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

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Running title: PrPC-dependent hematopoietic homeostasis radioprotection of hematopoietic progenitors.

Abstract

Highly conserved among species and expressed in various types of cells, numerous roles have been attributed to the cellular prion protein (PrPC). In hematopoiesis, PrPC regulates hematopoietic stem cells self-renewal but the mechanisms involved in this regulation are unknown. Here we show that PrPC regulates hematopoietic stem cells number during aging and their determination towards myeloid progenitors. Furthermore, PrPC protects myeloid progenitors against the cytotoxic effects of total body irradiation. This radioprotective effect was associated with increased cellular prion mRNA level and with stimulation of the DNA repair activity of the Apurinic/pyrimidinic endonuclease 1, a key enzyme of the base excision repair pathway. Altogether, these results show a previously unappreciated role of PrPC in adult hematopoiesis and indicate that PrPC mediated stimulation of BER activity might protect hematopoietic progenitors from the cytotoxic effects of total body irradiation.

Key words: prion protein; radiation; hematopoiesis; myeloid progenitors; base excision repair.

Introduction

Radiotherapy is commonly used alone or in combination with genotoxic drugs for treatment of numerous solid tumours. Despite progress in its targeting, radiotherapy can be deleterious to two tissues, the gastrointestinal tract and the bone marrow, and can lead to secondary effects commonly defined as the Acute Radiation Syndrome¹. Irradiation of the bone marrow damages hematopoietic stem and progenitor cells (HSPC) and perturbs the hematopoietic microenvironment^{2,3} resulting in radiation-induced acute myelosuppression^{4,5} and increased susceptibility to infections^{6,7}.

Numerous types of DNA lesions are induced by ionizing radiation exposure of cells. They include base modifications, apurinic/apyrimidic sites (AP sites), single- (SSB) and double (DSB)-strand breaks. DSBs are the main lesions affecting cell survival. They can arise not only directly by energy deposition on the DNA but also as a consequence of the formation of AP sites or SSBs^{8,9}. Indeed, base excision repair (BER) activities, and in particular the processing of abasic sites, have been shown to contribute to radiation-induced DNA damage^{10,11}.

AP endonuclease-1 (Ape1) is the unique enzyme that converts AP sites into single strand break intermediates during BER. Ape1 3'-phosphodiesterase and -phosphate activities (for review¹²) also contribute to the processing of radiation-induced DNA strand break extremities¹³ during the single strand break repair pathway (SSBR). Accordingly, protection of neuronal cells from radiation-induced damage requires Ape1^{14,15}.

The Prion protein (PrPC) is a highly conserved glycoprotein that, when structurally modified, plays a critical role in the pathogenesis of neurodegenerative disorders called prion diseases¹⁶. The normal Prion protein was shown to protect cells from oxidative stress¹⁷⁻²⁰. It also protects from DNA damage by promoting APE1 DNA repair activity and cell survival through an interaction with APE1²¹. During hematopoiesis, PrPC is highly expressed in HSC and hematopoietic progenitors²²⁻²⁴ and PrPC deficiency is associated with decreased HSC self-renewal²⁵. As oxidative stress and DNA damage act on HSC cell fate²⁶, PrPC might participate in the maintenance of the hematopoietic system and its response to cytotoxic stresses. To address these points, we used *Prnp* knockout mice to study the consequences of

PrPC deficiency on hematopoiesis of young and old adult mice and on the response of HSC and hematopoietic progenitors to gamma-irradiation.

Methods:

Mice

Mice experiments were carried out in compliance with the European Community Council Directive (EC/2010/63) and were approved by our Institutional ethics committee (CEtEA-CEA-DRF–N°17-096). The B6.129S7-Prnptm1Cwe/Orl mice were from the European Mutant Mouse Archive and bred in our animal facility. We also used Prnp^{ZH3/ZH3} mice provided by A. Aguzzi (Zurich, Switzerland) and C57BL/6 mice were purchased from Charles River.

Cell sorting and flow cytometry analysis of bone marrow cells.

Murine bone marrow cells were flushed out of femurs, tibiae, hipbone and humeruses using a syringe filled with DPBS and filtered through a 70 µm-cell strainer. After red blood cell lysis using NH₄Cl solution (STEMCELL Technologies), mononuclear cells were phenotyped using different antibody cocktails from Biolegend, e-Bioscience or Beckton Dickinson. Flow cytometry analysis was performed with a BD FACSLSR^{II}™ flow cytometer (BD Biosciences) and cell sorting with a FACS Influx cell sorter (Becton Dickinson). Data were analyzed with FlowJo software. Antibodies and gating strategies for hematopoietic subset analysis and sorting are described in supplementary methods. For RT-PCR and Ape1 endonuclease activity experiments, aliquots of 50 000 cells myeloid progenitors were sorted in PBS whereas aliquots of 10 000 HSC and MPP were sorted in PBS/1%BSA.

