Diminished apoptotic priming and ATM signalling confer a survival advantage onto aged haematopoietic stem cells in response to DNA damage

Paula Gutierrez-Martinez, Leah Hogdal, Manavi Nagai, Miriama Kruta, Rumani Singh, Kristopher Sarosiek, Andre Nussenzweig, Isabel Beerman, Anthony Letai and Derrick J. Rossi

Ageing of haematopoietic stem cells (HSCs) contributes to deficits in the aged haematopoietic system. HSC decline is driven in part by DNA damage accumulation; yet, how ageing impacts the acute DNA damage response (DDR) of HSCs is poorly understood. We show that old HSCs exhibit diminished ATM activity and attenuated DDR, leading to elevated clonal survival in response to a range of genotoxins that was underwritten by diminished apoptotic priming. Distinct HSC subsets exhibited ageing-dependent and subtype-dependent differences in apoptotic priming and survival in response to DNA damage. The defective DDR of old HSCs was non-cell autonomous, as ATM signalling and clonal survival in response to DNA damage could be restored to levels observed in young HSCs post-transplanted into young recipients. These data indicate that defective DDR and diminished apoptotic priming provide a selective advantage to old HSCs that may contribute to mutation accrual and disease predisposition.

Stem cells mediate tissue homeostasis and regeneration, and ageing-associated decline in stem cell compartments contributes to pathophysiology in multiple tissues and organ systems. Diminished haematopoietic stem cell (HSC) potential is a driver of ageing in the haematopoietic system. Numerous mechanisms underlie HSC ageing, including the accumulation of DNA damage, alterations in the transcriptional programme, epigenetic remodelling, cell polarity changes, altered lineage output and decreased regenerative potential. Adult HSCs are largely quiescent, which had been proposed to be a cytoprotective mechanism for preserving genome integrity and long-term function. However, it was recently shown that old HSCs have elevated levels of DNA damage at steady state that are, at least in part, attributable to prolonged periods of dormancy. Upon cell cycle entry, HSCs upregulate the DNA damage response and repair pathways and repair accrued strand breaks.

Results

Aged HSCs show increased survival upon DNA damage induction in vitro and in vivo. As many cancers are treated with genotoxic agents, we investigated how HSCs respond to diverse types of DNA damage and whether this response is differentially regulated during ageing. To address this, single HSCs from young and old mice were sorted via the immunophenotype Lin−c-kit+Sca-1+Flk2–CD34–Sca– ( Supplementary Fig. 2a), which are CD48+ regardless of age (Supplementary Fig. 1a,b), and exposed to different types of DNA-damaging agents. These included N-ethyl-N-nitrosourea (ENU) and ethyl methanesulfonate (EMS), which induce point mutations, doxorubicin and gamma irradiation (IR), which produce double-strand breaks, and hydroxyurea (HU), which induces replicative stress (Fig. 1a). In the absence of challenge, young and old HSCs produced similar numbers of colonies when cultured in minimal media (young HSCs: 64.7±14.3% and old HSCs: 62.9±12.4%) (Fig. 1b). Strikingly, old HSCs were invariably less sensitive to all genotoxic agents, exhibiting 2–6-fold elevated clonal survival compared to young HSCs depending on the type of DNA damage induced (Fig. 1b,c). The elevated clonal survival of old HSCs could not be attributed to differences in cell cycle, as both young and old HSCs showed similar cell cycle profiles when freshly isolated and after 18 h of culture (Supplementary Fig. 2b), as well as similar proliferation rates over the first 3 days of culture (Supplementary Fig. 2c). Colony size 10-days post-plating was diminished after DNA damage induction irrespective of age, indicating that the total proliferative output of surviving clones was ageing independent ( Supplementary Fig. 2d,e). The differential survival response to DNA damage induction was specific to old HSCs as single myeloid progenitors (MPs; Lin−c-kit+Sca–1) exposed to EMS, ENU and IR, and multipotent progenitors (MPP1s, Lin−c-kit+Sca–1+CD34+Flk2– and MPP2s, Lin−c-kit+Sca–1+CD34+Flk2+) exposed to IR gave rise to colonies at similar frequencies (Fig. 1d–f) and sizes (Supplementary Fig. 2f,g) regardless of age.

