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# TP53INP1 deficiency maintains murine B lymphopoiesis in aged bone marrow through redox-controlled IL-7R/STAT5 signaling

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**Bone marrow (BM) produces all blood and immune cells deriving from hematopoietic stem cells (HSCs). The decrease of immune cell production during aging is one of the features of immunosenescence. The impact of redox dysregulation in BM aging is still poorly understood. Here we use TP53INP1-deficient (KO) mice endowed with chronic oxidative stress to assess the influence of aging-associated redox alterations in BM homeostasis. We show that TP53INP1 deletion has no impact on aging-related accumulation of HSCs. In contrast, the aging-related contraction of the lymphoid compartment is mitigated in TP53INP1 KO mice. B cells that accumulate in old KO BM are differentiating cells that can mature into functional B cells. Importantly, this phenotype results from B cell-intrinsic events associated with defective redox control. Finally, we show that oxidative stress in aged TP53INP1-deficient mice maintains STAT5 expression and activation in early B cells, driving high Pax5 expression, which provides a molecular mechanism for maintenance of B cell development upon aging.**

hematopoiesis | aging | immunosenescence | oxidative stress | early B cell differentiation

**B**one marrow (BM) is the source of all of the cells that constitute the blood and immune system, and hematopoietic development depends on a complex succession of self-renewal, proliferation, and differentiation events. Thus, BM contains many different hematopoietic cell types, engaged in distinct differentiation pathways, all deriving from hematopoietic stem cells (HSCs). Production of blood cells by the BM occurs over the whole lifespan of an organism. However, with aging, hematopoietic homeostasis is not maintained properly, promoting immunosenescence, autoimmunity, and a high prevalence of hematological malignancies (1, 2). This functional decline is associated with and promoted by age-dependent deterioration in HSC functions, characterized by a decrease in regenerative capacity and a skewing of differentiation toward myeloid progenitors at the expense of lymphoid progenitors (3, 4). The decline in HSC functions is still poorly understood at the molecular level but is thought to result from both cell intrinsic changes and BM microenvironmental effects (2, 5).

Age-dependent impaired B lymphopoiesis favors defective antibody responses in the periphery, increased susceptibility to infections, and decreased vaccination response in aged individuals (6–9). The cellular compartment that drives lymphoid cell loss is not known, but studies have identified alterations at the common lymphoid progenitor or multilineage progenitor level (10, 11). Aging-associated changes also affect committed developing B cells, in particular maturation of pro-B cells to pre-B cells (12). Finally, molecular mechanisms of decreased B cell production in aged BM include reduced expression of transcription

factors primarily playing a role in B lineage commitment and differentiation (6, 9, 13–15).

Hematopoietic cell production can be drastically increased, particularly in stress situations such as radiation- or chemotherapy-induced BM ablation or infection-driven cytopenia; this increase allows the BM and the blood to be replenished (16). In many stress situations, including aging-related stress, the level of reactive oxygen species (ROS) is highly increased in the BM (8, 17). This excess of ROS production is closely associated with HSC senescence (18). However, at the physiological level, ROS act as second messengers in cell homeostasis, proliferation, and immune function (19). In the BM, homeostasis, differentiation, and functional properties of HSCs depend on intracellular ROS levels (17, 20). These data illustrate the dual role of ROS that needs to be precisely defined in each aspect of BM function.

The tumor suppressor p53 is one of the molecular actors in the regulation of HSC homeostasis, in part through its participation

## Significance

**Reactive oxygen species (ROS) play a role in signaling in immune cells, in particular in B cell activation and terminal differentiation in secondary lymphoid organs. Nevertheless, their role in B cell development in the bone marrow (BM) still remains poorly explored. TP53INP1 is a target of the tumor suppressor p53, which mediates its antioxidant activity. We report the surprising observation that chronic oxidative stress in TP53INP1-deficient mice rescues B lymphopoiesis in the BM during aging. ROS sustain IL-7R signaling in aged TP53INP1-deficient BM through maintenance of active STAT5 transcription factor, driving the expression of the B lineage Pax5 transcription factor. This work suggests that antioxidants cannot be in favor of antibody-producing cell development.**

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in redox control (17, 21). Our laboratory has previously shown that the tumor protein 53-induced nuclear protein 1 (TP53INP1) is one of the main p53-target genes mediating its antioxidant activity (22). TP53INP1 was initially identified as the thymus-expressed acidic protein highly expressed in lymphoid organs (23) and was thereafter shown to be overexpressed in inflamed tissues and stressed cells (reviewed in ref. 24). Our further work demonstrated that TP53INP1 performs a tumor suppressor activity through its activation during oxidative stress response (22, 25). In addition, we showed that TP53INP1 participates in the process of autophagy, more particularly mitophagy (mitochondria-specific autophagy), linking TP53INP1 regulation of bioenergetic metabolism to its tumor-suppressive activity (24).