Ape1 endonuclease activity

Cell extracts were obtained by sonication of pelleted bone marrow sorted cells in 20mM Tris-HCl, pH 7.5, 250mM NaCl, 1mM EDTA, 20mM sucrose, and protease inhibitor cocktail 0.1% (Sigma-Aldrich P2714). For progenitor analysis, 50 000 cells were suspended in 125µl extraction buffer. For HSC or MEP analysis, 10 000 sorted cells were suspended in 30µl extraction buffer. After sonication, the homogenate was centrifuged at 20 000 x g for 30 min at 4°C and aliquots of the supernatant were stored at -80°C. Ape1 endonuclease activity was measured using a 5'-end labelled 34-mer oligonucleotide containing a single tetrahydrofuranlyl artificial AP site at position 16 hybridized to its complementary oligonucleotide containing a cytosine opposite the lesion (Eurogentec). For measuring Ape1 activity in bone marrow

progenitors, 1 to 4 μ l of cell extract were incubated for 30min at 30°C in 10 μ l of reaction buffer containing 25mM Tris-HCl pH 7.5, 5mM MgCl₂, 5% glycerol, 52mM NaCl, BSA 1 μ g/ μ l and 150 fmoles of the hybridized oligonucleotide. For determining Ape1 activity in bone marrow HSC and MPP, 1 to 4 μ l of cell extract were incubated for 10min at 30°C in 10 μ l reaction buffer containing 25mM Tris-HCl pH 7.5, 5mM MgCl₂, 5% glycerol, 52mM NaCl, and 150 fmoles of the hybridized oligonucleotide. The reaction was stopped by adding 4 μ l of denaturing buffer (80 % formamide, 0.1% bromophenol blue, 10mM EDTA) followed by heating for 5 min at 95°C. The products of the reaction were resolved by denaturing 7M urea-20% polyacrylamide gel electrophoresis. Gels were scanned using a Typhoon 5 (GE Healthcare Life Sciences) and band intensities were quantified with ImageQuant TL 8.1 (GE Healthcare Life Sciences).

Statistical analyses

Quantitative data are presented as the mean \pm sem. Statistical significance was assayed using the non-parametric Mann Whitney U-test (GraphPad Prism software).

Additional material and methods are found in Supplementary Methods.

Results:

PrPC is differentially expressed in hematopoietic stem cells and myeloid progenitors and is involved in HSC expansion during aging

To address the potential functions of PrPC in hematopoietic stem and progenitor cells (HSPC), we first characterized the expression pattern of *Prnp* in different purified hematopoietic sub-subpopulations i.e. common Myeloid Progenitors (CMP), Granulocyte-Monocyte Progenitors (GMP), Megakaryocyte-Erythrocyte Progenitors (MEP), multi-potent progenitors (MPP) and hematopoietic stem cells (HSC). The highest level of *Prnp* mRNA was found in MEP while they were 2.7-fold and 4.3-fold lower in CMP and GMP, respectively (Fig 1A). These differences in mRNA expression were also found at the protein level (Fig 1B and Fig S1A). The *Prnp* mRNA level in purified HSC was 2.5 fold higher than in MPP (Fig 1C).

To determine if PrPC has a role in hematopoiesis, we first compared bone marrow (BM) from 3 months-old *Prnp*^{+/+} (WT) and *Prnp*^{-/-} (KO) mice. WT and KO mice showed similar peripheral blood counts (data not shown) and BM cellularity (Fig S1B). However, the frequency of CMP, GMP and MEP was significantly reduced in KO mice compared to WT mice (Fig 1D), whereas CLP, MPP, ST- and LT-HSC frequencies were similar (Fig 1E). The differences between KO and WT myeloid progenitor frequencies were not associated with increased apoptosis (Fig S1C) or cell cycle alteration (Fig S1D) but with a higher percentage of quiescent MPP and ST-HSC (Fig 1F). These results suggest a defect of determination of HSPC (MPP and ST-HSC) towards the myeloid lineage in *Prnp*^{-/-} mice. Finally, clonogenic assay using purified CMP and GMP showed a significantly decreased plating efficiency of *Prnp*^{-/-} CMP and GMP (Fig 1G). Taken together, these results show intrinsic myeloid differentiation deficiencies in *Prnp*^{-/-} HSC and progenitors and suggest that the reduction of cycling MPP and ST-HSC contributes to the lower myeloid progenitor content in the BM from KO compared to WT mice.