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To further investigate the functional impact of DNA damage induction on HSCs, we isolated young and old HSCs, exposed them to either ENU or IR, and then competitively transplanted them into lethally irradiated congenic recipients. As previously reported, old HSCs showed significantly reduced total regenerative potential (Fig. 2a,b). However, whereas young HSCs exposed to either ENU or IR showed significantly reduced long-term reconstitution compared to young untreated HSCs, old HSCs exposed to either ENU or IR maintained long-term reconstitution potential comparable to untreated old HSCs (Fig. 2a,b). Old HSCs showed myeloid-biased reconstitution as expected, and the lineage potentials of both young and old HSCs were unaltered by DNA damage induction (Fig. 2c,d).
Aged HSCs display elevated survival in response to DNA damage induction. The differential functional response of young and old HSCs observed in vitro (Fig. 1b,c) and in vivo (Fig. 2a,b) prompted us to explore clonal survival and growth dynamics in response to DNA damage induction. HSCs from young and old mice were clone sorted and were either untreated or exposed to IR, followed by culturing and analysis of viability and cell division daily for 8 days (n = 288 clones per condition) (Supplementary Fig. 2). As we had previously observed (Fig. 1b), young HSC clones exposed to IR gave rise to significantly fewer viable colonies than untreated controls, whereas irradiated old HSCs showed no differences in survival compared to untreated old HSCs at any time point (Fig. 2c–g and Supplementary Fig. 3). Strikingly, the greatest loss of viability of young clones occurred within the first 24 h post-IR, prior to the first cell division (Fig. 2e–g and Supplementary Fig. 1c), suggesting an intrinsic difference in the ability of young and old HSCs to respond to DNA damage. Consistent with clonal survival and transplantation data, old HSCs exhibited a diminished frequency of apoptotic (annexin V+propidium iodide (PI)+) cells compared to young HSCs 8-hours post-IR but not at steady-state (Fig. 2h,i). This differential apoptotic response was specific to old HSCs as young and old MPs exposed to IR showed similar frequencies of apoptotic cells albeit at higher levels than observed for HSCs (Fig. 2h,i).

Aged HSCs exhibit diminished apoptotic priming. Apoptosis is regulated through a balance between pro-apoptotic and anti-apoptotic members of the Bcl-2 family of proteins that controls mitochondrial outer membrane permeabilization (MOMP) upstream of cytochrome c release and caspase activation. This family consists of pro-apoptotic BH3-only proteins, pro-apoptotic effector proteins and anti-apoptotic proteins, which interact to control MOMP. The BH3-only proteins are divided into two groups based on function: the ‘activators’ (BIM and BID) and the ‘sensitizers’ (such as BAD, HRK and NOXA). The activators bind to and inhibit the anti-apoptotic proteins, and also directly interact with and activate the effector proteins (BAX and BAK), causing the proteins to promote apoptosis. The BH3-only sensitizers promote apoptosis by binding to the anti-apoptotic proteins, which allows for the release of the activator and effector proteins to initiate MOMP22,23,24 (Fig. 3a). Owing...
showed significantly diminished priming compared to all downstream progenitors that reached significance in comparison to MCP (Lin–c-kit–Sca-1–Flk2+IL7Rc–CD34+) and CLPs (Lin–c-kit–Sca-1–Flk2+IL7Rc+) (Fig. 3b). This pattern was exacerbated in the bone marrow of old mice with HSCs, which showed significantly diminished priming compared to all downstream progenitors (Fig. 3b). Comparison of young versus old stem and progenitor cells revealed that HSCs, MPP1s, MPP2s and oligo-potent CMPs all showed significantly diminished priming in old mice, with HSCs showing the greatest differential (Fig. 3b). This pattern was largely mirrored upon exposure to BID peptide with old HSCs and multi-potent progenitors that were significantly less primed than their young counterparts (Fig. 3c). Experiments with HSCs from Bax−/− and Bak−/− mice showed that HSCs can undergo apoptosis via BAX and/or BAK, as genetic deletion of either apoptosis effector diminished responses to their preferential activators, BIM and BID, respectively31, but did not completely eliminate responses (Supplementary Fig. 4b,c). Consistent with this, clonal survival upon IR was comparable in HSCs purified from Bax−/− or Bak−/− mice, indicating functional redundancy of BAX and BAK in HSCs (Supplementary Fig. 4d).

To assess the contribution of other Bcl-2 family members to apoptotic priming in young and old HSCs, we interrogated the dependency of HSCs on the anti-apoptotic proteins BCL-2, BCL-XL and MCL-1 using BH3 peptides of the pro-apoptotic sensitizers BAD, HRK and NOXA, which bind to BCL-2/BCL-XL, BCL-XL and MCL-1, respectively (Fig. 3a). As we had observed with BIM and BID, old HSCs showed a significantly diminished response to BAD (Fig. 3d). Interestingly, murine HSCs exposed to NOXA peptides did not undergo MOMP irrespective of age, indicating that dependency on the anti-apoptotic function of MCL-1 may be less critical in steady-state HSCs than in other contexts34,35. By contrast, MPs were responsive to BAD, HRK and NOXA irrespective of age (Supplementary Fig. 4e). Taken together, these data demonstrate that the apoptotic response in steady-state HSCs is BIM–BAX and BID–BAK dependent. Furthermore, these pathways, along with BAD–BCL2, are attenuated in old HSCs.