The gene encoding TP53INP1 (*Trp53inp1*) is highly expressed in murine lymphoid organs, including whole BM (23). It is expressed in all BM cell populations with higher expression in committed cells compared with stem cells ([www.immgen.org](http://www.immgen.org)). As BM function is highly dependent on stress signaling in which TP53INP1 plays a role, we addressed the question of TP53INP1 function in hematopoietic development using TP53INP1-deficient mice suffering from a chronic oxidative stress. We found that the absence of TP53INP1 favors the maintenance of redox-driven B lymphopoiesis in aged mice. We further unveiled the underlying molecular mechanisms by highlighting the primary role of the IL-7R/STAT5/Pax5 signaling cascade in this phenotype.

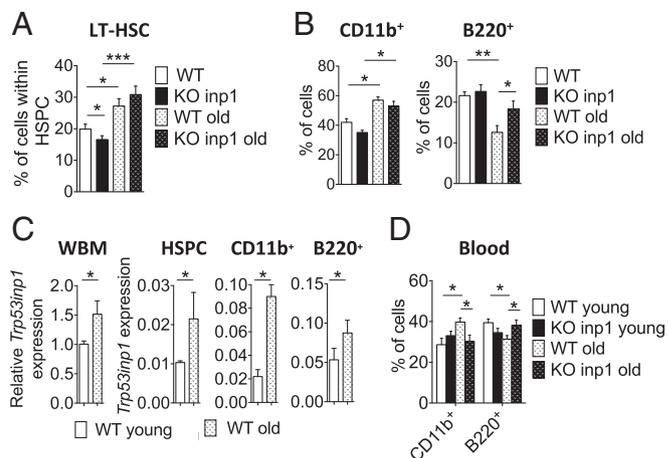
## Results

### Impact of TP53INP1 Deficiency on Hematopoietic Tissue upon Aging.

To gain insight into the role of TP53INP1 in hematopoietic development, we performed immunophenotypic characterization of the different BM hematopoietic cell compartments comparing young adult TP53INP1 KO and WT mice. HSC compartments of both genotypes were analyzed using the CD135/CD48/CD150 marker strategy (26) (*SI Appendix, Fig. S1*). We observed a low but significant decrease in the frequency of long-term HSC (LT-HSC) in KO mice, particularly in the LT-HSC population expressing high levels of CD150 (Fig. 1A and *SI Appendix, Fig. S2A–C*). However, this decrease had no consequence on more differentiated hematopoietic stem precursor cells (HSPCs) or committed lineages (Fig. 1B and *SI Appendix, Fig. S2A*).

As TP53INP1 is involved in the control of redox status and since BM aging is characterized by increased oxidative stress, we sought to analyze the effect of TP53INP1 deficiency on hematopoietic aging. First, we investigated whether *Trp53inp1* expression was modified in aged BM and different hematopoietic compartments from WT mice. We observed an increase of *Trp53inp1* mRNA level in aged whole BM as well as aged immature (HSPC), CD11b<sup>+</sup>, and B220<sup>+</sup> compartments (Fig. 1C). Next, we analyzed aged (12-mo-old; depicted as “old” in Fig. 1) TP53INP1 KO BM compartments in comparison with WT. Consistent with what was shown previously (2), the frequency of LT-HSC is significantly increased in the BM of aged WT mice (Fig. 1A). LT-HSCs from TP53INP1 KO mice were similarly accumulated upon aging (Fig. 1A), and the difference in the frequency of CD150<sup>high</sup> cells between KO and WT mice observed in young animals was abolished (*SI Appendix, Fig. S2C*). The relative increased frequency of myeloid cells that occurs upon aging was detectable in both WT and KO BMs (Fig. 1B, Left). In contrast, the reduced frequency of B cells observed in aged WT BM was considerably dampened in KO BM (Fig. 1B, Right). Maintenance of B cells was also observed in the blood of aged TP53INP1 KO mice relative to myeloid cells (Fig. 1D). Taken together, these data reveal a beneficial impact of TP53INP1 deficiency on the maintenance of the B lymphoid population in aged BM.

**TP53INP1 Is Involved in the Aging-Related Decrease in B Cell Differentiation.** To investigate further the maintenance of B cells observed during aging in TP53INP1 KO mice, we monitored the