Aging of HSC is associated with increased in the percentage of HSC in BM, decreased HSC self-renewal and accumulation of DNA damage²⁷. As PrPC has been implicated in HSC self-renewal²⁵ and in DNA repair²¹, we investigated the effect of PrPC loss in HSC numbers and DNA repair capacity during aging in *Prnp*^{+/+} and *Prnp*^{-/-} mice. *Prnp* mRNA level in HSC was 2.7-fold higher in 11 months-old

compared to 3 months-old mice (Fig 1H) but did not change in MPP (Fig 1H). BM from 11 months-old WT and KO mice displayed similar cellularity (Fig S1E). As in 3 months-old mice, a lower frequency of myeloid progenitors but not MPP (Fig 1I) was found in 11 months-old KO mice (Fig S1F). In contrast, compared to their 3 months-old counterparts, ST- and LT-HSC frequencies respectively increased 2- and 8.3-fold in 11 months-old WT mice but did not change (ST-HSC) or was only 3-fold increased (LT-HSC) in 11 months-old KO mice (Fig 1I). DNA repair was slightly dependent on PrPC in aged HSC. A 1.4 fold increased activity of Ape1 in WT HSC was found between 3 months and 11 months without any change in Ape1 mRNA level. In KO HSC, Ape1 activity also increased between 3 months and 11 months, but to a lesser extent (1.2-fold) than in WT HSC. Interestingly, this increased activity was associated with an increased Ape1 mRNA level (Fig 1J and Fig 1K).

Altogether, these results show that PrPC deficiency is associated with decreased HSC determination towards the myeloid lineage and decreased number of HSC and decreased APE1 activity in old mice.

Prnp expression is upregulated in myeloid progenitors and HSC subpopulations after in vivo radiation exposure

HSC aging is associated with increased oxidative stress²⁸ and PrPC has been shown to protect cells from oxidative stress¹⁷⁻²⁰. To characterize PrPC role during an oxidative stress of hematopoiesis, we performed total body irradiation (TBI) on WT and KO mice. Survival curves of WT and KO mice exposed to increasing gamma-radiation doses showed that a higher percentage of irradiated KO mice died earlier than irradiated WT mice even if statistical significance between both genotypes was not reached ($p=0.0792$ at 7 Gy) (Fig 2A). When KO mice were grafted with BM from non-irradiated WT mice 24h after a 7 Gy irradiation, they did not die, indicating that the higher sensitivity of KO mice to TBI was not due to the bone marrow microenvironment of KO mice and that they died from hematopoietic syndrome.

One hour after a 7 Gy TBI, a 1.5-fold increase of *Prnp* mRNA level was found in HSC, CMP and GMP but not in MPP and MEP (Fig 2B). These data are consistent with the observed *Prnp* upregulation in neuronal tissues after exposure to genotoxic stress²¹ and suggest a potential role of PrPC in response to radiation in GMP, CMP, and HSC.

PrPC-dependent increase in the DNA repair activity of Ape1 is associated with radioprotection of CMP and GMP

PrPC prevents cell death in response to alkylating agent or H₂O₂ exposure by directly stimulating the DNA repair activity of Ape1²¹. Without irradiation, Ape1 activity was similar in WT and KO progenitors (Fig 2C). One hour after a 7 Gy TBI, Ape1 activity increased in all WT irradiated myeloid progenitors analyzed (from 1.5 to 1.7 fold) but not in their KO counter parts (Fig 2D). In HSC and MPP Ape1 activity was similar in WT and KO mice (Fig 2E) but increased only in WT HSC after a 7 Gy TBI (Fig 2F). Whatever the subpopulation analyzed, the radiation-induced Ape1 activity was not associated with an increase in Ape1 mRNA level (Fig S2A and S2B). These data show that after irradiation, Ape1 activity in all myeloid progenitor subpopulations and in HSC is stimulated in a PrPC-dependent manner.