The primitive HSC compartment is heterogeneous37–40. HSC subsets that bear distinct lineage potentials can be discriminated based on the differential expression of CD150 (CD150hi, CD150lo and CD150–)41,44,45. As expected, the frequency of myeloid-biased HSCs (CD150hi) predominated the HSC compartment at the expense of lymphoid-biased CD150lo HSCs in the bone marrow of old mice, as previously shown41,44 (Fig. 4a and Supplementary Fig. 4f). Thus, we explored whether the diminished apoptotic priming observed in old HSCs (Fig. 3b,c) could be attributable to these HSC subsets. Myeloid-biased CD150hi HSCs were significantly less primed in response to BIM (old) and BID (young and old) than lymphoid-biased CD150lo HSCs, whereas lineage-balanced CD150lo HSCs generally exhibited intermediate responses regardless of age (Supplementary Fig. 4g). Comparison between young and old HSC CD150 subsets showed that apoptotic priming was significantly diminished with ageing in
response to BIM and BID in all cases, with the exception of CD150neg in response to BIM (Fig. 4bc). Clonal survival in response to IR and ENU revealed that lymphoid-biased CD150neg HSCs were more sensitive to DNA damage induction, showing reduced survival compared to myeloid-biased CD150hi HSCs regardless of age (Fig. 4d). By contrast, exposure to doxorubicin resulted in significant differences in clonal HSC subset survival (Fig. 4d). In all cases, the young HSC subsets were significantly more sensitive to doxorubicin treatment than their old counterparts, and myeloid-biased CD150hi HSCs were the least sensitive regardless of age (Fig. 4d). Competitive transplantation of the HSC subsets from young and old mice showed that IR did not significantly alter the reconstitution potential of these HSC subsets regardless of age, with the exception of lymphoid-biased CD150neg HSCs purified from young bone marrow (Fig. 4efg). Taken together, these data indicate that the differential response to DNA damage induction displayed by the old HSC compartment (Figs. 1,2) is in part underwritten by differences in apoptotic priming that are both age dependent and HSC-subtype dependent.

Transplantation into a young host resets the DNA damage response of aged HSCs. Factors contributing to the decline of HSCs during ageing have largely been linked to cell-autonomous mechanisms. To address whether the ageing-dependent differences in the DNA damage response of HSCs (Figs. 1–3) are cell intrinsic, we transplanted young or old bone marrow cells into young recipients (Supplementary Fig. 5a,b) and then assayed apoptotic priming 21–23 weeks post-transplantation in comparison to untransplanted controls. In contrast to the steady state in which old HSCs exhibited diminished apoptotic priming, the priming of old donor-derived HSCs post-transplantation into young recipients in response to BIM, BID and BAD was restored to levels that were comparable to those observed in young HSCs at steady state or post-transplantation (Fig. 5a). Apoptotic priming of MPs examined post-transplantation was comparable to steady state and unaltered regardless of donor age (Supplementary Fig. 5c). To test whether the normalization of apoptotic priming of old HSCs post-transplantation was concomitant with restoration of the DNA damage response,
we assessed the ability of donor-derived HSC clones to form colonies upon IR. In contrast to the differences observed between young and old HSCs examined at steady state (Fig. 1b), young and old donor-derived HSCs that were isolated post-transplantation into young recipients showed similar clonal survival in response to IR (Fig. 5b–d). We next asked whether exposure to an old microenvironment would reciprocally lower the apoptotic priming of young HSCs concomitant with a normalization of clonal survival upon exposure to DNA damage, and further suggest that the marrow microenvironment of old mice is altered post-IR, leading to markedly elevated levels of apoptotic priming of both donor-derived and host HSCs. However, at this stage, influences on apoptotic priming by the IR or transplantation procedure cannot be ruled out.

Aged HSCs have diminished ATM activity and DNA damage response. The differential survival of young and old HSCs upon DNA damage induction (Figs. 1,2) combined with the attenuated apoptotic priming (Fig. 3) prompted us to ask whether DNA damage signalling might be altered in old HSCs. We first analysed the expression of selected DNA damage response and sensor genes in young and old HSCs; of the 16 genes analysed, 5 (Trp53, Atm, Chk1, Mre11 and Mdc1) were age-regulated genes11 (Supplementary Fig. 6a,b). Of these, Trp53 was strongly upregulated in old HSCs, whereas all others were downregulated. Of the ageing-downregulated genes, Trp53 was strongly upregulated in old HSCs, whereas all others were downregulated.

