SUPPLEMENTARY APPENDIX

Prion protein deficiency impairs hematopoietic stem cell determination and sensitizes myeloid progenitors to irradiation

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Supplementary Data:

Supplementary Methods :

Mouse survival and retro-orbital bone marrow cells transplantation

Mice were exposed to total-body irradiation (TBI) using an Alcyon Cobalt60 irradiator (Varian), with a dose rate of 1Gy/min. Some series of mice were transplanted retro-orbitally under isoflurane anesthesia with 10^6 bone marrow cells from Prnp+/+ mice within the first 24 h after irradiation. For assessing survival rates, mice were monitored for at least 30 days to several months after TBI.

NFS analysis

Peripheral blood samples were collected by retro-orbital blleding with heparinized tubes under isoflurane anesthesia. Blood hematology profiles were determined using an automated hematology analyzer (CELL-DYN 3700CS, Abbott Diagnostics).

Antibodies used for cell sorting and flow cytometry analysis of bone marrow cells.

To study lymphoid and myeloid lineages, BM cells were stained directly with phycoerythrin (PE)-conjugated anti-CD11b, allophycocyanin (APC)-conjugated anti-CD3, phycoerythrin-cyanin 7 (PE-Cy7)-conjugated anti-B220, fluorescein isothiocyanate (FITC)-conjugated anti-Gr-1 antibodies.

The analysis of lymphoid and myeloid progenitor subsets was performed using biotin-conjugated anti-mouse lineage antibodies (Miltenyi Biotec) following with a staining with a BUV395-conjugated streptavidin, Pacific Blue (PB)-conjugated anti-Sca-1, APC-conjugated anti-CD117, PE-conjugated anti-CD135, FITC-conjugated anti-CD34, PE-Cy7-conjugated anti-CD16/32, and Alexa750-conjugated anti-CD127 antibodies.

To identify HSPCs, BM mononuclear cells were stained with biotin-conjugated anti-mouse lineage antibodies (Miltenyi Biotec) together with BUV395-conjugated streptavidin, and then incubated with PB-conjugated anti-Sca-1, allophycocyanin – cyanin 7 (APC-Cy7)-conjugated anti-CD117, PE-conjugated anti-CD135, PE-Cy7-conjugated anti-CD48, APC-conjugated anti-CD150 antibodies.

For cell sorting, bone marrow mononuclear cells were first depleted of mature hematopoietic cells using the cell Depletion kit (Mylteni Biotec) according to manufacturer's instructions to obtain Lin negative cells (Lin^-)

Lin^- biotin cells were then stained with eFluor450-conjugated streptavidin , PE-conjugated anti-Sca-1, APC-conjugated anti-CD117, PE-Cy7-conjugated anti-CD16/32, FITC-conjugated anti-CD34 antibodies. This staining allowed purifying CMP (LKS^-, $CD34^{int}$, $CD16/32^{int}$), GMP (LKS^-, $CD34^+$, $CD16/32^+$), MEP (LKS^-, $CD34^-$, $CD16/32^-$) and LSK cells. Staining Lin^- cells stained with PECy7-conjugated anti-Sca-1, APC-conjugated anti-CD117, and PE-conjugated anti-CD135 antibodies allowed purifying HSC (LSK, $CD135^-$) and MPP (LSK, $CD135^+$) subpopulations.

PrPC staining

Bone marrow cells were first stained to allow identification of hematopoietic subsets (see above). Then cells were either incubated with Mouse IgG2b isotype control antibody (ebioscience) or mouse monoclonal anti-PRP (8H4) (Sigma-aldrich) (1:300) in PBS containing 2.5% goat serum for 1h at RT. After wash, cells were incubated with APC-conjugated F(ab')2 anti-mouse IgG (ebioscience) (1:100) and analysed by flow cytometry with a BD FACSLSRII flow cytometer (BD Biosciences).