As the radiation-induced death of myeloid progenitors is dependent on apoptosis⁵, we quantified apoptosis in WT and KO myeloid progenitors one and twelve hours after TBI at 7 Gy. One hour after irradiation, apoptotic (Annexin V-positive cells) and dead cells (Annexin V-negative and Hoechst-positive cells) fractions increased only in CMP and were higher in KO compared to WT CMP (Fig 2G and S2C). Twelve hours after irradiation, both apoptotic (Fig 2H) and dead (Fig S2D) cells fractions increased in all myeloid progenitor subpopulations in both WT and KO mice. However, higher rates of apoptosis and cellular death were observed in irradiated KO compared to WT GMP. In accordance, significantly lower frequencies of CMP and GMP were found in KO versus WT irradiated mice 18h after irradiation (Fig 2I). Myeloid progenitors from *Prnp*^{ZH3/ZH3} mice exhibited the same radiation sensitivity than those from *Prnp*^{-/-} mice shown by a similar reduced number compared to mice in non-irradiated conditions (Fig S2 E). Altogether these results suggest that PrPC-dependent stimulation of the DNA repair activity of Ape1 is required for the radioprotection of myeloid progenitors.

Discussion

Despite numerous studies, the physiological role of PrPC remains elusive. Recently, we showed that PrPC can stimulate an important DNA repair pathway, the BER, in neuronal tissues through interaction with and stimulation of its key enzyme, APE1²¹. Here we show that the same mechanism can be proposed for the radioprotection of myeloid progenitors, HSC determination, and the expansion of the HSC compartment during aging.

Previous studies^{22–24,29} indicated a decreased *Prnp* expression during differentiation of hematopoietic cells. Here, we performed an extended study of *Prnp* expression in different hematopoietic sub-populations and showed a 3-fold higher *Prnp* expression level in MEPs compared to their progenitors CMP suggesting that the correlation between *Prnp* downregulation and cellular differentiation²⁴ may not be a general feature in hematopoiesis. Furthermore, and contrary to a previous study²⁵, we found that KO mice have less myeloid progenitors. This discrepancy could be explained by the fact that in the previous study younger mice were analyzed (7 to 10 weeks-old mice compared to 3 months- and 11 months-old mice used in the present study) and by the number of backcrosses (4 versus >10) that might influence the phenotype of *Prnp* knockout mice³⁰. Finally, we found a higher frequency of KO ST-HSC and KO MPP in the G0 phase. These populations being the direct precursors of myeloid progenitors, the increased quiescence of these cells might account for the decreased myeloid progenitor subpopulations³¹. Strikingly, both KO CMP and GMP exhibited a lower plating efficiency despite no significant change in their cell cycle *in vivo*. Whether the microenvironment of these cells could compensate *in vivo* for an intrinsic growth deficiency observed *in vitro* remains to be clarified.

Prnp expression in the HSC compartment increased 8-fold with age. This higher expression was associated with the known elevated frequency of both ST- and LT-HSC^{27,32}. PrpC deficiency was associated with no increase of ST-HSC and with a diminished increase of LT-HSC with age suggesting that PrPC plays a role in the age-dependent increase of HSC. Although aging-associated increase in HSC numbers has been known for a long time and is known to be cell-intrinsic^{33,34}, the underlying mechanisms are not fully understood. The results presented here indicate a significant involvement of PrPC in the age-dependent increase of HSC frequency. This increase may be accounted for by a PrPC-dependent up-regulation of Ape1

repair activity. Independent from its DNA repair activity^{35,36}. Ape1 has a redox activity shown to be necessary for normal embryonic hematopoiesis³⁷, stem cell pool maintenance^{38,39} and hematopoietic progenitors colony formation⁴⁰. Thus, the PrPC-dependent stimulation of the APE1 DNA repair activity might contribute to hematopoietic homeostasis.

We found a modest but recurrent radiation sensitization of *Prnp* KO mice that contrasts with a previous study showing that the absence of PrPC protected rather than sensitized mice to an 8 Gy TBI⁴¹. However, this work was performed on a mixed 129/C57BL6 background, whereas we used a pure C57BL6 background. Furthermore, that study used a dose of X-rays that was lethal for WT animals while we used different doses of non-lethal γ -rays. Bone marrow myeloid cells are particularly sensitive to chemical and radiation cytotoxicity^{4,42,43}. Accordingly, we found a dramatic decrease in bone marrow myeloid progenitors within the first 24h after radiation exposure that was exacerbated in *Prnp* KO irradiated mice. The reduced frequency of KO irradiated myeloid progenitors was associated with higher CMP and GMP apoptosis, within the first twelve hours after irradiation, as well as with an absence of stimulation of APE1 activity in these subpopulations one hour after irradiation. In contrast, in WT irradiated myeloid progenitors and HSC, upregulation of *Prnp* gene expression was associated with an increase in Ape1 activity in these subpopulations. PrPC has been shown to protect HSC from myelotoxic injury by 5-FU, commonly used in chemotherapy²⁵ and we now extend its myeloprotective role to radiotherapy.

