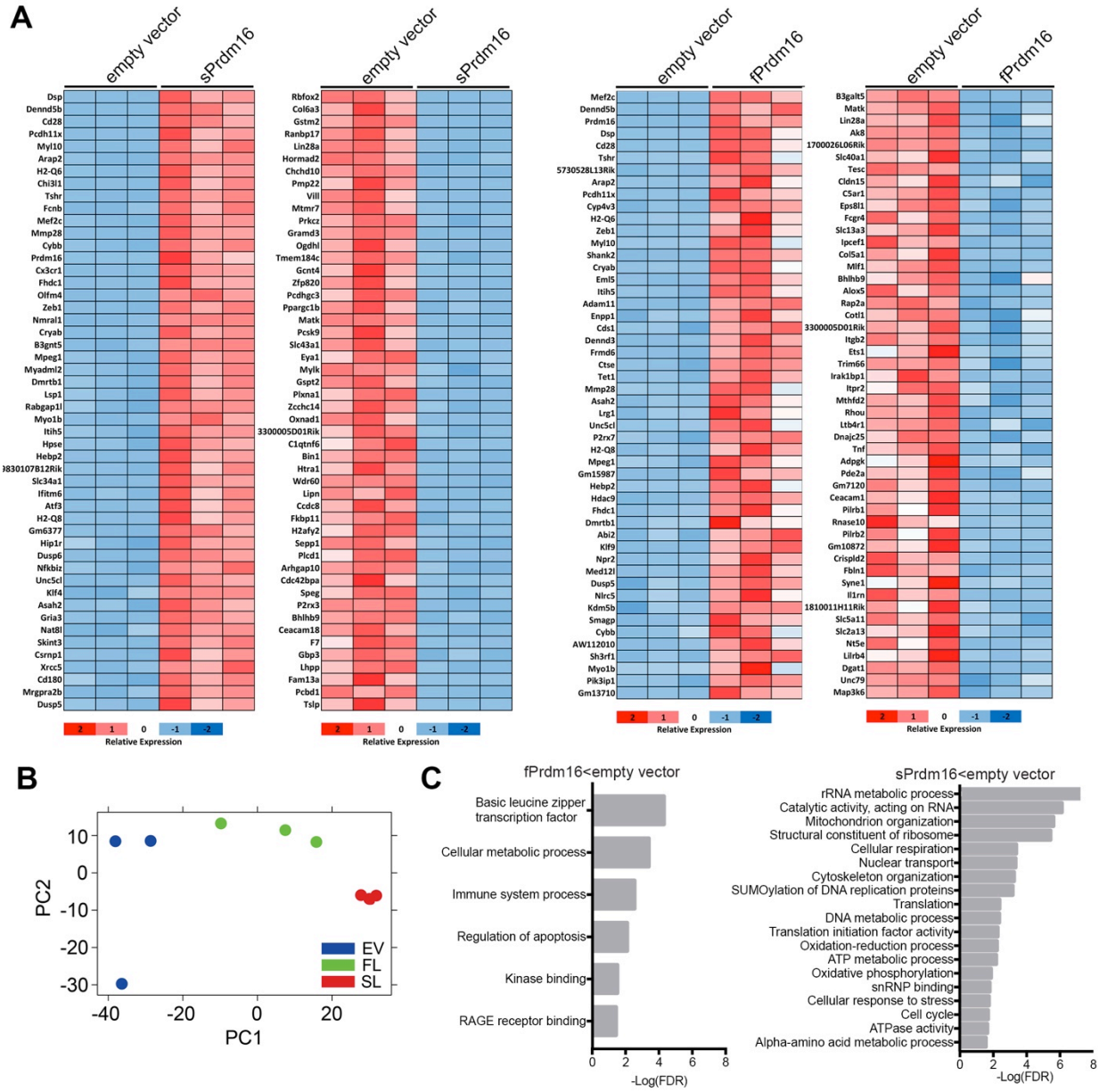

Figure S9: Genome-wide expression analysis in MLL-AF9 leukemia. (A) Heatmap of the top 50 genes (by *P*-value) in *Prdm16*$^{fl/fl}$*.Vav-Cre* MLL-AF9 cells expressing either empty vector, *fPrdm16*-GFP or *sPrdm16*-GFP and isolated *ex vivo*, as determined by RNAseq. (**B**) Principal component analysis (PCA) of RNAseq samples from *ex vivo* isolated *Prdm16*$^{fl/fl}$*.Vav-Cre* MLL-AF9 cells expressing either empty vector, *fPrdm16*-GFP or *sPrdm16*-GFP. **(C)** GO pathways significantly downregulated in *sPrdm16* or *fPrdm16*-expressing MLL-AF9 cells. Values expressed as -Log$_{10}$ of the P-value, determined by PANTHER analysis.