**Fig. 5 | Apoptotic priming and survival upon DNA damage induction are equalized upon transplantation.** a. Apoptotic priming of HSCs (LSK CD34−/loCD150−) in response to BIM (8 μM), BID (3 μM) and BAD (80 μM) in steady-state young and old bone marrow and in bone marrow transplant-derived young and old LSKs and MPs (21–23-weeks post-transplantation (post-Tx)); n=3–5 mice (each dot represents individual recipient mice). Data were pooled from five independent experiments. b. Clonal survival of donor-derived young and old HSCs measured as a percentage of viable clones of non-treated versus irradiated (2 Gy) HSCs. Each dot represents the per cent survival of 48 single HSCs (LSK CD34−/loFlk2− and LSK CD34−/loCD150−) purified from individual mice (n=4–5 mice). The numbers above the graphs indicate the total number of surviving clones (black) versus the total number of clones analysed (grey). Data were pooled from three independent experiments. c. Kaplan–Meier analysis showing the survival of donor-derived young and old HSCs (yHSC post-Tx, oHSC post-Tx) in steady-state young and old bone marrow and in bone marrow transplant-derived young HSCs (21–23-weeks post-transplantation) measured daily for 8 days. d. Quantification of the number of clonal colonies that died either at day 1 or between day 2 and day 8 post-plating. n=4 or n=5 mice per group (each dot represents the percentage of cells from individual recipient mice); 192–244 cells per condition were evaluated in three independent experiments. e. Apoptotic priming of HSCs (LSK CD34−/loCD150−) in response to BIM (8 μM) or BID (3 μM) in steady-state young and old bone marrow and in bone marrow transplant-derived young HSCs (21–23-weeks post-transplantation). n=3–6 mice per group (each dot represents individual recipient mice). Data were pooled from two independent experiments. *P<0.05, **P<0.005, ***P<0.0005 (two-tailed Student’s t-test); the centre bar represents the mean, and the error bars represent the s.e.m. †††P<0.0005 (log-rank test). Source data are included in Supplementary Table 1.
Fig. 6 | ATM activity is reduced in old HSCs and leads to a diminished apoptotic priming response. a–c. Representative images and the average number of γH2AX (a,b) and 53BP1 foci (a.c) in young and old HSCs upon 1h in culture with no treatment or IR (2 Gy). Data were pooled from four (γH2AX) and six (53BP1) independent experiments. d,e. The average number of γH2AX (d) and 53BP1 foci (e) in young and old HSCs upon 1h of in vivo IR (10 Gy). Data represent one experiment. f. Olive tail moment of young and old freshly isolated HSCs or upon 1, 6 and 24 h in culture post-IR with 2 Gy. Data were pooled from four (γH2AX) and γ of, 53BP1 foci of young and old freshly isolated HSCs or upon 1, 6 and 24 h in culture post-IR (2 Gy). Data represent one experiment. g, 53BP1 foci of young and old freshly isolated HSCs or upon 1, 6 and 24 h in culture post-IR with 2 Gy. Data represent one experiment. For f and g, comparisons are made against freshly isolated young or old HSCs. h, Apoptotic priming of young and old LSKs (yLSK and oLSK, respectively) and MPs (yMP and oMP, respectively) upon 4h of culture with treatments in response to BIM (3 μM), IR (2 Gy) and ATM inhibitor (ATMi; KU-55933, 10 μM). n = 3–12 mice per group (each dot represents individual mice). Data were pooled from five individual experiments. i, The clonal survival of young HSCs measured in terms of the number of viable clones (black) versus the total number of clones (grey) upon 4h of ATM inhibitor pre-treatment (yHSCATMi). Each dot represents the per cent survival of 48 single HSCs purified from individual mice (n = 3 mice). The numbers above the graphs indicate the total number of surviving clones (black) versus the total number of clones (grey). Data represent one experiment. j,k. The average number of γH2AX (j) and 53BP1 foci (k) in young and old HSCs 21–23-weeks post-transplantation upon 1h in culture with no treatment (yHSC post-TxPC, and oHSC post-TxPC, respectively) or IR (2 Gy) (yHSC post-TxIR, and oHSC post-TxIR, respectively). Data represent one experiment. For b–g,j,k, n numbers are stated below each panel and represent individual cells, indicated by dots. *P < 0.05, **P < 0.005, ***P < 0.0005, ****P < 0.0001 (two-tailed Student’s t-test); the centre bar represents the mean, and the error bars represent the s.e.m. Source data are included in Supplementary Table 1.
To explore whether the reduced ATM activity of old HSCs could contribute to diminished apoptotic priming, we analysed apoptotic responses to acute DNA damage induction in response to acute DNA damage induction in young and old HSCs. Young and old HSCs that were either untreated or exposed to 2 Gy IR were immunostained for γ-H2A histone, member X (γH2AX) whose phosphorylation is ATM-dependent. Consistent with previous reports in mice and in humans, old HSCs showed significantly greater numbers of γH2AX foci in the untreated samples (Fig. 6a,b). Strikingly, whereas young HSCs showed greater numbers of γH2AX foci post-IR, old HSCs showed no change (Fig. 6a,b) even though double-strand breaks were comparably induced by IR of both young and old HSCs, as shown by quantitation of p53-binding protein 1 (53BP1) IR-induced foci (Fig. 6a,c) and comet assays (Supplementary Fig. 6c,f). The deficit in ATM signalling in old HSCs was also observed in vivo, as HSCs purified from old irradiated mice failed to induce γH2AX foci (Fig. 6d,e). These data indicate that ATM signalling in response to acute DNA damage induction is compromised in old HSCs. However, this deficit in ATM signalling did not affect the ability of old HSCs to repair double-strand breaks, as young and old HSCs exhibited similar kinetics of repair as indicated by time-course resolution of comet tails and 53BP1 foci post-IR (Fig. 6f,g). To explore whether the reduced ATM activity of old HSCs could be linked to diminished apoptotic priming, we analysed apoptotic priming in LSKs (Lin−c-kit−Sca-1+) and MPs from young and old mice in response to IR in the presence or absence of the ATM inhibitor KU-55933 (ref. 45) at a dose that inhibited ATM-dependent γH2AX activity in LSK-enriched and c-kit-enriched cells (Supplementary Fig. 6d,e). ATM inhibition was sufficient to significantly reduce apoptotic priming in both young and old LSKs and MPs upon exposure to IR (Fig. 6h) and to increase the survival of young irradiated HSCs (Fig. 6i). These results suggest that ATM has a role in regulating apoptotic priming in haematopoietic progenitor cells, and raise the possibility that the diminished apoptotic priming associated with HSC ageing may be underwritten by diminished ATM signalling. As apoptotic priming was elevated upon transplantation of old HSCs into young irradiated recipients (Fig. 4a,b), we tested whether ATM signalling (Fig. 6a−c) was concomitantly restored post-transplantation into young hosts. To address this, we analysed the bone marrow of mice that had been transplanted 21–23 weeks previously with young or old whole bone marrow (Supplementary Fig. 4a). Strikingly, untreated young and old HSCs analysed post-transplantation showed comparable basal levels of γH2AX and 53BP1 (Fig. 6j,k) and a comparable ability to induce γH2AX and 53BP1 foci upon IR irrespective of donor age (Fig. 6j,k), indicating that ATM activity had been restored in old HSCs upon transplantation into a young host.

