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Mice with reduced expression of the telomere-associated protein Ft1 develop p53-sensitive progeroid traits

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Summary

Human AKTIP and mouse Ft1 are orthologous ubiquitin E2 variant proteins involved in telomere maintenance and DNA replication. AKTIP also interacts with A- and B-type lamins. These features suggest that Ft1 may be implicated in aging regulatory pathways. Here, we show that cells derived from hypomorph Ft1 mutant (Ft1kof/kof) mice exhibit telomeric defects and that Ft1kof/kof animals develop progeroid traits, including impaired growth, skeletal and skin defects, abnormal heart tissue, and sterility. We also demonstrate a genetic interaction between Ft1 and p53. The analysis of mice carrying mutations in both Ft1 and p53 (Ft1kof/kof; p53+/ko and Ft1kof/kof; p53+/ko) showed that reduction in p53 rescues the progeroid traits of Ft1 mutants, suggesting that they are at least in part caused by a p53-dependent DNA damage response. Conversely, Ft1 reduction alters lymphomagenesis in p53 mutant mice. These results identify Ft1 as a new player in the aging process and open the way to the analysis of its interactions with other progeria genes using the mouse model.

KEYWORDS

aging, AKTIP, DNA damage, lamins, progeria, telomeres

INTRODUCTION

Human AKTIP, mouse Ft1, and Drosophila Pendolino (Peo) are orthologous ubiquitin E2 variant proteins involved in telomere maintenance (Burla et al., 2015; Cenci, Ciapponi et al., 2015). AKTIP mediates proper telomere replication, binds telomeric DNA and the shelterins TRF1 and TRF2, and interacts with the DNA replication machinery components PCNA and RPA70. We have previously suggested that AKTIP works in concert with TRF1 to facilitate telomeric DNA replication (Burla et al., 2015).

AKTIP also interacts with A- and B-type lamins and is enriched at the nuclear rim (Burla, Carcuro, et al., 2016). Reduction in AKTIP
in human fibroblasts results in senescent phenotypes, including the activation of the p53 pathway, nuclear deformity, heterochromatin alterations, and senescence. In addition, AKTIP reduction affects lamin A expression in human cells (Burla, Carcuro, et al., 2016). Altogether, the properties of AKTIP place this protein at the crossroad of multiple pathways that have been associated with progeroid phenotypes.

The Hutchinson–Gilford progeria syndrome (HGPS) is the best-characterized example of progeria, caused by a mutation in exon 11 of the LMNA gene leading to the production of a truncated form of lamin A (De Sandre-Giovannoli et al., 2003). Patients with HGPS develop multiorgan abnormalities, including skeletal defects and absence of subcutaneous fat. They show a limited growth and die in the teenage years, prevalently due to cardiovascular problems leading to infarction or stroke (Hennekam, 2006). Mouse models reflect several aspects of the human disease; the LAKI model, for example, carrying the G608G mutation in the LMNA gene, is characterized by reduced lifespan and body weight, and skeletal and skin defects (Osorio, Navarro, et al., 2011).

The idea that lamins play a pivotal role in determining premature aging is also supported by the discovery of progeroid disorders different from HGPS. For example, restrictive dermopathy patients carry recessive mutations in the ZMPSTE24 gene, which encodes the proteolytic enzyme involved in lamin A maturation (Barrowman, Wiley, Hudon-Miller, Hrycyna & Michaelis, 2012). Also, in this case, a mouse model replicates the progeroid phenotype of the disease (Osorio, Ugalde, et al., 2011). A partial recovery of the ZMPSTE24−/− phenotype is obtained by depletion of the tumor suppressor protein p53, pointing to a role of DNA damage in the pathophysiology of this progeria (Varela et al., 2005).

In addition to the LMNA gene, several genes involved in DNA metabolism have been implicated in progeroid syndromes. They include the WRN and BLM genes, which encode members of the RecQ DNA helicase family and are responsible for the Werner and Bloom syndromes, respectively (Bachrati & Hickson, 2003).

Telomere dysfunctions have also been linked to progerias. Dyskeratosis congenita, which is caused by mutations in telomere-related genes, has progeroid phenotypic traits (Dokal, 2011).

