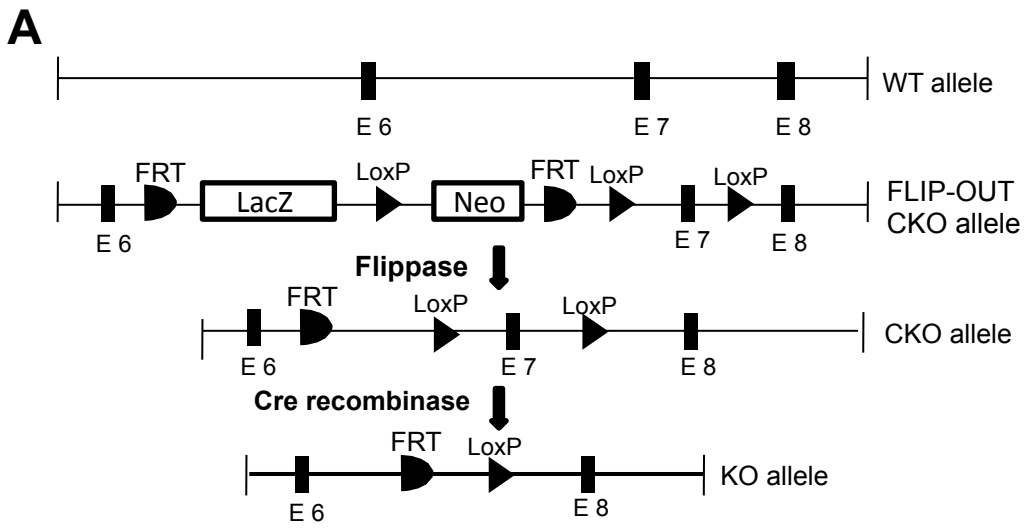
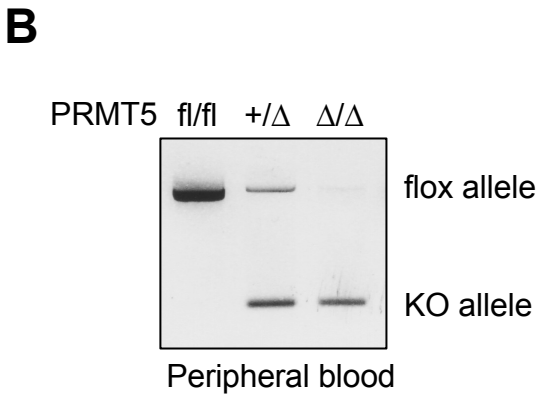
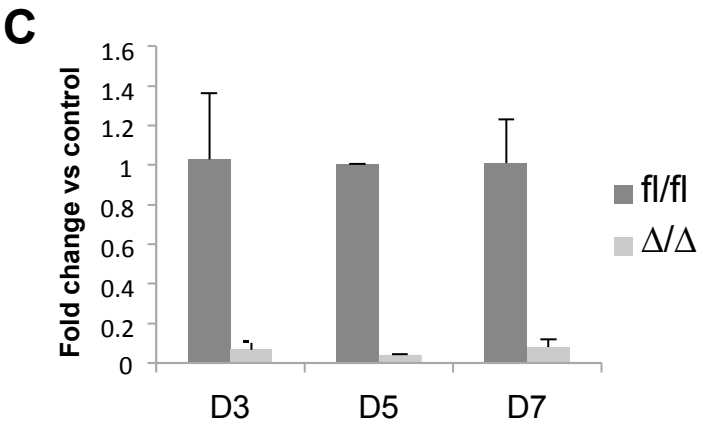

A
WT allele
E 6
E 7
E 8
FRT
LacZ
LoxP
Neo
FRT
LoxP
LoxP
FLIP-OUT
CKO allele
E 6
E 7
E 8
Flippase
FRT
LoxP
LoxP
CKO allele
E 6
E 7
E 8
Cre recombinase
FRT
LoxP
KO allele
E 6
E 8


B
PRMT5 fl/fl +/Δ Δ/Δ
flox allele
KO allele
Peripheral blood


C
Fold change vs control
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
D3
D5
D7
fl/fl
Δ/Δ

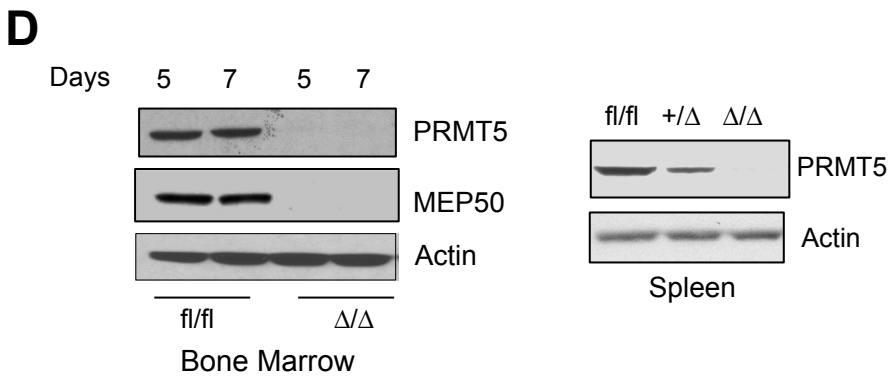

D
Days 5 7 5 7
PRMT5
MEP50
Actin
fl/fl
Δ/Δ
Bone Marrow
fl/fl +/Δ Δ/Δ
PRMT5
Actin
Spleen

Figure S1: Generation of *Prmt5* conditional KO mice. **A)** Strategy used to generate *Prmt5* conditional KO mice. In the original version of the allele (FLIP-OUT CKO), gene function was inactivated by splicing the upstream endogenous exons to a splice acceptor site in the targeting cassette. To render this allele a conditional allele, the targeting cassette was removed using Flp recombinase, leaving loxP sites flanking a critical exon (exon 7, which encodes the catalytic domain of PRMT5). Mice homozygous for the *Prmt5* floxed allele were then crossed to *Mx1Cre* or *ERCre* transgenic mice to generate $Mx1Cre^{+}$ or $ERCre^{+}PRMT5^{fl/+}$ mice ($ERCre^{+}PRMT5^{fl/fl}$ mice were used for the experiments shown in Figure 5F). These mice were bred to homozygosity for the floxed *Prmt5* allele, but with only one copy of the *Mx1Cre* or *ERCre* transgene. **B)** Genomic DNA was purified from peripheral blood at 5 d.p.i. and PCR used to confirm the deletion of exon 7 of *Prmt5* in $Mx1Cre^{+}Prmt5^{fl/+}$ and $Mx1Cre^{+}Prmt5^{fl/fl}$ mice. **C)** Quantitative Real-time PCR demonstrates the loss of *Prmt5* mRNA in mouse bone marrow cells at 3, 5 and 7 d.p.i. The expression of *Prmt5* was normalized to *Hprt1*. **D)** Lack of PRMT5 and MEP50 proteins in mouse bone marrow and spleen cells was confirmed by western blot, 5 and 7 d.p.i.