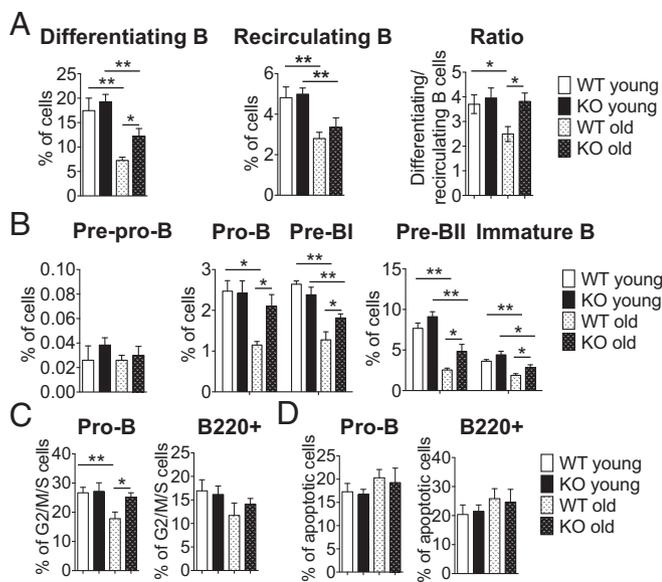


**Fig. 1.** Impact of TP53INP1 deficiency on hematopoietic tissue. For BM immunophenotyping, we used 3-mo-old (referred to as “young”) and 12-mo-old (referred to as “old”) sex-matched mice. (A) Percentage of LT-HSCs within the HSPC compartment in young and old WT and KO mice. (B) Percentage of myeloid (CD11b<sup>+</sup>) and lymphoid (B220<sup>+</sup>) cells in young and old WT and KO BM. In A to C  $n = 5$  for young WT and KO mice;  $n = 8$  and  $n = 9$  for old WT and old KO mice, respectively. (C) *Trp53inp1* expression analysis by quantitative RT-PCR in whole BM (WBM) and in HSPC, CD11b<sup>+</sup>, and B220<sup>+</sup> compartments. mRNA levels from 3-mo-old compared with 9-mo-old (for WBM) or 16-mo-old (for sorted cells) C57BL/6J mice were normalized to *36B4* expression ( $n = 3$  for each group). Results are expressed as the mean  $\pm$  SEM. \* $P < 0.05$ . (D) Percentage of myeloid (CD11b<sup>+</sup>) and B (B220<sup>+</sup>) cells in the blood of 3-mo-old (WT and KO young;  $n = 3$ ) and 12-mo-old mice (WT and KO old;  $n = 8$ ). In A, B, and D data are presented as the mean  $\pm$  SEM; \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ . Data are representative of three independent experiments.

frequency of the different B cell populations in young (3-mo-old) and old (16-mo-old) KO in comparison with WT BM (Fig. 2A and B). Overall, despite the existence of a significant decrease in CD19<sup>+</sup>CD23<sup>-</sup> differentiating B cells in the BM of both old TP53INP1 KO and WT mice compared with young mice, the decrease was milder in TP53INP1-deficient mice, leading to a higher frequency of CD19<sup>+</sup>CD23<sup>-</sup> differentiating B cells (Fig. 2A). The frequency of CD19<sup>+</sup>CD23<sup>+</sup> recirculating B cells was, however, similar between TP53INP1 KO and WT mice (Fig. 2A). As a consequence, in opposition to WT mice, the ratio between differentiating and recirculating BM B cells was maintained during aging in KO mice at levels similar to those of young adults (Fig. 2A). This result suggests that the turnover of mature B cells in periphery may be more efficiently sustained during aging in KO animals.

By dissecting the different stages of B cell differentiation (schematized in *SI Appendix, Fig. S3*), we found that TP53INP1 deletion did not impact the earliest pre-pro-B stage but restored the frequency of pro-B cells in aged BM close to the one observed in young mice (Fig. 2B). This accumulation was observed throughout later stages of B cell differentiation (Fig. 2B). Interestingly, the aged KO pro-B cells specifically (not the total B220<sup>+</sup> population) showed a higher proliferation rate compared with their WT counterparts (Fig. 2C), without any difference in apoptosis rate (Fig. 2D). This suggests that increased proliferation of B precursors in the absence of TP53INP1 sustains the maintenance of the B compartment in aged mice. It further shows that B cell impairment during aging is related to a developmental defect and not to increased apoptosis.

Given the potential importance of keeping a high level of B cell development during aging, we addressed the question of whether these numerous B cells produced in aged TP53INP1-deficient mice were functional. To this aim, we first performed immunization assays at different ages (3-, 8-, and 16-mo-old



**Fig. 2.** Maintenance of B cell differentiation in aged TP53INP1 KO mice. (A and B) B cell populations were analyzed in the BM from 3-mo-old ("young") WT ( $n = 6$ ) and KO ( $n = 6$ ) mice and from 16-mo-old ("old") WT ( $n = 5$ ) and KO ( $n = 6$ ) mice. Percentages in total bone marrow are shown. Data are presented as the mean  $\pm$  SEM and are representative of four independent experiments. (A) Differentiating B cells are defined as B220<sup>+</sup>CD19<sup>+</sup>CD23<sup>-</sup>IgM<sup>-</sup>, and recirculating B cells are defined as B220<sup>+</sup>CD19<sup>+</sup>CD23<sup>+</sup>IgM<sup>+</sup>. The ratio between differentiating B cells and recirculating B cells is shown on the Right. (B) The different B cell populations were analyzed with appropriate markers described in *SI Appendix*. (C and D) Proliferation and apoptosis rates were monitored in pro-B and total B220<sup>+</sup> cells by flow cytometry. Data are presented as the mean  $\pm$  SEM ( $n = 3$ –6 for each group) and are representative of two independent experiments. \* $P < 0.05$ , and \*\* $P < 0.01$ .