Cell cycle analysis of bone marrow populations

Cell surface staining was performed as described above. Samples were incubated in Cytofix/cytoperm (BD Biosciences) for 20 min. Cells were washed using Permwash and incubated in Cytoperm Plus buffer for 15 min before staining with FITC-conjugated anti-Ki67 (BD Pharmingen) in PermWash supplemented with BSA 0.5% for 30 min. After washing in PermWash buffer, cells were stained with 1 mg/ml Hoechst 33258 and analyzed by flow cytometry with a BD FACSLSRII TM flow cytometer (BD Biosciences).

Colony assay on methylcellulose

Flow-sorted CMP and GMP cells were plated in triplicate onto dishes containing Methocult M3434 (STEMCELL Technologies). Colonies were scored 8 days post-plating. Determination of plating efficiency (PE) was performed by dividing the mean number of colonies by the number of plated cells. Data are mean ± SEM.

Apoptosis

To assess apoptosis, BM mononuclear cells from recipient mice were stained with biotin-conjugated anti-mouse lineage antibodies (Miltenyi Biotec) together with PE-Texas-Red-conjugated streptavidin, and then incubated with PE- conjugated anti-Sca-1, APC-eFluor® 780-conjugated anti-CD117 (eBiosciences), APC-conjugated annexin V (BD Pharmingen) and 2 µg/ml Hoechst 33258 (Sigma). Apoptotic cells were defined as annexin V-positive cells and dead cells as annexinV-negative and Hoechst 33258-positive cells.

RT-QPCR

For the analysis of gene expression, total RNAs were extracted from 50 000 sorted progenitor subpopulations and 10 000 sorted HSC (defined as LSK $CD135^-$ cells) and MPP (LSK $CD135^+$ cells) using the RNeasy microkit Plus (Qiagen). First-strand cDNA synthesis was performed using the superscript VILO cDNA synthesis kit (Invitrogen). Quantitative RT-PCR was performed using an Applied Biosystems 7300 sequence detector system (Applied Biosystems) with Itaq Universal Probes SuperMix (Biorad) for TaqMan probe system. Probes used were Rplp0 (Mm00725448) ; Actb (Mm02619580) ; Prnp (Mm00448389) ; Ape1 (Mm01319526). PCR comprised initial denaturation (95°C for 10 minutes), followed by 40 cycles at 95°C for 15 seconds and 60°C for one minute.

Supplementary Figures :

Fig S1: (A) Flow cytometric analysis of the PrPC protein expression in the indicated bone marrow subpopulations. Representative histograms of PrPC expression in the indicated WT (open histogram) and KO (filled histogram) bone marrow subpopulations. Dotted open histograms correspond to IgG2a isotype controls.
(B) Bone marrow cellularity in WT (black bars) and KO (white bars) 3 months old mice. Data are presented as the mean±SEM of 7-8 mice.
(C) Percentage of apoptotic (AnnexinV-positive cells) myeloid progenitors in WT (black bars) and KO (white bars) 3 months-old mice (n=7).

(D) Percentage of WT (black bars) and KO (white bars) myeloid progenitors in quiescence and in each phase of the cell cycle. Data are presented as the mean±SEM of 5 mice.
(E) Bone marrow cellularity is similar in WT (black bars) and KO (white bars) 11 months old mice. Data are presented as the mean±SEM of 6-8 mice.
(F) Frequencies of myeloid progenitors in the bone marrow from WT (black bars) and KO (white bars) 11 months-old mice. Data are presented as the mean±SEM of 6-8 mice. Statistically significant differences between WT and KO are indicated by asterisks (* P<0.05; ** P<0.01).

Fig S2:

(A) qRT-PCR analysis of *Ape1* expression, normalized to *Rplp0* in myeloid progenitors from 3 months-old mice. Non-irradiated KO and WT cells are presented as white and black bars, respectively. Irradiated (1h after 7 Gy) KO (KOIR) and WT (WTIR) cells are presented as light gray and dark grey bars, respectively. Data are presented as the mean±SEM of 4-16 mice. Means with different letters indicate are significantly different for WT (a;b) and KO (a';b') mice, respectively.