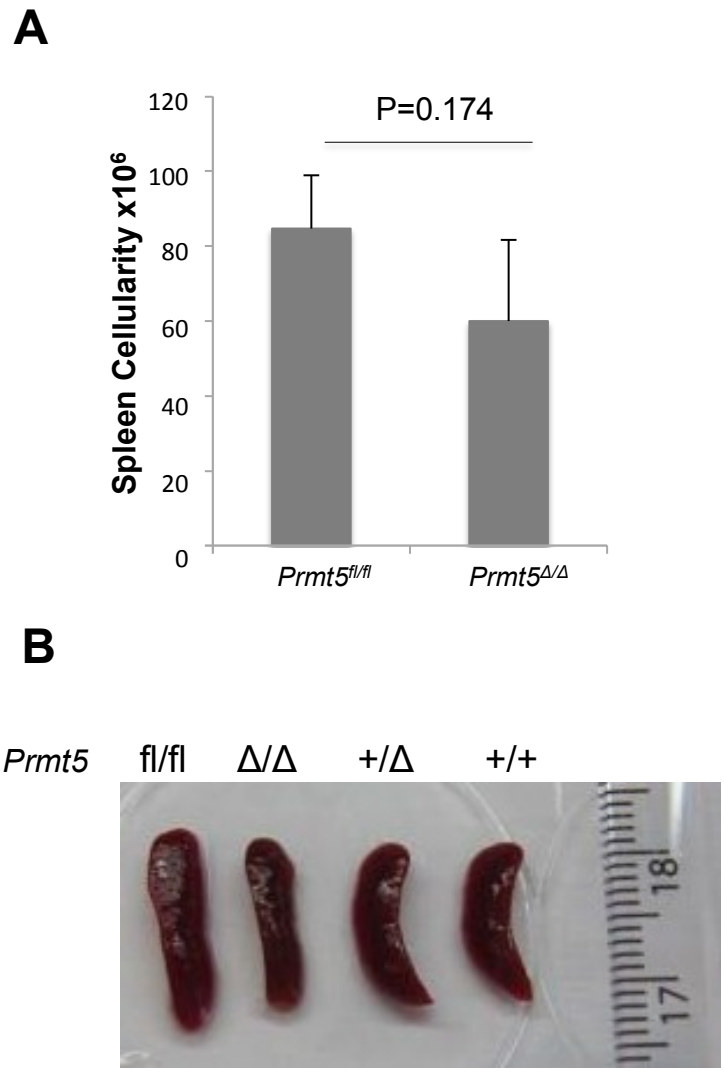
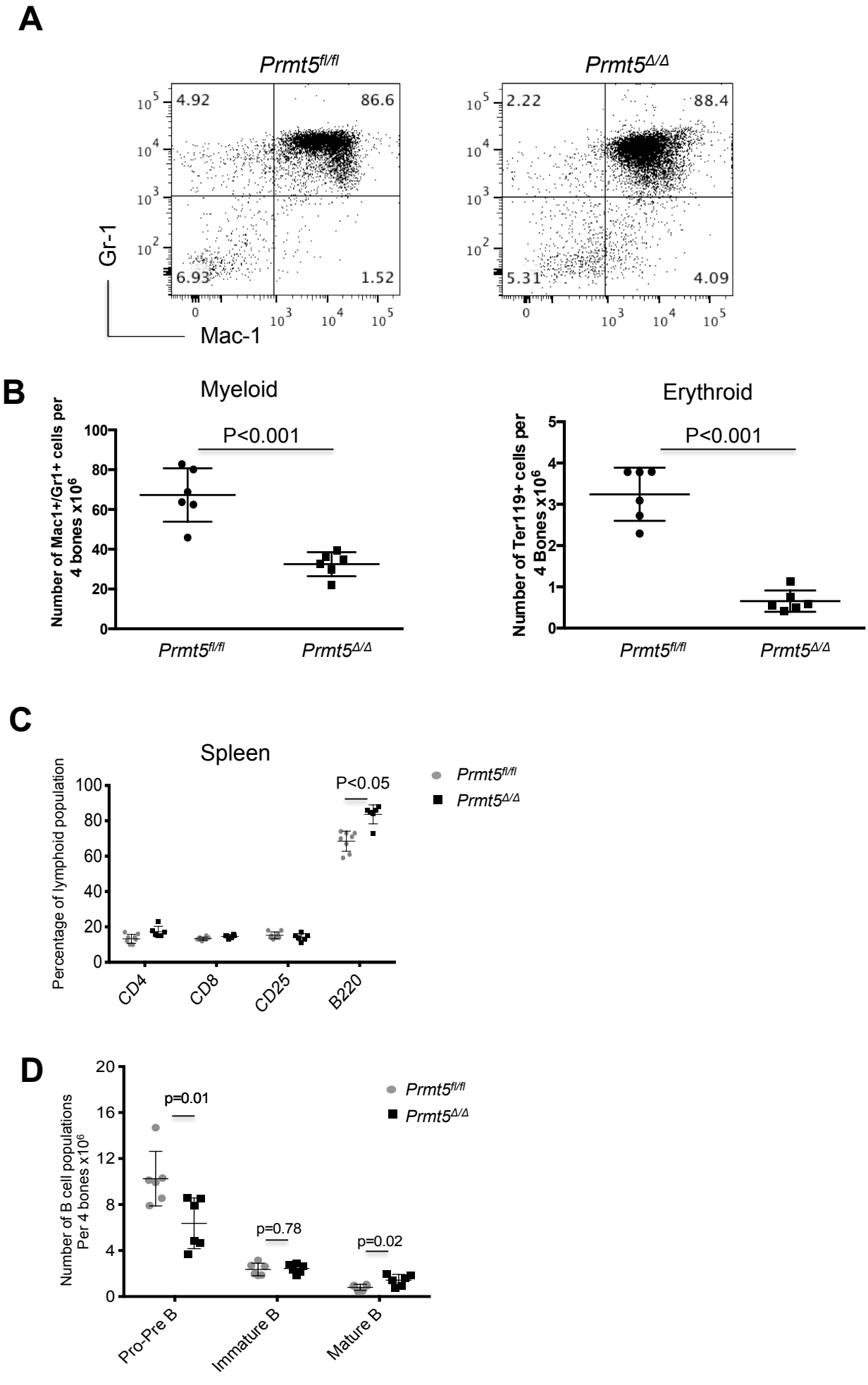
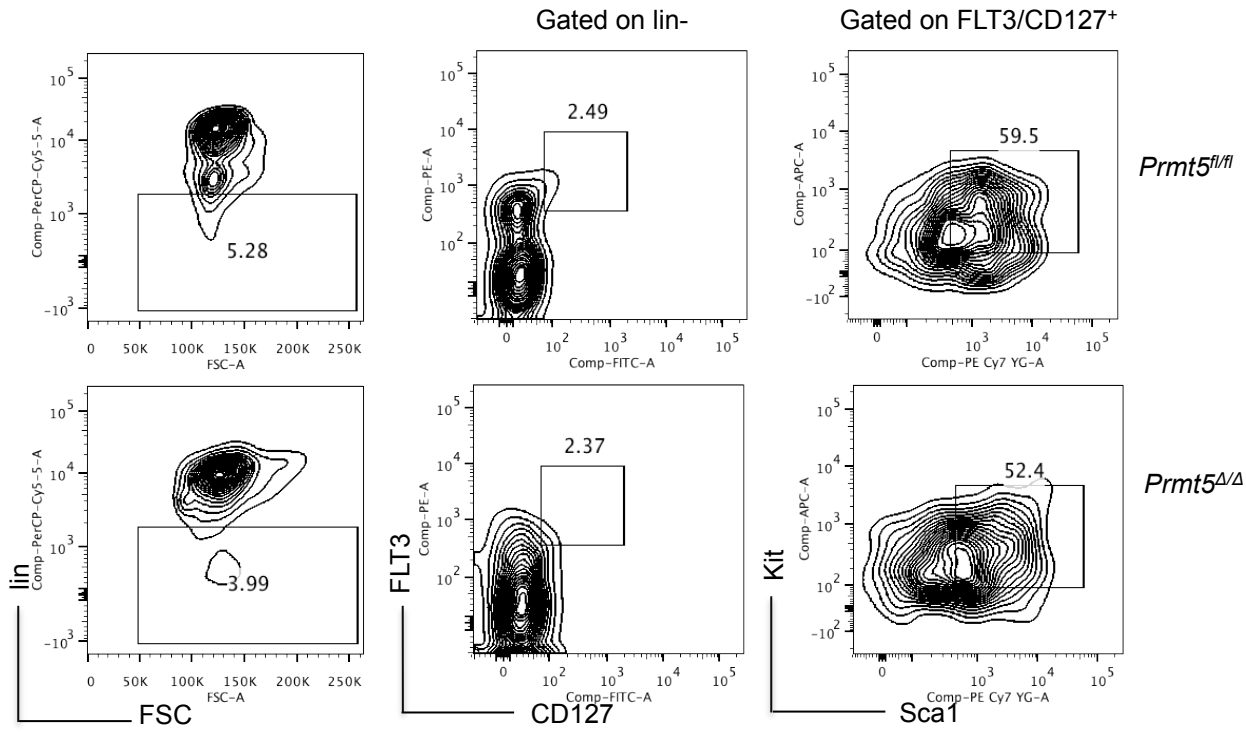