**Discussion**

In this study, we show that old HSCs are more resistant to DNA-damage-induced apoptosis than young HSCs regardless of the type of DNA lesion induced. This generalized response points towards a common mechanism underlying the survival advantage of old HSCs rather than differential activity of specific DNA repair pathways. Indeed, using BH3 profiling, we show that, during ageing, HSCs become less primed for apoptosis underwritten by diminution of BIM–BAX, BID–BAK and BAD–BCL-2 responses. We discovered that the survival advantage of old HSCs in response to DNA damage induction could be reset to a level comparable with young HSCs upon transplantation into young hosts, which suggests an important role for the microenvironment in regulating apoptotic priming. In addition, we uncovered a role for ATM in regulating apoptotic priming and survival in haematopoietic progenitors and showed that old HSCs have diminished ATM activity in response to acute DNA damage induction. Whether ageing-associated cellular alterations in the niche, differences in downstream blood progenitors/effectors or humoral signals emanating from the microenvironment underlie the differential regulation of apoptosis of HSCs during ageing impinge directly on ATM activity or represent independent mechanisms is still unclear. Nonetheless, the fact that transplantation into a young host restored ATM activity in old HSCs concomitant with normalization of apoptotic priming and survival of HSCs in response to DNA damage induction suggests that the aged niche may have a critical role in regulating ATM activity and the DNA damage response during HSC ageing. Taken together, these results suggest that the diminished apoptotic priming observed in old HSCs could underlie the perpetuation of the stem cell pool even in the face of DNA damage by providing a selective advantage to old HSCs that are rendered insensitive to DNA lesions that would otherwise kill their younger counterparts. Consistent with this idea, p53 has been shown to act as a critical mediator of cell competition in stem and progenitor cells with levels of DNA damage that do not reach a threshold high enough to activate apoptosis, allowing damaged stem cells to survive and propagate (44–46). In a comparable manner, diminution of apoptotic priming provides a mechanism that allows damaged HSCs, particularly myeloid-biased HSCs, to survive and propagate in the aged marrow, thereby contributing to the oligoclonal expansion of the HSC compartment observed in mice and humans (47–49). Such a selective advantage may also underlie the accrual of DNA damage in the HSC compartment (50–52), ultimately leading to ageing-associated haematopoietic malignancies.

**Methods**

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41556-018-0054-y.

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Author contributions

P.G. designed, performed and analysed most of the experiments and wrote the manuscript. I.H. designed and performed the BH3 profiling experiments. M.N. provided technical help. M.K. performed the immunostainings, U. Rajarajacholi for help with dot blots and all the members of the D.J.R. laboratory for help. The A.N. laboratory was supported by the Intramural Research Program of the NIH, the National Cancer Institute, the Center for Cancer Research and the Alex Lemonade Stand Foundation Award. I.H. was supported by the NIH fellowship F31CA186301. The A.L. Laboratory was supported by the NIH grant PI1 CA66996 and the Leukemia and Lymphoma Society Grant TRP6387-13. D.J.R. is supported by grants from the NIH (ROIHL107630, RO0AG29760 and U01DK072473-01) as well as grants from The Leona M. and Harry B. Helmsley Charitable Trust, The New York Stem Cell Foundation, The Harvard Stem Cell Institute and the American Federation for Aging Research.

Competing interests

The authors declare no competing interests.

Additional information

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**Methods**

Results presented in this study are drawn from each and every experiment that did not fail for technical reasons, and only experiments that failed for technical reasons were excluded. No other exclusion criteria were applied.