Finally, a similar basal reduced number of myeloid progenitors and a similar radiation sensitivity of myeloid progenitors were found in the co-isogenic *Prnp*^{ZH3/ZH3} mouse line⁴⁴ and the *Prnp*^{-/-} mouse line. These results rule out the involvement of any *Prnp* flanking genes polymorphism previously described by Nuvolone et al⁴⁵.

Altogether, these results suggest that PrPC is involved in the homeostasis of steady-state hematopoiesis and that PrPC dependent activation of base excision repair contributes to the radioprotection of the myeloid progenitors of the mouse bone marrow.

Author contributions: CS performed experiments and analyzed data. ND performed cell sorting. VB performed bone marrow grafting. NG performed experiments, analyzed data, and reviewed the manuscript. PH R and JP R reviewed the manuscript. AA provided *Prnp*^{ZH3/ZH3} mice. JBS and AB designed and performed experiments, analyzed data, and wrote the manuscript.

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References

1. Dörr H, Meineke V. Acute radiation syndrome caused by accidental radiation exposure - therapeutic principles. *BMC Med.* 2011;9(1):126.
2. Simonnet AJ, Nehmë J, Vaigot P, Barroca V, Leboulch P, Tronik-Le Roux D. Phenotypic and functional changes induced in hematopoietic stem/progenitor cells after gamma-ray radiation exposure. *Stem Cells.* 2009;27(6):1400–1409.
3. Shao L, Luo Y, Zhou D. Hematopoietic Stem Cell Injury Induced by Ionizing Radiation. *Antioxid Redox Signal.* 2014;20(9):1447–1462.
4. Down JD, Boudewijn A, van Os R, Thames HD, Ploemacher RE. Variations in radiation sensitivity and repair among different hematopoietic stem cell subsets following fractionated irradiation. *Blood.* 1995;86(1):122–127.
5. Mohrin M, Bourke E, Alexander D, et al. Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis. *Cell Stem Cell.* 2010;7(2):174–185.
6. Kim JH, Thimmulappa RK, Kumar V, et al. NRF2-mediated Notch pathway activation enhances hematopoietic reconstitution following myelosuppressive radiation. *J Clin Invest.* 2014;124(2):730–741.
7. Zachman DK, Leon RP, Das P, et al. Endothelial cells mitigate DNA damage and promote the regeneration of hematopoietic stem cells after radiation injury. *Stem Cell Res.* 2013;11(3):1013–1021.
8. Ensminger M, Iloff L, Ebel C, Nikolova T, Kaina B, Löbrich M. DNA breaks and chromosomal aberrations arise when replication meets base excision repair. *J Cell Biol.* 2014;206(1):29–43.
9. Bauer NC, Corbett AH, Doetsch PW. The current state of eukaryotic DNA base damage and repair. *Nucleic Acids Res.* 2015;43(21):10083–10101.
10. Fung H, Demple B. Distinct roles of Ape1 protein in the repair of DNA damage induced by ionizing radiation or bleomycin. *J Biol Chem.* 2011;286(7):4968–4977.
11. Wang H, Wang X, Chen G, et al. Distinct roles of Ape1 protein, an enzyme involved in DNA repair, in high or low linear energy transfer ionizing radiation-induced cell killing. *J Biol Chem.* 2014;289(44):30635–30644.
12. Laev SS, Salakhutdinov NF, Lavrik OI. Inhibitors of nuclease and redox activity of apurinic/apyrimidinic endonuclease 1/redox effector factor 1 (APE1/Ref-1).