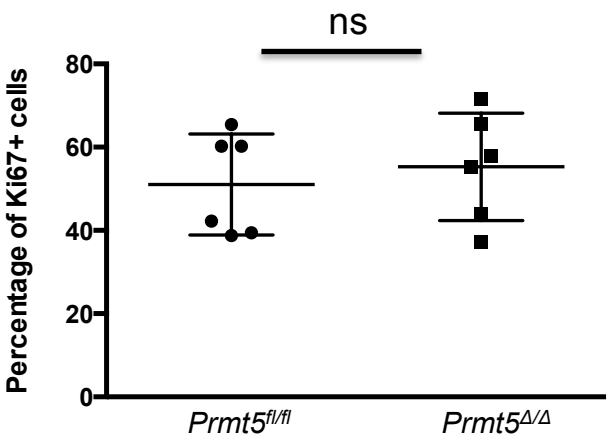
Figure S2 Minimal effect of PRMT5 loss on spleen cellularity. A) Numbers of spleen cells isolated from *Prmt5* control and knockout mice 15 days post poly (I:C) injection are plotted (n=5). P value is calculated by student's *t*-test. B) a representative image of spleens isolated from day 15 *Prmt5* control and heterozygous/homozygous knockout mice.


A
Prmt5fl/fl
Prmt5Δ/Δ
4.92
86.6
6.93
1.52
2.22
88.4
5.31
4.09
Gr-1
Mac-1
B
Myeloid
P<0.001
Number of Mac1+/Gr1+ cells per 4 bones x10^6
Erythroid
P<0.001
Number of Ter119+ cells per 4 Bones x10^6
Prmt5fl/fl
Prmt5Δ/Δ
C
Spleen
P<0.05
Percentage of lymphoid population
CD4
CD8
CD25
B220
D
p=0.01
p=0.78
p=0.02
Number of B cell populations Per 4 bones x10^6
Pro-Pre B
Immature B
Mature B

E


Gated on lin-
Gated on FLT3/CD127+
Prmt5fl/fl
Prmt5Δ/Δ
5.28
2.49
59.5
3.99
2.37
52.4
lin
FLT3
Kit
FSC
CD127
Sca1

F


ns
Percentage of Ki67+ cells
Prmt5fl/fl
Prmt5Δ/Δ

G

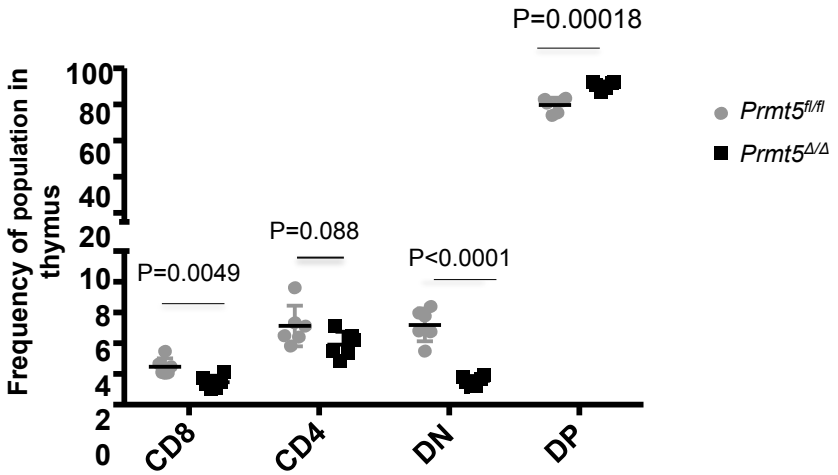

P=0.00018
P=0.088
P<0.0001
P=0.0049
Frequency of population in thymus
CD8
CD4
DN
DP
Prmt5fl/fl
Prmt5Δ/Δ

Figure S3 Myeloid, erythroid, B and T cell development in PRMT5 knockout mice. **A)** BM cells were isolated from day 7 control and PRMT5-deleted mice and cell surface expression of Mac1 and Gr1 was examined by FACS analysis. **B)** The absolute numbers of day 7 Mac1/Gr1 double-positive myeloid cells (left) and Ter119-positive erythroid cells (after red blood cell lysis, right) were calculated based on the frequencies and BM cellularities. P values: student's *t*-test. **C)** Frequencies of CD4, CD8, CD25 and B220-positive cells were determined in day 7 control and *Prmt5* KO spleen cells. P value: student's *t*-test. **D)** B cell development in day 7 BM was determined by cell surface expression of IgM and B220. Frequencies of pro/pre-B cells (IgM^- and $B220^{low}$), Immature B cells ($IgM^-$and $B220^{high}$) and mature B cells (IgM^+ and $B220^{high}$) are plotted. P values: student's *t*-test. **E)** The frequency of CLP was determined in day 7 BM. Purified BM cells were first gated on lin–negative population (excluding the CD127 marker), then gated on CD127 and FLT3 double-positive cells, and finally gated on Sca1/c-Kit intermediate cells. Representative FACS plots from 2 independent experiments, with 3 mice per group in each experiment, are shown. **F)** The proliferation of CLP was determined by Ki67 staining (n=6). P value: student's *t*-test. **G)** Thymocytes were isolated from control and PRMT5-null mice 9 days after cre induction. Frequencies of CD4, CD8 single positive, double positive (DP) and double negative (DN) were determined by FACS analysis and plotted here (n=6). P values: student's *t*-test.