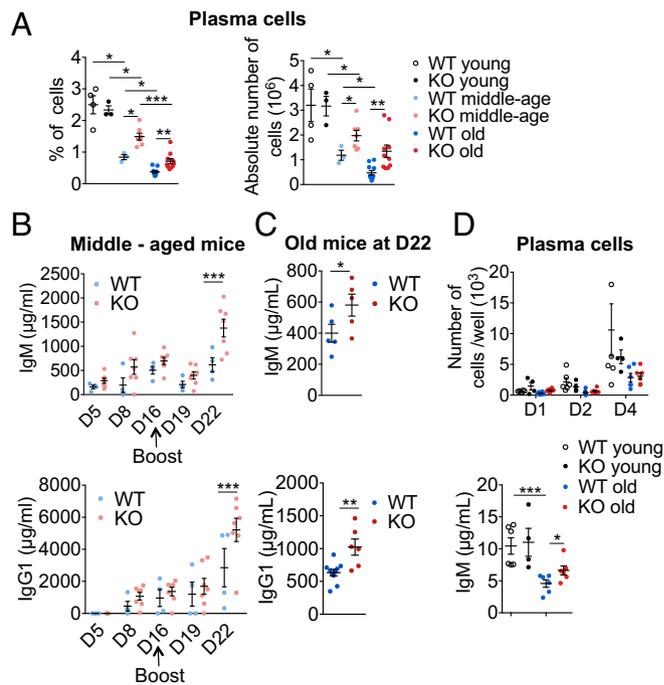
mice, Fig. 3A–C). Fig. 3A shows (i) a decrease in the frequency and number of antibody-secreting plasma cells (PCs) upon aging in immunized WT mice, as expected, and (ii) higher frequency and number of PCs in immunized 8- and 16-mo-old KO spleen compared with WT, suggesting a better B cell maturation into PCs upon immunization in aged KO mice. This was paralleled by a higher Ig production in immunized aged KO mice compared with WT (Fig. 3B and C). These data demonstrate that B cells that are produced in aged TP53INP1-deficient mice are functional and even more responsive to immunization than WT. Furthermore, we showed that ex vivo differentiating plasma cells from aged KO splenic B cells secrete a higher amount of IgM compared with WT without a difference in cell number (Fig. 3D), suggesting that the absence of TP53INP1 favors the intrinsic functional capacity of B cells to produce antibodies.

**TP53INP1 Dampens B Cell Development Through Intrinsic Redox Control.** Considering that TP53INP1 is involved in the oxidative stress response which is increased during aging in mammals (27), we next addressed the question of whether TP53INP1 antioxidant activity could impact B cell differentiation as an intrinsic event. First, we observed an increase in *Trp53inp1* mRNA level in all aged BM B cell subsets compared with young ones (*SI Appendix*, Fig. S4). We next performed BM transplantation experiments under antioxidant treatment by *N*-acetylcysteine (NAC) supplementation (Fig. 4A–D). After reconstitution, the percentage of lymphoid (B220<sup>+</sup>) cells was higher when mice were reconstituted with KO compared with WT BM, in contrast to myeloid (CD11b<sup>+</sup>) cells (Fig. 4B). Measurement of the lymphoid versus myeloid cells emphasizes that development was in favor of lymphoid cells in mice reconstituted with KO BM (Fig. 4C). Treatment with the antioxidant NAC abolished this phenotype,

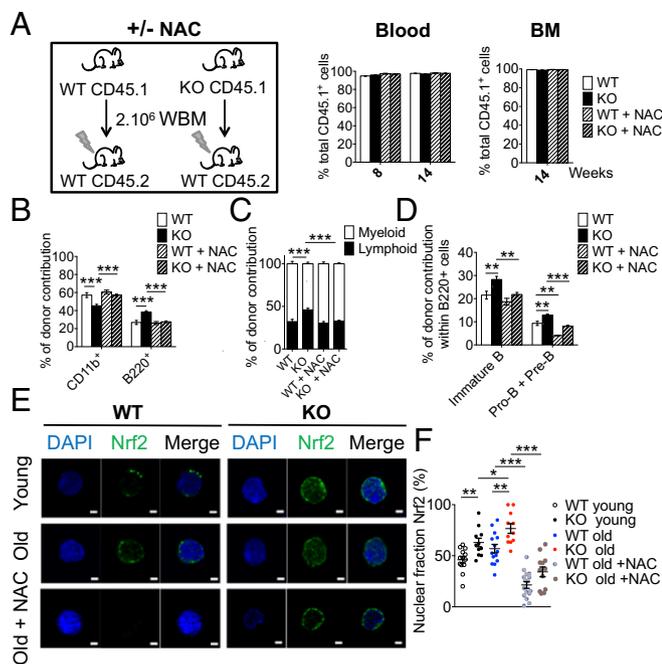
restoring a BM in NAC-treated KO BM transplanted mice similar to the BM isolated from WT BM transplanted mice (treated or not treated) (Fig. 4B and C). Noticeably, both precursors (pro-B and pre-B cells) and immature populations were similarly increased in the absence of TP53INP1 but diminished upon NAC treatment (Fig. 4D), suggesting an antioxidant activity of TP53INP1 in independent B cell differentiation stages. Taken together, these data suggest that TP53INP1 antioxidant activity is involved in restricting B cell development.