**Mice.** All mice used were C57BL/6 males. Young mice were 12–14 weeks old. Old mice were obtained from the National Institute on Aging and were 24–26 months old. All mice were maintained according to protocols approved by the Harvard Medical School Animal Facility Administrative Panel on Laboratory Animal Care, and all procedures were performed with consent from the local ethics committees (Institutional Animal Care and Use Committee [IACUC]; Harvard Protocol 94428).

**Purification of cells.** Adult bone marrow cells were extracted by crushing the bones of donor mice. Cells were enriched using a kit magnetic beads (Miltenyi) and stained with the following cell surface antibodies for 1.5 h on ice:

- Lin cocktail (Mac-1, Gr-1, Ter119, B220, CD3, CD4 and CD8), c-kit, Sca-1, CD34, Flk2, FcRy, IL7Ra, CD150, CD45.1 and CD45.2. All cells were sorted on a FACSAria II (Becton, Dickinson) and PI was used to exclude dead cells.

**Cell culture.** Cells were clone sorted into 96-well round-bottom plates. HSCs were cultured in 5-Culture (Iwai North America Inc.) supplemented with 0.75% AlbuMAX-1 (Gibco), 1x penicillin/streptomycin, 50 mM 2-mercaptoethanol (Invitrogen) and the following cytokines: 20 ng per ml mouse stem cell factor, 20 ng per ml mouse thrombopoietin, 40 ng per ml mouse IL-12. MPs and c-kit-enriched cells were cultured in Dulbecco’s modified Eagle’s medium and F-12 medium (Gibco and Invitrogen) supplemented with 10% fetal calf serum (Hyclone and Thermo Scientific), 1x penicillin/streptomycin, 2 mM GlutaMAX, 50 mM 2-mercaptoethanol and the following cytokines: 20 ng per ml mouse stem cell factor, 20 ng per ml mouse thrombopoietin, 40 ng per ml mouse IL-3 and 20 ng per ml mouse granulocyte-macrophage colony-stimulating factor (all purchased from PeproTech). c-kit-enriched bone marrow (2x10^6 cells per ml) was exposed for 4 h to 10μM ATMi inhibitor (KU55933, Selleckchem). All cells were kept in a 5% CO2 incubator.

**Survival assays.** For each experiment, 24–48 individual cells from individual mice were analysed. Cells were either irradiated with 3 Gy or treated for 18 h with 0.2 ng per ml ENU, 0.2 ng per ml ethyl-methanesulfonate, 0.5 mM HU or 250 ng per ml doxorubicin (all from Sigma). For HSC survival upon ATM inhibition, single cells were pre-treated with 10μM ATMi inhibitor (KU55933, Selleckchem) for 4 h and irradiated with 2 Gy. After the incubation time, media was refreshed with drug-free media. Cells were incubated at 37°C in a humidified atmosphere with 5% CO2. At 8 days of culture, colonies were counted under the microscope. At 10 days of culture, the size of each colony was measured under a microscope. For the cell growth dynamics assay, either freshly isolated young and old or donor-derived young and old HSCs were counted under the microscope every 24 h for 8 days. Viability was determined by appearance.

**Annexin V assays.** HSCs and MPs were isolated and either stained immediately after purification or irradiated and kept in culture for 8 h. Cells were stained following the manufacturer’s protocol (annexin V Apoptosis Detection kit, BD Pharmingen).

**Transplantation.** For competitive transplantation upon DNA damage induction, 500 CD45.2 HSCs or CD150+, CD150+ or CD150+iSccs HSCs were transplanted into lethally irradiated (10 Gy) CD45.1 8–12-week-old female recipients along with 3 x 10^5 competitor congenic bone marrow cells. For non-competitive transplantation, 5 million whole bone marrow cells were transplanted into lethally irradiated (10 Gy) CD45.1 8–12-week-old female recipients or 17-month-old CD45.2 males. Peripheral blood analysis was performed at 4-week intervals post-transplantation using antibodies against Ter119, B220, Mac1, Gr-1, CD3, CD45.1 and CD45.2. PI was used to exclude dead cells.

**BH3 profiling.** Bone marrow cells from young and old mice or from transplanted mice were isolated, c-kit enriched using separation columns (Miltenyi) and stained with antibodies to identify bone marrow populations as described above. For BH3 profiling, cells were resuspended at 2 x 10^6 cells per ml and incubated with 20 μg per ml oligomycin, 0.0005% digitonin and BH3 peptides at indicated concentrations (for amino acid peptide sequences for BH3 peptides, see ref. 1) in DTEB buffer (135 mM Tris(hydroxymethyl)aminomethane (Tris[HCl]) pH 6.8, 8% SDS and 5% β-mercaptoethanol) at 5 x 10^5 cells per ml and incubated for 10 min at 99°C. 30 x 10^5 LKSs were spotted on a 0.2-μm nitrocellulose membrane (BioRad), dried, washed with PBST (0.1% Tween 20 in PBS) and blocked overnight at 4°C in 5% BSA in PBST. Primary antibodies (anti-H2A.X Phospho-Ser139, anti-53BP1 and anti-gamma H2AX (BioLegend) and anti-53BP1 (Novus Biologicals)) at 1:1,000 dilution at 36°C at 4°C. Then, HSCs were incubated with the secondary antibodies anti-rabbit Alexa Fluor 594 (A21207) and anti-mouse Alexa Fluor 488 (A10667) for 1 h at room temperature and incubated with the primary antibodies anti-γ H2AX (BioLegend) and anti-53BP1 (Novus Biologicals) at 1:1,000 dilution for 36 h at 4°C. Then, HSCs were transferred to slides and mounted on Vecta-Mount. Images were acquired on a Carl Zeiss Observer Z.1 fluorescence microscope and processed by ImageJ 1.49v. Several hundreds of HSCs from four (53BP1) and six (γH2AX) experiments were scored blindly. Foci were counted manually, and statistics were done using Graph Pad Prism.