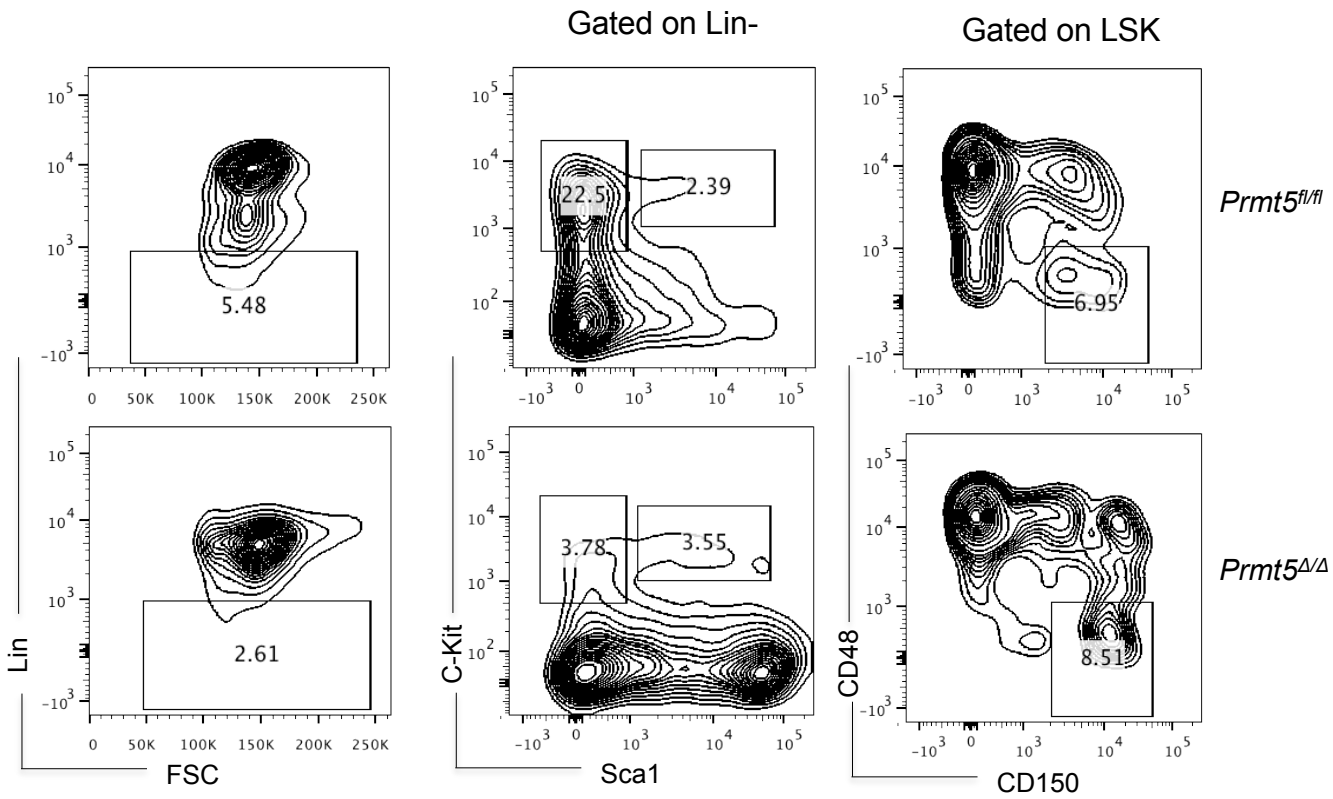
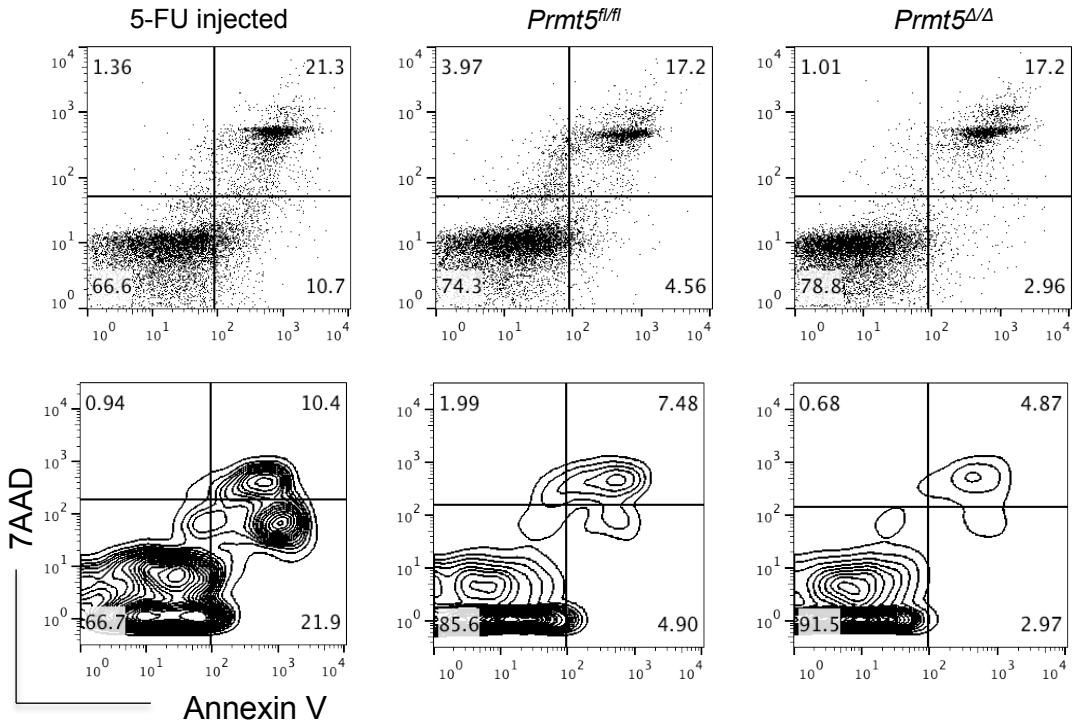
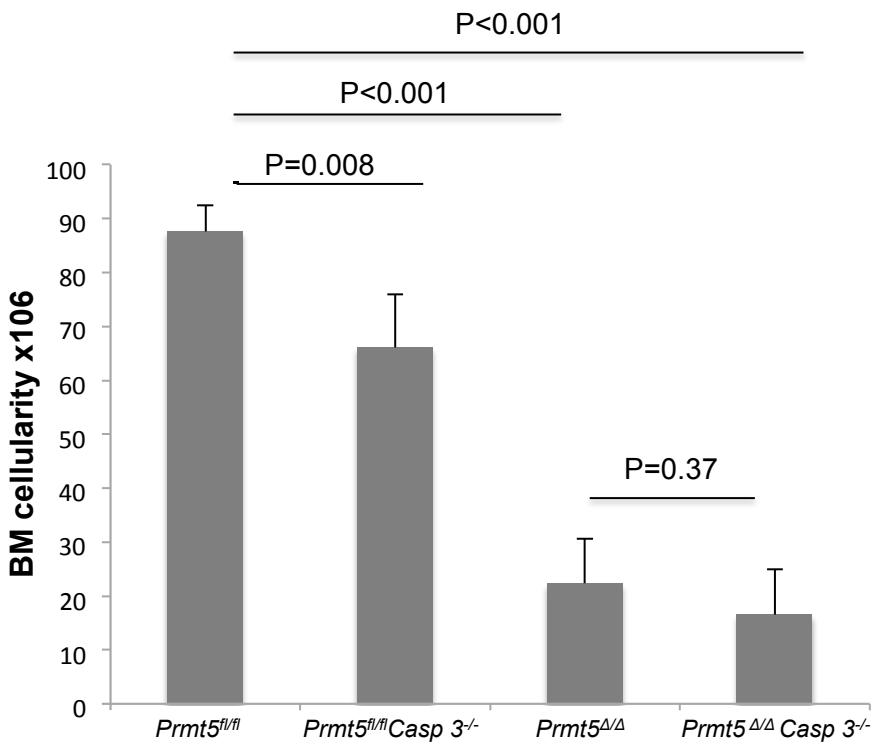