To assess oxidative stress in early B cells, we monitored by immunocytofluorescence Nrf2 (nuclear factor E2-related factor 2) subcellular localization (ROS-activated Nrf2 translocates into the nucleus). The Nrf2 nuclear fraction in pro-B cells was higher in KO cells compared with WT whatever their age and increased upon aging (Fig. 4E and F). As expected, NAC treatment decreases Nrf2 nuclear fraction in both genotypes by alleviating oxidative stress. Together, our data show (i) a high level of Nrf2 activation in KO pro-B cells (Fig. 4E and F) and (ii) rescue of the difference between KO and WT B cell development by NAC treatment (Fig. 4B–D), thus strongly arguing in favor of a primary role of oxidative stress sustaining B cell development in the absence of TP53INP1.

**Oxidative Stress in TP53INP1-Deficient B Cell Precursors Sustains B Differentiation Through the STAT5 Pathway.** To gain further insight into the molecular mechanisms underlying sustained B cell



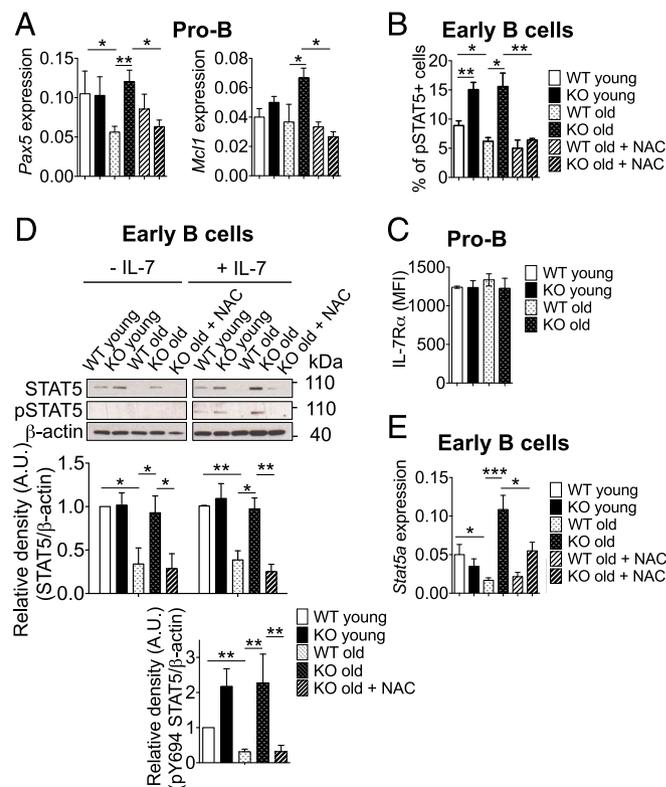
**Fig. 3.** Maintenance of functional B cells in aged TP53INP1 KO mice. (A–C) Immunization experiments in young (3-mo-old), middle-aged (8-mo-old), and old (16-mo-old) mice. (A) At day 22 after the first antigen injection, the percentage of plasma cells in the spleen was quantified by flow cytometry, and absolute number was calculated. Each dot represents one mouse. The line shows the median  $\pm$  SEM. (B) IgM and IgG1 levels were determined by ELISA in the serum at different time points (D = day) in immunized 8-mo-old mice. Each dot represents one mouse, and the line shows the median. (C) IgM and IgG1 levels in the serum of immunized 16-mo-old mice at D22. (D) Ex vivo differentiation assay. Splenocytes were treated with 1  $\mu$ g/mL LPS in vitro, plasma cells were quantified at different time points (D = day) by flow cytometry, and IgM level was monitored in the supernatant at D4. Each dot represents one mouse, and the line shows the median. \* $P < 0.05$ , \*\*\* $P < 0.01$ , and \*\*\*\* $P < 0.001$ . Data are representative of two independent experiments.