- Bioorganic Med Chem. 2017;25(9):2531–2544.
13. Parsons JL, Dianova II, Dianov GL. APE1 is the major 3'-phosphoglycolate activity in human cell extracts. *Nucleic Acids Res.* 2004;32(12):3531–3536.
 14. Vasko MR, Guo C, Thompson EL, Kelley MR. The repair function of the multifunctional DNA repair/redox protein APE1 is neuroprotective after ionizing radiation. *DNA Repair (Amst).* 2011;10(9):942–952.
 15. Ströbel T, Madlener S, Tuna S, et al. Ape1 guides DNA repair pathway choice that is associated with drug tolerance in glioblastoma. *Sci Rep.* 2017;7(1):1–13.
 16. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science.* 1982;216(4542):136–144.
 17. Milhavet O, Lehmann S. Oxidative stress and the prion protein in transmissible spongiform encephalopathies. *Brain Res Brain Res Rev.* 2002;38(3):328-339.
 18. Rachidi W, Vilette D, Guiraud P, et al. Expression of prion protein increases cellular copper binding and antioxidant enzyme activities but not copper delivery. *J Biol Chem.* 2003;278(11):9064–9072.
 19. McLennan NF, Brennan PM, McNeill A, et al. Prion protein accumulation and neuroprotection in hypoxic brain damage. *Am J Pathol.* 2004;165(1):227–235.
 20. Krebs B, Wiebelitz A, Balitzki-Korte B, et al. Cellular prion protein modulates the intracellular calcium response to hydrogen peroxide. *J Neurochem.* 2007;100(2):358–367.
 21. Bravard A, Auvré F, Fantini D, et al. The prion protein is critical for DNA repair and cell survival after genotoxic stress. *Nucleic Acids Res.* 2015;43(2):904–916.
 22. Kent DG, Copley MR, Benz C, et al. Prospective isolation and molecular characterization of hematopoietic stem cells with durable self-renewal potential. *Blood.* 2009;113(25):6342–6350.
 23. Liu T, Li R, Wong B-S, et al. Normal Cellular Prion Protein Is Preferentially Expressed on Subpopulations of Murine Hemopoietic Cells. *J Immunol.* 2001;166(6):3733–3742.
 24. Panigaj M, Glier H, Wildova M, Holada K. Expression of prion protein in mouse erythroid progenitors and differentiating murine erythroleukemia cells. *PLoS One.* 2011;6(9):e24599.
 25. Zhang CC, Steele AD, Lindquist S, Lodish HF. Prion protein is expressed on long-term repopulating hematopoietic stem cells and is important for their self-

- renewal. *Proc Natl Acad Sci U S A*. 2006;103(7):2184–2189.
26. Weiss CN, Ito K. DNA damage: A sensible mediator of the differentiation decision in hematopoietic stem cells and in leukemia. *Int J Mol Sci*. 2015;16(3):6183-6201.
 27. Rossi DJ, Seita J, Czechowicz A, Bhattacharya D, Bryder D, Weissman IL. Hematopoietic stem cell quiescence attenuates DNA damage response and permits DNA damage accumulation during aging. *Cell Cycle*. 2007;6(19):2371–2376.
 28. Chen F, Liu Y, Wong NK, Xiao J, So KF. Oxidative Stress in Stem Cell Aging. *Cell Transplant*. 2017;26(9):1483-1495.
 29. Dodelet VC, Cashman NR. Prion protein expression in human leukocyte differentiation. *Blood*. 1998;95(5):1556–1561.
 30. Schmitz M, Greis C, Ottis P, et al. Loss of Prion Protein Leads to Age-Dependent Behavioral Abnormalities and Changes in Cytoskeletal Protein Expression. *Mol Neurobiol*. 2014;50(3):923-936.
 31. Susek KH, Korpos E, Huppert J, et al. Bone marrow laminins influence hematopoietic stem and progenitor cell cycling and homing to the bone marrow. *Matrix Biol*. 2018;6747–62.
 32. Kowalczyk MS, Tirosh I, Heckl D, et al. Single-cell RNA-seq reveals changes in cell cycle and differentiation programs upon aging of hematopoietic stem cells. *Genome Res*. 2015;25(12):1860–1872.
 33. Rossi DJ, Bryder D, Zahn JM, et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc Natl Acad Sci U S A*. 2005;102(26):9194–9199.
 34. Geiger H, De Haan G, Carolina Florian M. The ageing haematopoietic stem cell compartment. *Nat Rev Immunol*. 2013;13(5):376-389.
 35. Tell G, Quadrioglio F, Tiribelli C, Kelley MR. The Many Functions of APE1/Ref-1: Not Only a DNA Repair Enzyme. *Antioxid Redox Signal*. 2008;11(3):601–620.
 36. Xie J, Zhang L, Li M, et al. Functional analysis of the involvement of apurinic/apyrimidinic endonuclease 1 in the resistance to melphalan in multiple myeloma. *BMC Cancer*. 2014;14:11.
 37. Zou GM, Luo MH, Reed A, Kelley MR, Yoder MC. Ape1 regulates hematopoietic differentiation of embryonic stem cells through its redox