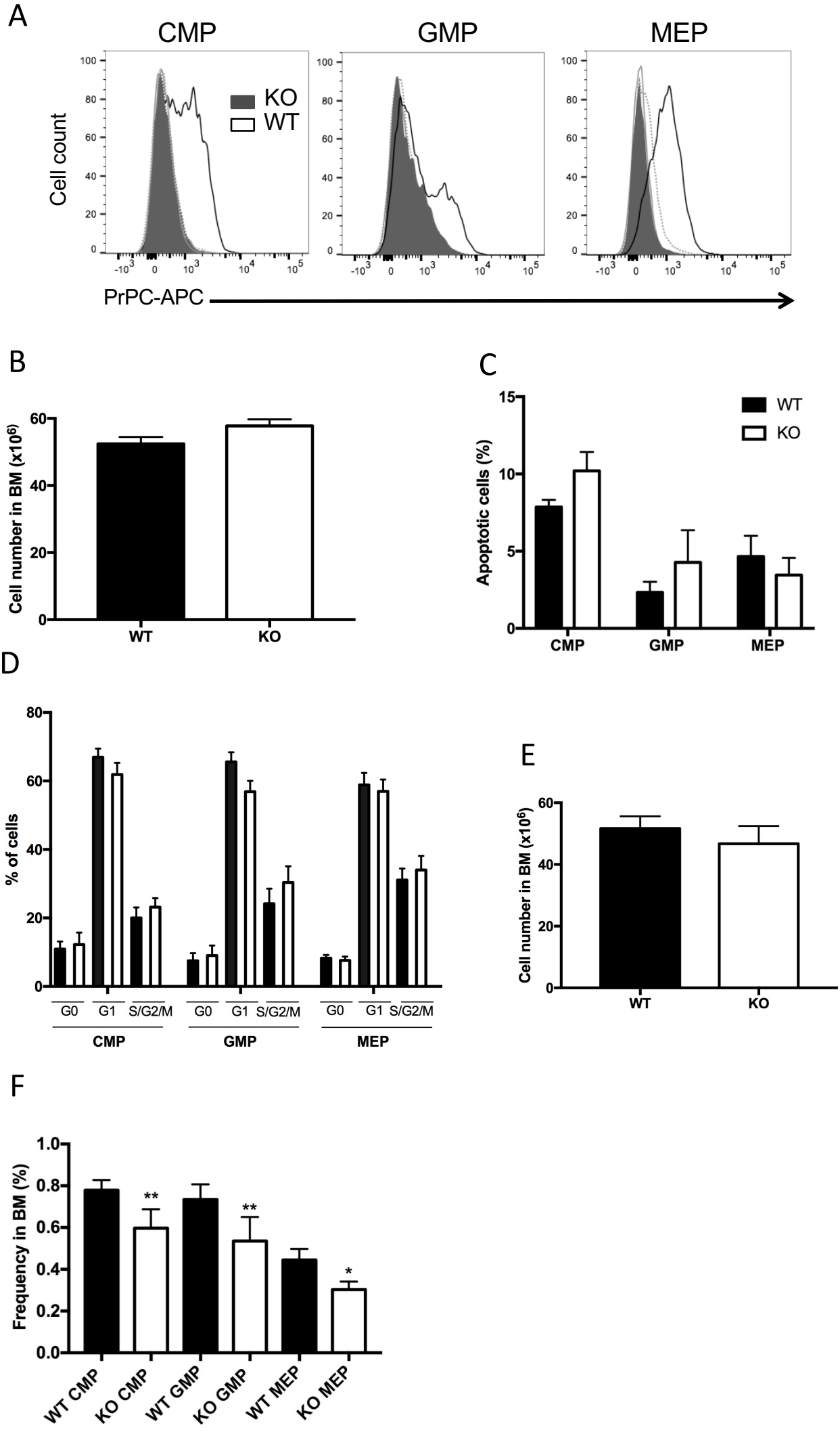
(B) qRT-PCR analysis of *Ape1* expression, normalized to *Actb* in HSC and MPP. Non-irradiated KO and WT cells are presented as white and black bars, respectively. Irradiated (1h after 7 Gy) KO (KOIR) and WT (WTIR) cells are presented as light gray and dark grey bars, respectively. Data are presented as the mean±SEM of 3 mice.