**Fig. 4.** TP53INP1 controls redox status in BM cells upon aging in a cell-autonomous manner. (A) Experimental scheme of BM transplantation experiments. Total BM cells from 2-mo-old male WT or KO *inp1* donor mice (CD45.1) were transplanted into irradiated 2-mo-old male CD45.2 recipient mice. When indicated, NAC was added to the drinking water of donor mice (from the pairs) and of recipient mice starting the day of transplantation. Analyses were performed 14 wk posttransplantation. The percentage of chimerism was analyzed in blood and bone marrow. (B) Contribution of WT or KO BM cells, pretreated or not with NAC, to myeloid (CD11b<sup>+</sup>) and lymphoid (B220<sup>+</sup>) lineages. (C) Ratio between myeloid and lymphoid cells in the BM after reconstitution with the indicated mice. (D) Contribution of WT or KO BM cells, pretreated or not with NAC, to immature B cells and pro-B + pre-B cells (B220<sup>+</sup>CD23<sup>+</sup>IgM<sup>+</sup> and B220<sup>+</sup>IgM<sup>-</sup>, respectively). In B–D, data are presented as the mean  $\pm$  SEM ( $n = 10$  for WT and KO;  $n = 7$  for WT + NAC, and  $n = 9$  for KO + NAC). \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ . All these experiments were performed twice independently. (E) Immunofluorescence assay of subcellular localization of Nrf2 protein using fluorescence confocal microscopy. Enriched fractions in early B cells (Pro-B + Pre-B) from 3-mo-old (referred to as “young”) and 16- to 23-mo-old (referred to as “old”) mice were costained with anti-Nrf2 and anti-CD2 (to distinguish CD2<sup>-</sup> pro-B cells from CD2<sup>+</sup> pre-B cells) antibodies and DAPI. Pictures of pro-B cells (B220<sup>+</sup>CD19<sup>+</sup>IgM<sup>-</sup>IgD<sup>-</sup>CD2<sup>-</sup>) are shown. (Scale bars: 1  $\mu$ m.) (F) Quantification of nuclear Nrf2 (activated Nrf2). Each dot represents one cell, and the line shows the mean  $\pm$  SEM. Data are representative of three independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ .

development in aged TP53INP1-deficient BM, we looked at the IL-7R/STAT5 signaling cascade which controls B lymphopoiesis through induction of pro-B cell proliferation as well as up-regulation of *Pax5* (a major B cell transcription factor) and *Mcl1* (antiapoptotic gene) (14, 28–30). First, we compared the expression of *Pax5* in pro-B cells isolated from young and old WT and KO mice (Fig. 5A, Left). In contrast to WT pro-B cells that show a twofold decrease upon aging, *Pax5* expression was maintained at a high level in aged KO pro-B cells (Fig. 5A). Interestingly, NAC treatment completely abolished the difference between old KO and WT mice, suggesting a direct link between ROS accumulation and high *Pax5* expression in TP53INP1-deficient B cell precursors. When assessing *Mcl1* expression in pro-B cells (Fig. 5A, Right), we observed stable *Mcl1* expression in old WT cells compared with young ones, while there was a significant up-regulation in old KO cells, which was reversed upon NAC treatment.

Next, we investigated the level of phosphorylated STAT5 (phospho-STAT5) in CD19<sup>+</sup>IgM<sup>-</sup>IgD<sup>-</sup> fractions enriched in early B cells (composed of both pro-B and pre-B) stimulated in vitro with IL-7. IL-7-dependent signaling is switched off at the pre-B cell stage (31), and STAT5 is mainly expressed by pro-B cells (32), supporting the relevance to study IL-7R/STAT5 signaling in early B cell fractions as a readout of events in pro-B cells. We observed less phosphorylation of STAT5 upon IL-7 stimulation in old compared with young WT mice (Fig. 5B and SI Appendix, Fig. S5). In contrast, the absence of TP53INP1 completely abolishes the aging-associated decreased phosphorylation of STAT5, as we found a higher percentage of phospho-STAT5 positive cells following IL-7 stimulation in KO early B cells compared with WT cells, independent of their age (Fig. 5B). Once again, NAC treatment completely reversed high STAT5