Figure S4 Loss of hematopoietic stem/progenitor cells (HSPCs) in PRMT5 null mice. BM cells were isolated from control and *Prmt5* knockout mice 9 days after poly (I:C) injection. Frequencies of HSPCs were determined by surface expression of lineage marks, c-Kit, Sca1 and SLAM marks (CD150 and CD48). Representative FACS plots from 5 independent experiments are shown.

A


5-FU injected
Prmt5fl/fl
Prmt5Δ/Δ
1.36
21.3
66.6
10.7
3.97
17.2
74.3
4.56
1.01
17.2
78.8
2.96
0.94
10.4
66.7
21.9
1.99
7.48
85.6
4.90
0.68
4.87
91.5
2.97
7AAD
Annexin V

B


P<0.001
P<0.001
P=0.008
P=0.37
BM cellularity x106
100
90
80
70
60
50
40
30
20
10
0
Prmt5fl/fl
Prmt5fl/flCasp 3-/-
Prmt5Δ/Δ
Prmt5 Δ/Δ Casp 3-/-

C

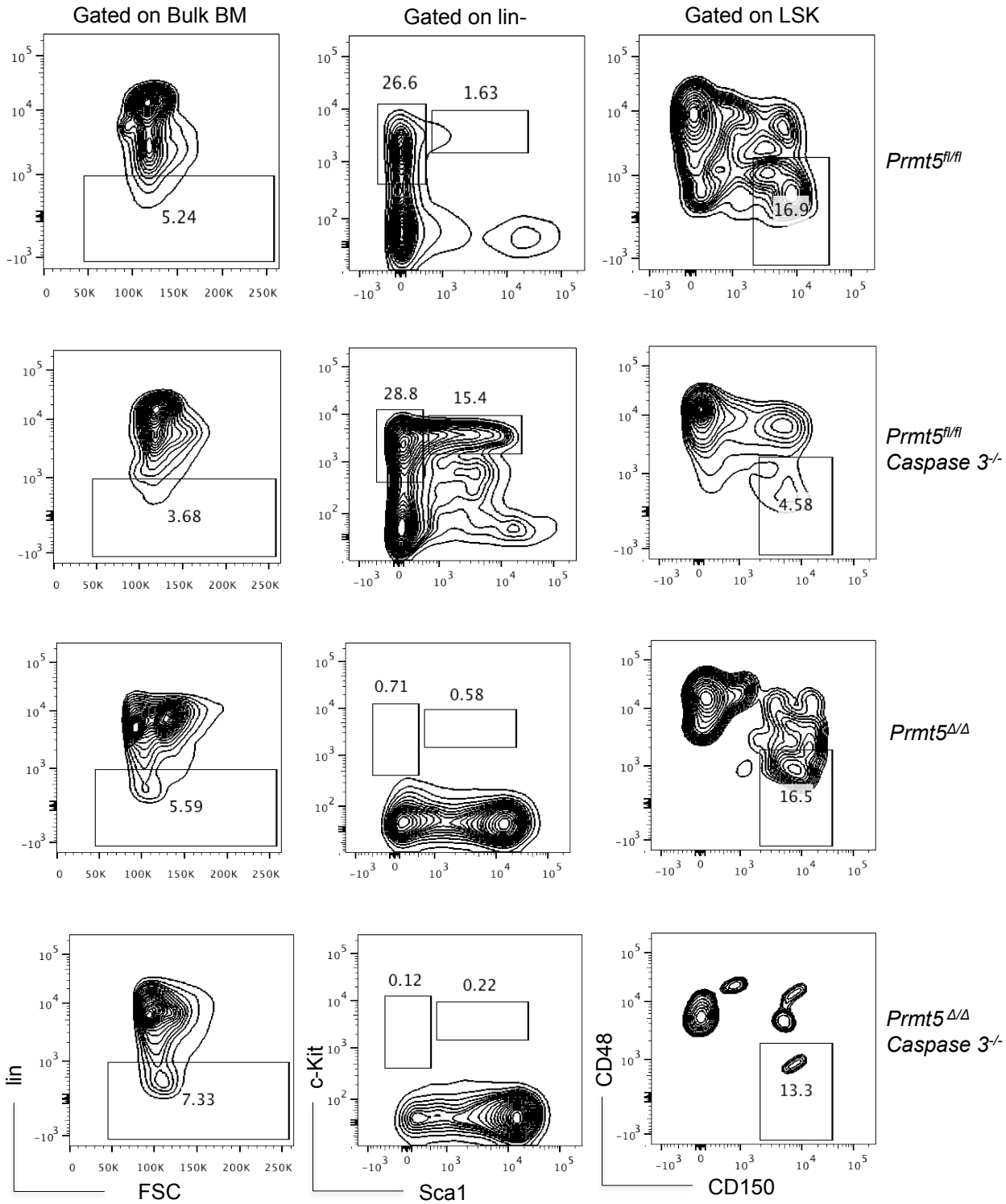
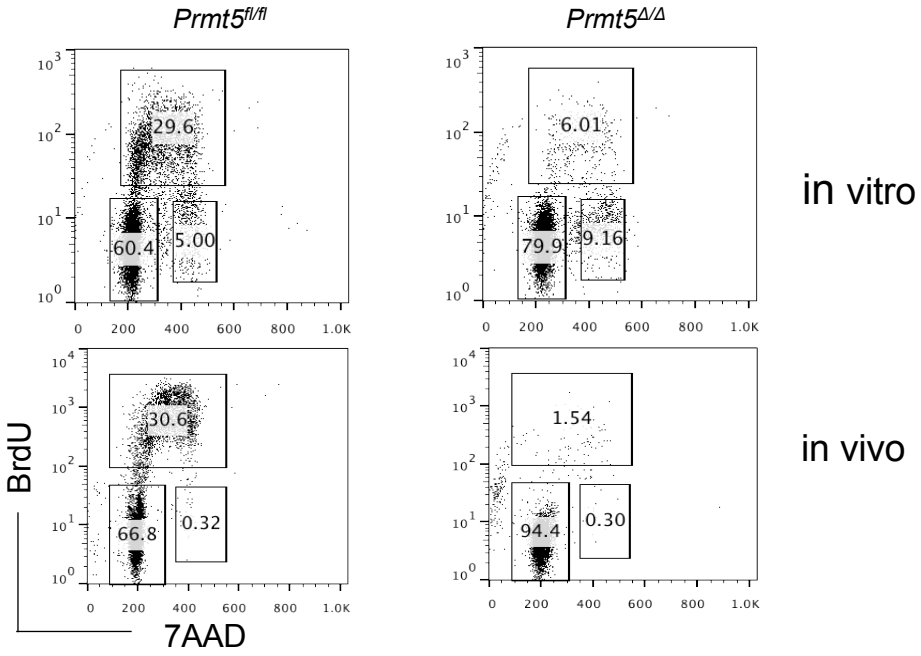
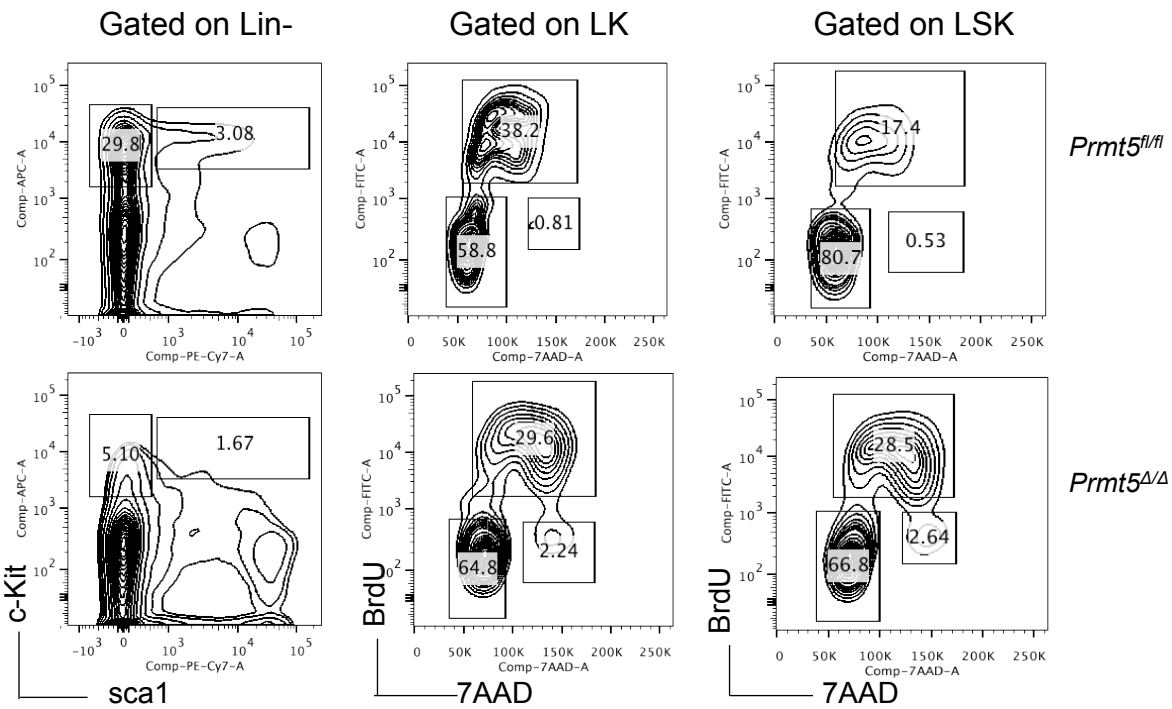