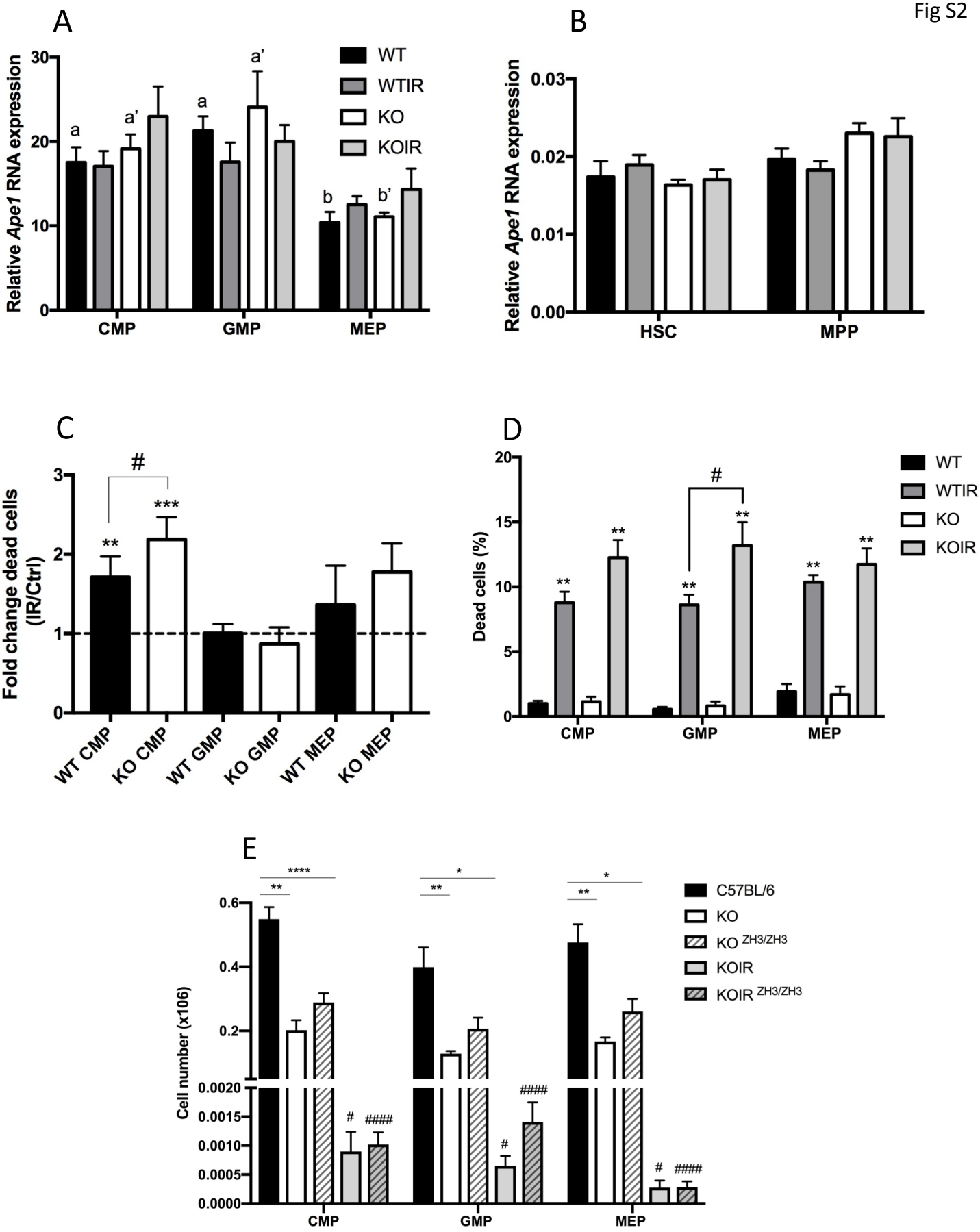
(C) Percentage of dead myeloid progenitors (AnnexineV-negative and Ho33258-positive cells) 1h after irradiation (7 Gy). WT and KO subpopulations are presented as black and white bars, respectively. Data are presented as the mean±SEM fold change of the percentage dead cell in irradiated relative to non-irradiated progenitors in the BM from 6-8 mice. Statistically significant differences non-irradiated and irradiated mice or between WT and KO are indicated by asterisks (*) and hash signs (#), respectively (** P<0.01; *** P<0.001; # P<0.05).