**Dot blot.** LSKs were sorted, irradiated and cultured in F12 complete media for 1 h. Then, cells were lysed in modified Laemmli buffer (240 mM Tris/HCl pH 6.8, 8% SDS and 5% β-mercaptoethanol) at 5 x 10^5 cells per ml and incubated for 10 min at 99°C. 30 x 10^5 LKSs were spotted on a 0.2-μm nitrocellulose membrane (BioRad), dried, washed with PBST (0.1% Tween 20 in PBS) and blocked overnight at 4°C in 5% BSA in PBST. Primary antibodies (anti-γH2A.X Phospho-Ser139 (BioLegend) and anti-γH2AX (BioLegend)) were used at 1:1,000 for 2 h at room temperature in 0.5% BSA in PBST. Secondary antibodies (anti-mouse HRP (Santa Cruz) and anti-goat HRP (Jackson)) were incubated at 1:2,000 in 0.5% BSA in PBST. Membranes were washed 3 times for 15 min in PBST. Dot blot was developed using SuperSignal West Fermo (ThermoFisher Scientific) and Amershaw Hyperfilm ECL (GE Healthcare).

**Comet assay.** Young and old HSCs were sorted, irradiated (2 Gy) and cultured in S-Cline media and then fixed with 4% paraformaldehyde for 30 min at room temperature. Cells were permeabilized (0.1% Triton, 2% goat serum in PBS) for 15 min at room temperature, blocked (2% goat serum with 0.03% Tween in PBS) for 1 h at room temperature and incubated with the primary antibodies anti-γ H2AX (BioLegend) and anti-53BP1 (Novus Biologicals) at 1:1,000 dilution for 36 h at 4°C. Then, HSCs were incubated with the secondary antibodies anti-rabbit Alexa Fluor 594 (A21207) and anti-mouse Alexa Fluor 488 (A10667) for 1 h at room temperature and visualized the nuclei, the cells were counterstained with 4,6-diamidino-2-phenylindole (DAPI). To remove the permeabilization solution, primary and secondary antibodies, respectively, the cells were washed several times in 0.03% Tween in PBS. Finally, the cells were transferred to slides and mounted on Vecta-Mount. Images were acquired on a Carl Zeiss Observer Z.1 fluorescence microscope and processed by ImageJ 1.49v. Several hundreds of HSCs from four (53BP1) and six (γH2AX) experiments were scored blindly. Foci were counted manually, and statistics were done using Graph Pad Prism.

**Statistics and reproducibility.** Statistical analysis (two-tailed Student’s t-test and log rank (test) was performed using GraphPad Prism. Experiments were repeated at least three times, except for those in Figs. 2a, c, 3d, 5e and Supplementary Figs. 2d–g and 3e, which were repeated twice and Figs. 2h, d, 4e–g, 6d–g and Supplementary Figs. 5a,b,e and 6c,e, which were performed once. Experiments that were only performed once or twice include multiple biological replicates (survival, transplants and BH3 profiling after ATM inhibition) or are assays in which different treatments should not be pooled and compared (comet assay). When data were pooled from different experiments, all replicates were done in the same conditions.

**Life Sciences Reporting Summary.** Further information on experimental design is available in the Life Sciences Reporting Summary.

**Code availability.** The script used to generate Supplementary Fig. 3 has been developed by J. A. Garcia-Martin and is available from the corresponding authors upon request.