**Fig. 5.** Oxidative stress in TP53INP1-deficient B cell precursors sustains B differentiation through the STAT5 pathway. Three-month-old (“young”) and 16- to 23-mo-old (“old”) mice were used. Cells were FACS sorted for pro-B cells (A) or magnetic sorted for early B cells (CD19<sup>+</sup>IgM<sup>-</sup>IgD<sup>-</sup>) (B, D, and E). (A) Expression of *Pax5* and *Mcl1* in sorted pro-B cells, normalized to *36B4* expression. The mean  $\pm$  SEM of different mice for each genotype is shown (young WT,  $n = 4$ ; young and old KO,  $n = 5$ ; old WT and old WT + NAC,  $n = 8$ ; old KO + NAC,  $n = 6$ ). (B) Early B cells (pro-B + pre-B) from young WT ( $n = 7$ ), young KO *inp1* ( $n = 8$ ), old WT ( $n = 4$ ), old KO *inp1* ( $n = 8$ ), old WT + NAC ( $n = 4$ ), and old KO *inp1* + NAC ( $n = 5$ ) mice were stimulated ex vivo with IL-7, and then phosphoY694-STAT5<sup>+</sup> cells were quantified by flow cytometry. In A and B \* $P < 0.05$ , and \*\* $P < 0.01$ . (C) Mean fluorescence intensity (MFI) of cell surface expression of IL7-R $\alpha$  in pro-B (B220<sup>+</sup>CD19<sup>+</sup>IgM<sup>-</sup>BP1<sup>-</sup>CD25<sup>-</sup>) cells from WT and KO young and old mice ( $n = 3$  for each genotype). (D) Western blots showing total STAT5, phosphoY694-STAT5 (pSTAT5), and  $\beta$ -actin as loading control in early B cells (pro-B + pre-B). (Upper) One mouse representative of four mice is shown for each group. (Lower) Quantifications of total STAT5/ $\beta$ -actin and pSTAT5/ $\beta$ -actin ( $n = 4$  for each group). \* $P < 0.05$ , and \*\* $P < 0.01$ . A.U., arbitrary unit. (E) *Stat5a* expression in early B cells (pro-B + pre-B), normalized to *36B4* (young WT and old KO + NAC,  $n = 8$ ; young KO and old WT,  $n = 6$ ; old KO and old WT + NAC,  $n = 7$ ). \* $P < 0.05$ , and \*\*\* $P < 0.001$ . These data are representative of at least two independent experiments.

phosphorylation in the absence of TP53INP1, suggesting a primary role of oxidative stress in STAT5 activation. Importantly, IL-7R $\alpha$  levels on the surface of pre-pro-B and pro-B cells were maintained independently of the genotype or the age (Fig. 5C), ruling out the possibility that higher activation of STAT5 could be linked to a higher level of IL-7R at the surface of KO early B cells. Western blotting experiments confirmed the high level of phospho-STAT5 in old KO early B cells (Fig. 5D), in contrast to old WT early B cells in which the phospho-STAT5 level was almost undetectable, as expected (30). When assessing the total STAT5 level in the same cells, we observed its reduction in old WT early B cells (Fig. 5D). In contrast, total STAT5 was maintained at a high level in old KO early B cells but was almost absent upon NAC treatment, suggesting a key role of ROS in STAT5 production (Fig. 5D). Accordingly, *Stat5a* mRNA level was greatly enhanced in old KO early B cells compared with old WT (Fig. 5E), this difference being partly abolished in NAC-treated mice. Altogether, these results demonstrate a connection between oxidative stress, active STAT5 production, and early B cell development in aged mice.

## Discussion

The study presented here highlights the maintenance of B cell development in the bone marrow of aged TP53INP1-deficient mice, thus revealing an important role for the stress protein TP53INP1 in early B lymphopoiesis during aging. We showed that expression of the gene encoding TP53INP1 is increased upon aging in the whole BM, as reported previously in other tissues during stressful conditions (reviewed in ref. 24). Aging-associated overexpression of TP53INP1 is observed in several BM compartments and in the different B cell subtypes. Additionally, up-regulation of TP53INP1 in aged BM resident antibody-secreting cells was reported previously (33). Those data point to the implication of TP53INP1 in aging-associated stress in the BM.

BM aging is characterized by two main features: accumulation of HSCs that reflects abnormal self-renewal properties of old HSCs and expansion of the myeloid compartment at the expense of the lymphoid compartment (3, 4). Remarkably, we demonstrated here that TP53INP1 has no impact on age-related accumulation of HSCs. Moreover, the aging-related expansion of the myeloid compartment is maintained in the aged TP53INP1 KO BM. Notwithstanding, our data revealed an unexpected maintenance of B cells in aged KO BM, hence preventing the aging-associated contraction of the lymphoid compartment (composed mainly of B cells). Thus, the absence of TP53INP1 has a beneficial impact on the maintenance of the lymphoid population in aged mice without affecting the accumulation of HSCs or the increase of myeloid differentiation.

To go deeper into the maintenance of B cells observed during aging in TP53INP1 KO mice at the cell level, we showed that these cells are differentiating B cells and not recirculating B cells from the periphery. We further characterized which stage of the BM B cell was rescued by TP53INP1 deletion upon aging. Our data showed that the pro-B cells and later differentiation stages are maintained in aged KO BM and that KO pro-B cells have a proliferative advantage. These data suggest that aging-related overexpression of TP53INP1 could dampen the proliferation of B cell precursors, consistent with the reported antiproliferative role of TP53INP1 (22). Moreover, immunization and ex vivo differentiation assays demonstrate that B cells that develop in aged TP53INP1-deficient mice are functional and even more responsive to immunization than WT. The antigen used in this study (NP) induces a T-dependent antibody response, which is known to decline with age (34). We indeed observed this decline in WT mice upon aging, but it was strongly attenuated in aged TP53INP1-deficient mice, highlighting an unexpected contribution of TP53INP1 to aging of the immune system.