- functional domain. *Blood*. 2007;109(5):1917–1922.
38. Wang K, Zhang T, Dong Q, Nice EC, Huang C, Wei Y. Redox homeostasis: The linchpin in stem cell self-renewal and differentiation. *Cell Death Dis*. 2013;4:e537.
 39. Domenis R, Bergamin N, Gianfranceschi G, et al. The redox function of APE1 is involved in the differentiation process of stem cells toward a neuronal cell fate. *PLoS One*. 2014;9(2):e89232.
 40. Rohrabough SL, Hangoc G, Kelley MR, Broxmeyer HE. Mad2 haploinsufficiency protects hematopoietic progenitor cells subjected to cell-cycle stress in vivo and to inhibition of redox function of Ape1/Ref-1 in vitro. *Exp Hematol*. 2011;39(4):415-423.
 41. Strup-Perrot C, Vozenin MC, Monceau V, et al. PrP^c deficiency and dasatinib protect mouse intestines against radiation injury by inhibiting of c-Src. *Radiother Oncol*. 2016;120(1):175–183.
 42. Pilzecker B, Buoninfante OA, van den Berk P, et al. DNA damage tolerance in hematopoietic stem and progenitor cells in mice. *Proc Natl Acad Sci U S A*. 2017;114(33):E6875–E6883.
 43. Roth RB, Samson LD. 3-Methyladenine DNA glycosylase-deficient Aag null mice display unexpected bone marrow alkylation resistance. *Cancer Res*. 2002;62(3):656–660.
 44. Nuvolone M, Hermann M, Sorce S, et al. Strictly co-isogenic C57BL/6J- Prnp^{-/-} mice: A rigorous resource for prion science. *J Exp Med*. 2016;213(3):313–327.
 45. Nuvolone M, Kana V, Hutter G, et al. SIRP α polymorphisms, but not the prion protein, control phagocytosis of apoptotic cells. *J Exp Med*. 2013;210(12):2539–2552.

Figure legends:

Fig1: PrPC contributes to mouse hematopoietic homeostasis.

(A) qRT-PCR analysis of *Prnp* expression, normalized to *Rplp0* in the indicated bone marrow sub-populations: CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; MEP, megakaryocyte-erythrocyte progenitor purified by flow cytometry from BM of 3 months-old mice (n=7-9). Data are presented as mean±SEM. Means with different letters are significantly different ($P<0.05$).

(B) Flow cytometry analysis of the PrPC protein expression in the indicated bone marrow subpopulations. The graph depicts ratio of median fluorescence intensity (MFI) in WT and KO control cells (n=5-6). Data are presented as mean±SEM. Means with different letters are significantly different ($P<0.05$) (CMP versus MEP $p=0.06$).

(C) qRT-PCR analysis of *Prnp* expression, normalized to *Actb* in HSC, hematopoietic stem cell (LSK CD135⁻) and in MPP, multipotent progenitor (LSK CD135⁺) purified by flow cytometry from BM of 3 months-old mice (n=9); Data are presented as mean±SEM. Statistically significant difference between HSC and MPP is indicated by asterisks (** $P<0.001$).

(D) Frequencies of myeloid progenitors in WT (black bars) and KO (white bars) bone marrow from 3 months-old mice (n=6-10). Data are presented as mean±SEM. Statistically significant differences between WT and KO are indicated by asterisks (* $P<0.05$; ** $P<0.01$).

(E) Frequencies of lymphoid progenitors (CLP), MPP, ST- and LT-HSC in the bone marrow from WT (black bars) and KO (white bars) mice (n=6). Data are presented as mean±SEM.

(F) Distribution of WT (black bars) and KO (white bars) HSC and MPP in each phase of the cell cycle. Data are presented as mean±SEM. Statistically significant differences between WT and KO are indicated by asterisks (* $P<0.05$; ** $P<0.01$).

(G) In vitro plating efficiency of CMP and GMP purified by flow cytometry from BM of WT (black bars) and KO (white bars) mice (n=6-9). Data are presented as mean±SEM. Statistically significant differences between WT and KO are indicated by asterisks (** $P<0.01$).