Gated on Bulk BM
Gated on lin-
Gated on LSK
Prmt5fl/fl
Prmt5fl/fl Caspase 3-/-
Prmt5Δ/Δ
Prmt5Δ/Δ Caspase 3-/-
5.24
26.6
1.63
16.9
3.68
28.8
15.4
4.58
5.59
0.71
0.58
16.5
7.33
0.12
0.22
13.3
lin
FSC
c-Kit
Sca1
CD48
CD150

Figure S5 Apoptosis is not increased in PRMT5-null HSPCs or bulk BM cells. **A)** FACS analysis (with 5 to 6 mice per group) of 7-AAD and Annexin V staining was performed either on lin^+ BM cells (top) or $lin^-c\text{-}kit^+$ stem/progenitor cells (bottom) isolated from day 9 control and PRMT5-deleted mice. BM cells isolated from 5-FU treated mice were used as the positive control. **B)** Cellularity was determined 9 d.p.i. for BM cells isolated form *Prmt5*$^{fl/fl}$, *Prmt5*$^{fl/fl}$ *Caspase3*$^{-/-}$, *Prmt5*$^{\Delta/\Delta}$ and *Prmt5*$^{\Delta/\Delta}$ *Caspase3*$^{-/-}$ mice (n=4). P values: student's *t*-test. **C)** Representative FACS plots from 3 independent experiments show the frequencies of lin^-, LK, LSK and LT-HSC cells ($CD48^-$ $CD150^+$ LSK) in *Prmt5*$^{fl/fl}$, *Prmt5*$^{fl/fl}$ *Caspase3*$^{-/-}$, *Prmt5*$^{\Delta/\Delta}$ and *Prmt5*$^{\Delta/\Delta}$ *Caspase3*$^{-/-}$ mice 9 d.p.i.

A


Prmt5fl/fl
Prmt5Δ/Δ
29.6
60.4
5.00
6.01
79.9
9.16
in vitro
30.6
66.8
0.32
1.54
94.4
0.30
in vivo
BrdU
7AAD

B


Gated on Lin-
Gated on LK
Gated on LSK
29.8
3.08
38.2
0.81
58.8
17.4
80.7
0.53
Prmt5fl/fl
5.10
1.67
29.6
64.8
2.24
28.5
66.8
2.64
Prmt5Δ/Δ
c-Kit
sca1
BrdU
7AAD
Comp-APC-A
Comp-PE-Cy7-A
Comp-FITC-A
Comp-7AAD-A