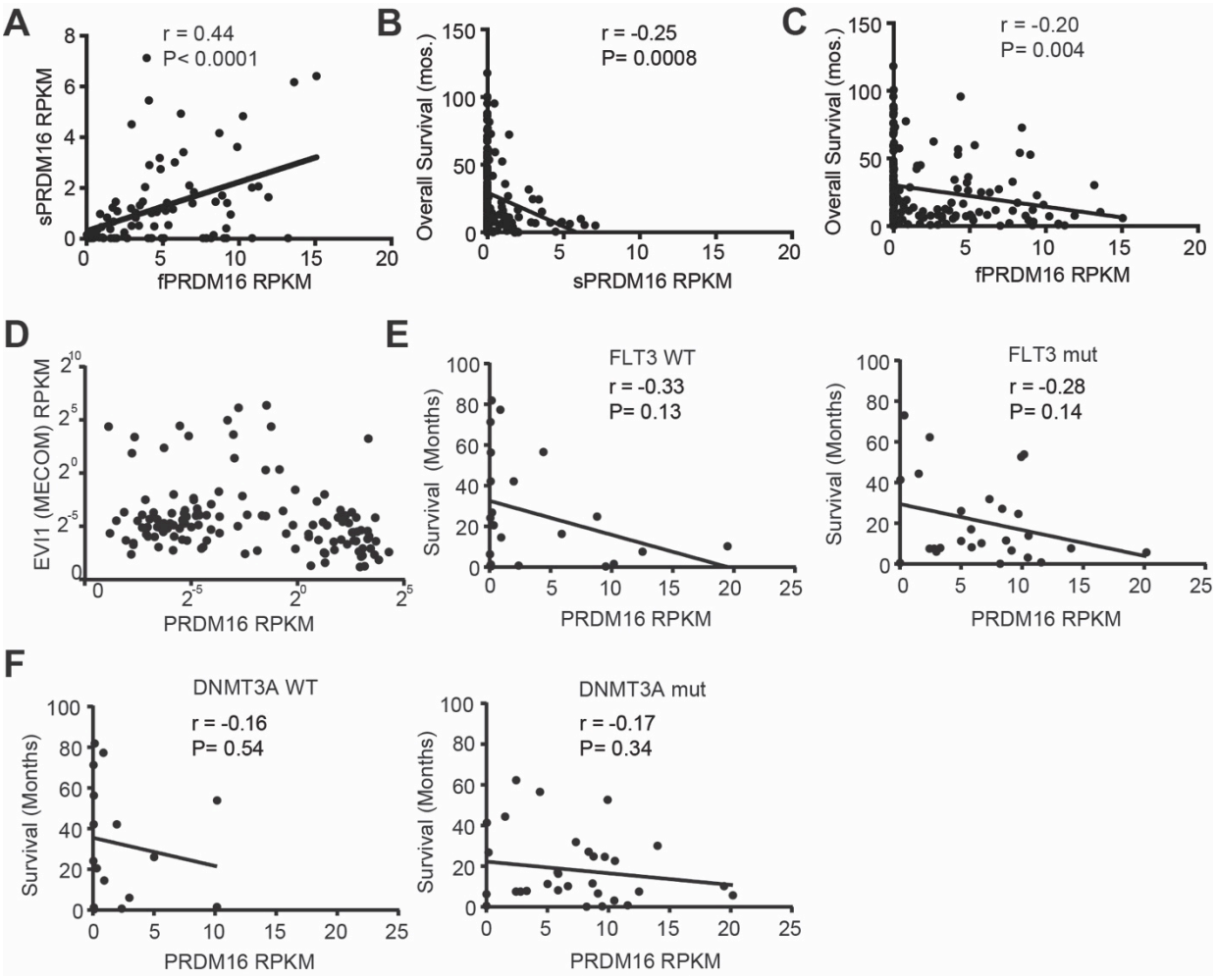

Figure S10: Expression of *sPRDM16* and *fPRDM16* in CGA. (**A**) Correlation between *fPRDM16* and *sPRDM16* mRNA expression (RPKM) among the set of PRDM16-expressing human AML cases in the Cancer Genome Atlas (CGA) cohort (corresponding to Q3 and Q4, or RPKM >0.1, as described in the manuscript and in Figure 7B) (*n* = 90). (**B,C**) Negative correlation between both *sPRDM16* **(B)** and *fPRDM16* **(C)** mRNA expression (RPKM) and overall survival among 179 AML in the CGA. **(D)** Absence of correlation between *PRDM16* and *EVI1* (*MECOM*) RPKM among the 179 CGA cases (P = 0.44). **(E,F)** Correlation between survival and *PRDM16* expression among NPM1-mutant AML with wt or mutant *FLT3* **(E)** or *DNMT3a* **(F)**.