(H) qRT-PCR analysis of *Prnp* expression, normalized to *Actb* in WT and KO HSC and MPP purified by flow cytometry from bone marrow of 3 months and 11 months

old mice (n=6-9). Data are presented as the mean±SEM. Statistically significant differences between 3 and 11 months-old mice and between HSC and MPP subpopulations are indicated by hash signs and asterisks, respectively (** or ### $P<0.001$)

(I) Frequencies of MPP, ST- and LT-HSC in BM from 3 months and 11 months old WT (black bars) and KO (white bars) mice. Data are presented as the mean±SEM fold change of the frequencies in 11 months relative to 3 months-old mice. Statistically significant differences according to age or genotype are indicated by asterisks and hash signs, respectively (n=6-10; ** or ## $P<0.01$).

J) *Ape1* endonuclease activity in 3 (opened) and 11 (hatched) months-old WT (dark) and KO (light) HSC. Data are presented as the mean±SEM. Statistically significant differences according to age or genotype are indicated by asterisks and hash signs, respectively (n=4-5; * or # $P<0.05$).

K) qRT-PCR analysis of *Ape1* expression, normalized to *Actb* in WT (dark) and KO (light) HSC purified by flow cytometry from bone marrow of 3 (opened bars) and 11 (hatched bars) months-old mice. Data are presented as the mean±SEM. Statistically significant differences according to age or genotype are indicated by asterisks and hash signs, respectively (n=7-9; * or # $P<0.05$).

Fig 2: PrPC favours survival of mice exposed to moderate doses of γ -rays and protects CMP from radiation-induced death.

(A) Kaplan-Meier survival plots of WT (solid lines) and KO (dashed lines) mouse overall survival after whole-body irradiation at indicated doses (n= 5 (10 Gy); n=13 (6.5 Gy); n= 28 (7 Gy) for each genotype). The arrow points to the 100% survival of both KO and WT irradiated mice (7 Gy) after transplantation of WT bone marrow cells (n=5).

(B) qRT-PCR analysis of *Prnp* expression in HSC, MPP and myeloid progenitors one hour after irradiation (7 Gy) (n=6). *Prnp* RNA levels were normalized to *Actb* (HSC and MPP) or *Rplp0* (myeloid progenitors). Data are presented as the mean±SEM fold change of normalized *Prnp* RNA levels in irradiated relative to control cells. Statistically significant differences between irradiated and control are indicated by asterisks (* $P<0.05$; ** $P<0.01$).

(C) Ape1 endonuclease activity in myeloid progenitor subpopulations from WT (black bars) and KO (white bars) mice (n=5-8). Data are presented as the mean±SEM. Means with different letters are significantly different ($P<0.05$).

(D) Ape1 endonuclease activity in myeloid progenitors from WT (black bars) and KO (white bars) mice (n=5-8) one hour after irradiation (7 Gy). Data are presented as the mean±SEM fold change of Ape1 endonuclease in irradiated relative to non-irradiated control cells. Statistically significant differences between non-irradiated and irradiated cells are indicated by asterisks (* $P<0.05$; ** $P<0.01$).

(E) Ape1 endonuclease activity in HSC and MPP from WT (black bars) and KO (white bars) mice (n=5-8). Data are presented as the mean±SEM.

(F) Ape1 endonuclease activity in HSC and MPP from WT (black bars) and KO (white bars) mice (n=4-5) one hour after irradiation (7 Gy). Data are presented as the mean±SEM fold change of Ape1 endonuclease in irradiated relative to non-irradiated control cells. Statistically significant differences according to treatment or genotype are indicated by an asterisk and hash sign, respectively (* or # $P<0.05$).

(G) Percentage of apoptotic myeloid progenitors (AnnexineV-positive cells) in BM from WT (black bars) and KO (white bars) mice (n=6-8) 1h after irradiation (7 Gy). Data are presented as the mean±SEM fold change of percentage of apoptotic cells in irradiated relative to non-irradiated control myeloid progenitors. Statistically significant differences according to treatment or genotype are indicated by asterisks (*) and hash signs, respectively (** $P<0.01$; *** $P<0.001$; # $P<0.05$).

(H) Percentage of apoptotic myeloid progenitors (AnnexineV-positive 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 (** $P<0.01$; # $P<0.05$).

(I) Percentage of myeloid progenitors in BM from WT (black bars) and KO (white bars) mice (n=6-7) 18h after irradiation (7 Gy). Data are presented as the mean±SEM of the percentage of cells remaining in BM 18h after irradiation compared to non-irradiated control. Statistically significant differences between WT and KO mice are indicated by asterisks (** $P<0.01$).