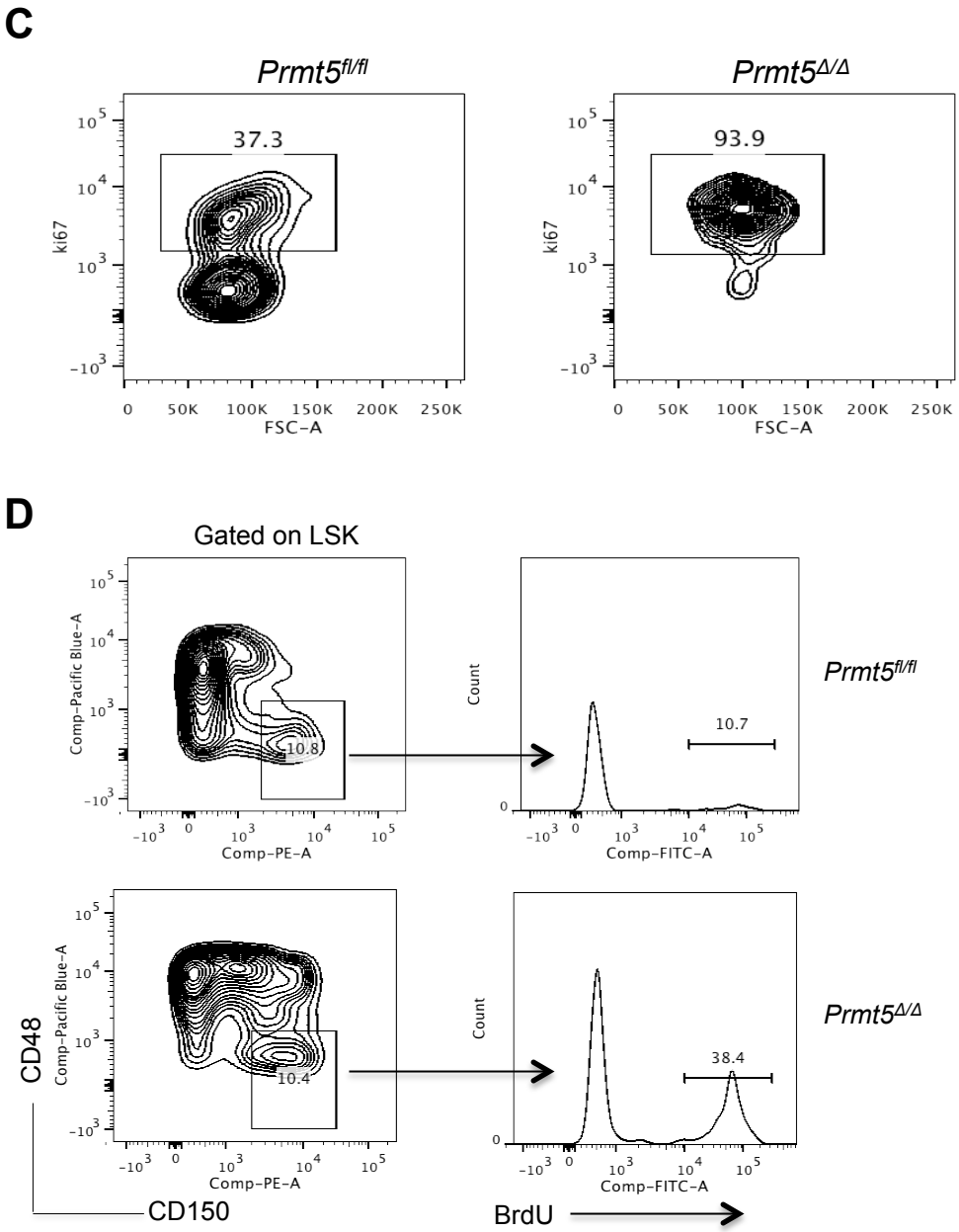


Figure S6 PRMT5 deletion confers distinct effects on the cell cycle regulation of hematopoietic stem vs. progenitor cells. **A)** BrdU incorporation was measured by FACS analysis in vitro (top) and in vivo (bottom) using BM cells isolated from day 9 control and PRMT5-deleted mice. Representative plots from 3 independent experiments show the flow profile gated on lin^- cells. B) The in vivo BrdU incorporation in day 9 LK and LSK cells was determined by FACS analysis. The gating strategy and frequency of each gate are shown on each representative plot. Two experiments with 2 mice per group in each experiment. C) BM cells were isolated from day 9 control and PRMT5-deleted mice and Ki67 expression was determined within the $CD34^-$ $FLT3^-$ LSK population. Two experiments with 2 mice per group in each experiment. D) Representative FACS plots show the increased percentage of $BrdU^+$ cells in the PRMT5-null LT-HSCs. Two experiments with 3 mice per group in each experiment.

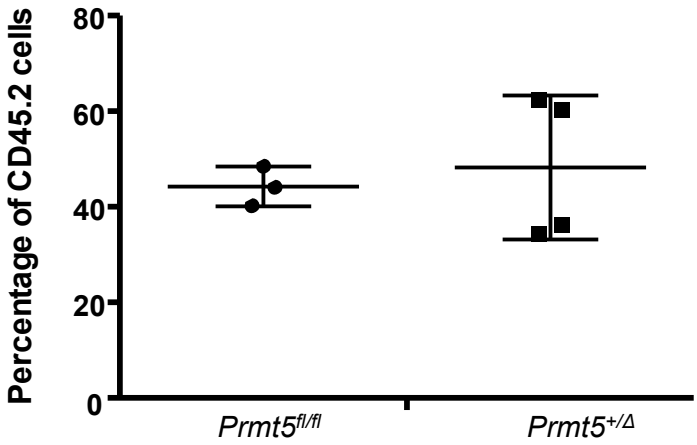


Figure S7 Heterozygous deletion of *Prmt5* does not affect the repopulating capacity of HSCs. Equal number of CD45.2 BM cells isolated from either $Prmt5^{fl/fl}$ or $Prmt5^{+/\Delta}$ mice and wild-type CD45.1 BM cells were transplanted into lethally irradiated recipient mice. PRMT5 deletion was induced by poly(I:C) injection 4-month post transplantation and the percentage of CD45.2 cells in BM was determined by FCAS analysis 14 d.p.i.