Redox changes were shown to be involved in terminal B cell differentiation in the periphery (35). We therefore addressed the possibility that redox dysregulation in the absence of TP53INP1 could impact B cell development in the BM. Treatments with the antioxidant NAC provide the demonstration that chronic oxidative stress associated with TP53INP1 deficiency is a leading promoter of B cell accumulation. NAC is a widely used antioxidant since it efficiently alleviates oxidative stress mainly through replenishment in reduced glutathione (36), in particular in TP53INP1-deficient mice in which the level of reduced glutathione is low (25). In addition, we monitored nuclear Nrf2 that provides a direct demonstration of chronic oxidative stress. Nrf2 translocates to the nucleus upon activation by ROS to control gene expression involved in ROS detoxification, which is a major mechanism in the cellular defense against oxidative stress (37). We showed that nuclear Nrf2 is higher in aged KO pro-B cells compared with WT, suggesting a defect in redox regulation in these cells. Thus, our work emphasizes that ROS are involved in the regulation of B cell differentiation in mammalian BM, which to our knowledge has not been reported yet, contrary to the crucial role of ROS in physiological B cell activation and terminal differentiation in secondary lymphoid organs (38–40). By contrast, B lymphocyte activation is rather suppressed in the context of pathological severe oxidative stress (41). Moreover, we provide evidence that, contrary to what was expected, ROS limit the aging-related decline of B cell development. As reported for Ink4a (42), TP53INP1 has tumor suppressor activity through the control of ROS levels preventing malignant transformation (cancer) but favoring growth defects of lymphoid progenitors upon aging.

At the molecular level, we showed that the well-known aging-associated decreased IL-7-driven activation of STAT5 (phospho-STAT5) in WT early B cells (43) could be mostly related to a decreased level of STAT5 at both protein and gene expression levels, which to our knowledge has not been reported previously. We further showed that chronic oxidative stress in the absence of TP53INP1 rescues the aging-associated decrease in active STAT5. This is in favor of a primary role of oxidative stress in STAT5 activation, consistent with previous reports (44, 45). Conserved redox-promoted STAT5 expression could result from the direct impact of ROS on transcription factors regulating *Stat5a* expression or indirectly through epigenetic control by promoter methylation (46). Finally, by sustaining STAT5 production and activation, chronic oxidative stress sustains the expression of the STAT5 targets *Pax5* and *Mcl1* in TP53INP1-deficient pro-B cells, thus promoting B cell development. Even if we did not observe any apoptosis defect in pro-B cells whatever the genotype (Fig. 2D), we cannot rule out that increase in survival could participate in the maintenance of these B precursors.

Thus, this work reveals a mechanism involving ROS in the protection from B cell decline upon aging. STAT5 is lost in early B cells in the BM during aging, and oxidative stress associated with TP53INP1 deficiency can prevent this loss. We thus propose the following model (*SI Appendix, Fig. S6*). Oxidative stress in the absence of TP53INP1 favors accumulation of STAT5 and phospho-STAT5 (active STAT5), triggering *Pax5* and *Mcl1* expression, thus sustaining pro-B cell proliferation and B cell development (*SI Appendix, Fig. S6, Left*). Treatment with NAC that scavenges ROS reverses the maintenance of the pro-B cell pool observed in the BM of old TP53INP1-deficient mice through decreasing active STAT5 (*SI Appendix, Fig. S6, Right*). Thus, we suggest that a high level of ROS in the absence of TP53INP1 can sustain B lymphopoiesis through positively regulating the IL-7R/STAT5 signaling pathway.

To conclude, our work adds to the current understanding of why B cell genesis declines with age. Mutating a tumor suppressor with antioxidant activity will not necessarily accelerate hematopoietic aging; on the contrary, this counteracts the age-related

immune deficiency (mimicking rejuvenation) by favoring ROS-dependent functional B cell production. Deciphering the molecular actors of the balance between aging and cancer is an active field of research. Tumor-suppressive processes such as senescence and apoptosis are protective against tumor development but can contribute to aging features of the immune system such as the loss of B production (42, 47). This work enhances the understanding of the proaging versus antiaging roles of tumor suppressor activation.

## Materials and Methods

TP53INP1 WT and KO mice on C57BL/6 background originate from heterozygous (*Trp53inp1*<sup>+/-</sup>) pairs. All animal care and experimental procedures were performed in accordance with French Guidelines for animal handling and were approved by the Aix-Marseille University Institutional Animal Care and Use Committee. Experiments were performed on age- and sex-matched cohorts. Immune cell populations were analyzed using a BD-LSRII SORP flow cytometer or sorted using either a BD FACS Aria cell sorter or by magnetic-activated cell separation with an AutoMACS cell sorter. Gene expression was quantified by real-time RT-PCR, protein abundance was monitored by

Western blotting, and Nrf2 subcellular localization was analyzed by confocal microscopy.

Detailed methods and other procedures are given in *SI Appendix, Supplemental Materials and Methods*.

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