(D) Percentage of dead myeloid progenitors (AnnexineV-negative and Ho33258-positve cells) in BM from WT (dark gray bars - WTIR) and KO (light gray bars - KOIR)

mice (n=6-8) 1h after irradiation (7 Gy). Data are presented as the mean±SEM. Non-irradiated control WT (black bars) and KO (white bars) myeloid progenitors are shown. Statistically significant differences according to treatment or genotype are indicated by asterisks (*) and hash signs, respectively (** *P*<0.01; # *P*<0.05).

(E) Number of myeloid progenitors remaining in BM 18h after irradiation (7 Gy). Non-irradiated control C57BL/6, KO, and *Prnp*$^{ZH3/ZH3}$ mice are presented as black, white, and hatched white bars, respectively (n=4-10). KO and *Prnp*$^{ZH3/ZH3}$ irradiated mice are presented as light gray and dark gray hatched bars, respectively (n=5-11). Data are presented as the mean±SEM. Statistically significant differences between WT and either KO or *Prnp*$^{ZH3/ZH3}$ mice non-irradiated mice are indicated by asterisks. Statistically significant differences between non-irradiated and irradiated KO or *Prnp*$^{ZH3/ZH3}$ mice are indicated by hash signs (#). (**** or #### *P*<0.0001; ** P<0.01; * or # P<0.05).


A
CMP
GMP
MEP
KO
WT
Cell count
PrPC-APC
B
Cell number in BM (x10^6)
WT
KO
C
Apoptotic cells (%)
WT
KO
CMP
GMP
MEP
D
% of cells
G0
G1
S/G2/M
CMP
GMP
MEP
E
Cell number in BM (x10^6)
WT
KO
F
Frequency in BM (%)
WT CMP
KO CMP
WT GMP
KO GMP
WT MEP
KO MEP


A
Relative Ape1 RNA expression
30
20
10
0
a
a'
a
a'
b
b'
WT
WTIR
KO
KOIR
CMP
GMP
MEP
B
Relative Ape1 RNA expression
0.03
0.02
0.01
0.00
HSC
MPP
C
Fold change dead cells (IR/Ctrl)
3
2
1
0
#
**
***
WT CMP
KO CMP
WT GMP
KO GMP
WT MEP
KO MEP
D
Dead cells (%)
20
15
10
5
0
#
**
CMP
GMP
MEP
WT
WTIR
KO
KOIR
E
Cell number (x106)
0.6
0.4
0.2
0.0020
0.0015
0.0010
0.0005
0.0000
****
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*
#
####
CMP
GMP
MEP
C57BL/6
KO
KO ZH3/ZH3
KOIR
KOIR ZH3/ZH3