**Data availability.** Source data have been provided as Supplementary Table 1. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

**References**

60. Ryan, J. & Letai, A. BH3 profiling in whole cells by fluorimeter or FACS. Methods 61, 156–164 (2013).
Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

### Experimental design

1. **Sample size**
   
   Describe how sample size was determined.

   | For experiments involving transplanted mice, 4 to 12 mice were used. For experiments involving freshly purified populations, all stem and/or progenitors cells were used from 3 to 12 mice. For experiments where single cells were assayed, several hundred individual cells were analyzed (100-800) per condition. No sample size calculation was performed, experiments were repeated as many times as possible with as many mice or cells that could be handled per experiment. |

2. **Data exclusions**
   
   Describe any data exclusions.

   | Only experiments failed for technical reasons were excluded from data analysis |

3. **Replication**
   
   Describe whether the experimental findings were reliably reproduced.

   | All replication experiments were successful. |

4. **Randomization**
   
   Describe how samples/organisms/participants were allocated into experimental groups.

   | Not applicable as samples did not have to be allocated in different groups for each experiment. |

5. **Blinding**
   
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   | All experiments were analyzed blinded. |

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- n/a Confirmed
- □ The exact sample size \( n \) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- □ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- □ □ A statement indicating how many times each experiment was replicated
- □ □ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- □ □ □ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- □ □ □ The test results (e.g. \( P \) values) given as exact values whenever possible and with confidence intervals noted
- □ □ □ □ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- □ □ □ □ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Policy information about availability of computer code

Describe the software used to analyze the data in this study.

FACSDiva V8.0.1, FlowJo V9.5.3, GraphPad Prism 5, Photoshop CS4, PowerPoint 2008, Excel 2008, Word 2008, CometScore

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Aged mice were obtained from the NIA (restricted access), all other materials used for this study have no restrictions on availability.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Ter119-PacBlue, PacOrange or PerCP-Cy5.5 (clone Ter119)-BioLegend. Used: 1/200
B220-PacBlue, PacOrange or APC-Cy7 (clone RA3-6B2)-BioLegend. Used: 1/200
Mac1-PacBlue, PacOrange or PE-Cy7 (clone M1/71)-BioLegend. Used: 1/200
Gr1-PacBlue, PacOrange or FITC (clone RB6-8C5)-BioLegend. Used: 1/200
CD3-PacBlue, PacOrange or PE (clone 17A2)-BioLegend. Used: 1/200
CD8a-PacBlue, PacOrange (clone 53.6.7)-BioLegend. Used: 1/200
CD4-PacBlue, PacOrange (clone RM4-5)-BioLegend. Used: 1/200
IL7Rca-PacBlue, PacOrange or FITC (clone A7R34)-BioLegend. Used: 1/200
CD34-FITC (clone RAM34)-BioLegend. Used: 1/33
Fk2-PE or APC (clone A2F10)-BioLegend. Used: 1/200
ckit-APC-Cy7 (clone 2B8)-BioLegend. Used: 1/200
Sca1-APC or PE-Cy7 (clone D7)-BioLegend. Used: 1/200
CD150-PE-Cy7 or PacOrange (clone TC15-12F12.2)-BioLegend. Used: 1/200
FcgRc-PE or PerCP-Cy5.5 (Clone 2.4G2)-eBioscience. Used: 1/200
CD45.1-APC (clone A20)-BioLegend. Used: 1/200
CD45.2-PacBlue (clone 104)-BioLegend. Used: 1/200
AnnexinV-PacBlue (lot B192797)-BioLegend. Used: 1/500
H2A.X Phospho Ser139 (clone 2F3, lot B194731)-BioLegend. Used: 1/500
actin (C-11, lot J311)-SantaCruz. Used: 1/1000

Concentrations are based on previous knowledge in the lab and according to manufacturer's instructions.
10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used.
   No cell lines were used for this study
   b. Describe the method of cell line authentication used.
   No cell lines were used for this study
   c. Report whether the cell lines were tested for mycoplasma contamination.
   No cell lines were used for this study
   d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.
   No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
    Provide details on animals and/or animal-derived materials used in the study.
    young male (13 weeks +/- 1 week) or female recipients (9 weeks +/- 1 week) BL6 mice were obtained from JAX.
    old male mice were obtained from the aged colony of the NIA (24 months +/- 2 months)
    Bax and Bak KO were kindly provided by the Sarosiek Lab, ages range between 8 and 17 weeks
    bone marrow stem and progenitor cells were obtained by crushing leg bones and hips, filtering the resulting material and staining and sorted for the populations of interest.

Policy information about studies involving human research participants

12. Description of human research participants
    Describe the covariate-relevant population characteristics of the human research participants.
    This study does not involve human research participants
Flow Cytometry Reporting Summary

Data presentation

For all flow cytometry data, confirm that:

1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
3. All plots are contour plots with outliers or pseudocolor plots.
4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation. Murine bone marrow cells were obtained by crushing bones, filtering resulting material and subsequent enrichment, staining and sorting

6. Identify the instrument used for data collection. ARIA Standard, SORP and FACSCanto

7. Describe the software used to collect and analyze the flow cytometry data. FACSDiva, FlowJo

8. Describe the abundance of the relevant cell populations within post-sort fractions. Purity of primary sort was analyzed by checking purity of sorted sample immediately post-sorting. Sorted cells were used for experiments when sorting purity was above 90% (purity of bulk sorts assessed by re-analysis of sorted samples).

9. Describe the gating strategy used. All events were analyzed on FSC-A/SSC-A dot blots, G1 gate was analyzed on FSC-A/FSC-W dot blot, G2 gate was analyzed on PI/FSC-A dot blot. Positive and negative populations were established based on previous knowledge, published data and FMO controls

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