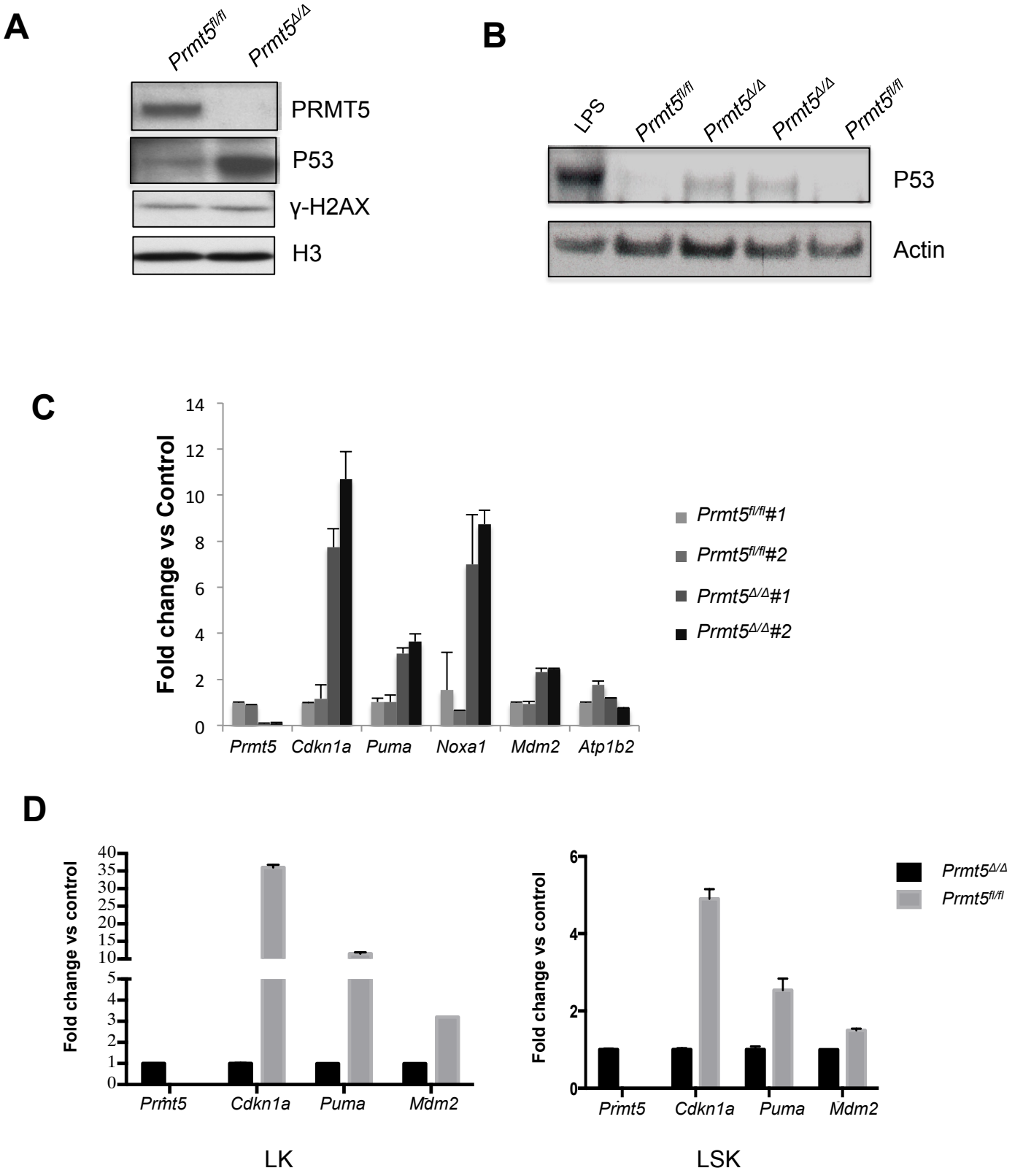


Figure S8: Up-regulation of p53 protein and its target genes**. A)** HSPCs were purified from day 7 control and PRMT5-null BM cells using flow cytometry. Blots were probed with antibodies indicated in the figure. **B)** Total BM cells isolated from day 7 control and PRMT5-null mice were lysed and resolved on SDS-page gel. Expression of p53 and β-actin was determined as Figure 6A. BM cells from LPS treated mice were used as positive control for p53 expression. **C)** and **D)** Real-time PCR for p53 target genes in total BM cells (C) or LK and LSK cells (D) isolated from day 7 control and *Prmt5* knockout mice. The representative PCR results from 3 independent experiments are shown.

Supplementary Methods:

Western blotting and antibodies:

BM or spleen cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors (Pierce). The protein samples were separated by electrophoresis on denaturing 10% or 4-12% premade polyacrylamide gels (Invitrogen) and blotted to PVDF membranes (Millipore). The following antibodies used in western blotting were purchased from Cell Signaling Technology: MEP50 (2823s), γH2AX (9718p), total ERK1/2 (9102s), p-ERK1/2 (4377s); p-STAT5 (9351s), total AKT (4060s), p-AKT (2938p), Caspase 3 (9665s) and symmetric di-methylated arginine (13222s). Antibodies purchased from Santa Cruz are: PRMT5 (sc-22132), β-actin (sc-47778) and total STAT5 (sc-835). The antibody specific for symmetric dimethyl H4R3 was obtained from Epigentek (p-3090-96) and antibody for detecting mouse p53 (CM5) was from Leica Biosystems. All primary antibodies were used at 1:1000 dilutions.

Real-time PCR

Total RNA was purified using Qiagen RNeasy Mini Kit or Micro Kit (if the cells used for RNA purification are less then 1 x 10^5) and was reverse transcribed using the SuperScript III First-strand cDNA Synthesis System (Invitrogen) with random hexamers. Real-time PCR for determining the gene expression levels was performed using the following Taqman probes (Invitrogen): *Prmt5* (Mm01319219-m1), *Cdkn1a* (Mm04205640_g1), *Bbc3* (Mm00519268_m1), *Mdm2* (Mm01233136_m1), *Noxa1* (Mm00549172_m1), *Il-3ra* (Mm00434273_m1), *c-Kit* (Mm00445212_m1), *Flt3* (Mm00439016_m1), *Il-6ra* (Mm00438653_m1) and *Hprt1* (Mm01545399_m1).

To measure intron retention in the *Flt3*, *c-Kit* and *Il-6rα mRNA*, we designed PCR primers flanking each exon-intron junction (intron PCR) to detect the un-spliced introns, and normalized these intron PCR results to total mRNA levels (using PCR primers within one exon). All PCR reactions were performed using total RNA pre-treated with DNase I, to eliminate residual genomic DNA.

Cell surface markers for HSC and their differentiated progeny (in Figure 1A):

To purify cells used for PCR in Figure 1A, the myeloid progenitor cells (LK cells) and stem cell-enriched LSK population was defined by cell surface expression of Lineage markers (Ter119, B220, Mac1, Gr1, CD3e, CD4, CD5, CD8 and CD127), c-Kit and Sca1. LK cells are Lineage$^-$, c-Kit$^+$ and Sca1$^-$, and LSK cells are Lineage$^-$, c-Kit$^+$ and Sca1$^+$ cells. Within the LSK population, the cell surface expression of FLT3 and CD34 was used to further define the sub-populations. LT-HSCs are CD34$^-$ FLT3$^-$ LSK; ST-HSCs are CD34$^+$ FLT3$^-$ LSK cells; MPP cells are CD34$^+$ FLT3$^+$ LSK cells. Within the LK population, CD34 and FcRII/III markers were used to further define the sub-populations. CMP cells are CD34$^+$ and FcRII/III$^-$ LK cells; GMP cells are CD34$^+$ and FcRII/III$^+$ LK cells; and MEP cells are CD34$^-$ and FcRII/III$^-$ LK cells.

Within the differentiated cells, myeloid cells are the Mac1 and Gr1 double-positive cells, erythroid cells are Ter119$^+$ cells, and B cells are B220$^+$ cells.

In vivo BrdU incorporation assay:

Bromodeoxyuridine (BrdU, BD Pharmingen) was given to mice by intraperitoneal injection at 150mg/kg. BM cells were isolated 90 min post-injection and stained for cell surface markers of HSCs (SLAM LSK or CD34$^-$ FLT3$^-$ LSK). Incorporated BrdU was

then detected using the FITC BrdU Flow Kit (BD Pharmingen) according to the manufacturer's instructions, and subjected to FACS analysis.