The involvement of AKTIP in telomere maintenance and regulation of lamin A (Burla et al., 2015; Burla, Carcuro, et al., 2016) prompted us to investigate whether this protein contributes to preventing premature aging. We thus generated mice bearing a single mutants, Ft1+/kof mice exhibit multiple telomeric foci. We next investigated whether MEFs from Ft1+/kof mice exhibit the same phenotypes as those previously observed in RNAi cells depleted of AKTIP or Ft1 (Burla et al., 2015; Burla, Carcuro, et al., 2016). We first checked the Ft1 subcellular localization by immunostaining MEFs with an anti-Ft1 antibody. In human cells, AKTIP is depleted of AKTIP or Ft1 (Burla et al., 2015; Burla, Carcuro, et al., 2016). We then asked whether MEFs from Ft1+/kof mice activate the DNA damage response (DDR) and exhibit telomere defects. Compared to wt MEFs, Ft1+/kof MEFs displayed substantial increases in 53BP1 and γH2AX foci, indicating that Ft1 is required for the maintenance of genome integrity (Figure 2a–f). In addition, double immunofluorescence staining of γH2AX and TRF1 showed that Ft1+/kof MEFs exhibit a significant increase in γH2AX/TRF1 co-labeled foci (Telomere Dysfunction induced Foci, TIFs) compared to control MEFs, suggesting that the DDR of mutant cells was at least in part linked to telomere dysfunction (Figure 2c,d). To determine the nature of telomere defects in Ft1+/kof MEFs, we performed in situ hybridization with a TTAGGG probe. The analysis of metaphase spreads showed that Ft1+/kof MEFs exhibit multiple telomeric signals (MTS, also known as fragile telomeres) and sister telomere associations (STA) (Figure 2g–i and Figure S1e). These types of telomere aberrations are considered hallmarks of defective telomere replication (Sfeir et al., 2009). Ft1+/kof MEFs showed a small but not

2 | RESULTS

2.1 | Generation of Ft1 kof mice and characterization of derived MEFs

Given that AKTIP is required for DNA replication and cell proliferation (Burla et al., 2015), we reasoned that full knockout (ko) of Ft1 would cause physiological damage incompatible with mouse survival. Thus, we produced animals with reduced Ft1 levels using the knockout first (kof) strategy, based on the insertion into the target gene (referred as kof allele) of the jgeok cassette (Testa et al., 2004) (Figure 1a), which traps and truncates Ft1 nascent transcript reducing the expression of the gene (Figure 1a). Transgenic founders and subsequent generations were screened by PCR (Figure 1b), and two independent kof lines (lines 107 and 588) were selected and analyzed for mRNA reduction. q-PCR on tail biopsies from Ft1kof/kof and Ft1+/kof animals showed that Ft1 expression was significantly reduced compared to wild-type (wt) mice (Figure 1c). In Ft1kof/kof mice from lines 107 and 588, Ft1 expression was reduced to 18% and 12%, respectively; in Ft1+/kof animals from the same lines, Ft1 expression was reduced to 64% and 52% (Figure 1c). The analysis of 573 F1 progeny from crosses between Ft1+/kof and wild-type mice showed Mendelian ratios, although we observed a slight nonsignificant trend of embryonic lethality of Ft1+/kof animals (Figure 1d).

We next investigated whether MEFs from Ft1+/kof mice exhibit the same phenotypes as those previously observed in RNAi cells depleted of AKTIP or Ft1 (Burla et al., 2015; Burla, Carcuro, et al., 2016). We first checked the Ft1 subcellular localization by immunostaining MEFs with an anti-Ft1 antibody. In human cells, AKTIP is enriched at the nuclear rim where it partially co-localizes with lamins (Burla, Carcuro, et al., 2016). Consistent with these results, Ft1+/kof MEFs displayed a Ft1 signal at the nuclear periphery (Figure 1e), while the signal was undetectable in the Ft1kof/kof MEFs (Figure 1e,f). In line with these results, Western blotting showed a strong reduction of Ft1 in Ft1+/kof MEF extracts (Figure 1g).

We then asked whether MEFs from Ft1+/kof mice activate the DNA damage response (DDR) and exhibit telomere defects. Compared to wt MEFs, Ft1+/kof MEFs displayed substantial increases in 53BP1 and γH2AX foci, indicating that Ft1 is required for the maintenance of genome integrity (Figure 2a–f). In addition, double immunofluorescence staining of γH2AX and TRF1 showed that Ft1kof/kof MEFs exhibit a significant increase in γH2AX/TRF1 co-labeled foci (Telomere Dysfunction induced Foci, TIFs) compared to control MEFs, suggesting that the DDR of mutant cells was at least in part linked to telomere dysfunction (Figure 2c,d). To determine the nature of telomere defects in Ft1+/kof MEFs, we performed in situ hybridization with a TTAGGG probe. The analysis of metaphase spreads showed that Ft1+/kof MEFs exhibit multiple telomeric signals (MTS, also known as fragile telomeres) and sister telomere associations (STA) (Figure 2g–i and Figure S1e). These types of telomere aberrations are considered hallmarks of defective telomere replication (Sfeir et al., 2009). Ft1+/kof MEFs showed a small but not
statistically significant increase in telomere fusions compared to matched control MEFs. In addition, \(Ft1^{kof/kof}\) MEFs displayed a frequency of telomeres with a TTAGGG signal comparable to that of control, confirming (Burla et al., 2015) that an impairment of the \(Ft1\) function does not result in telomere loss (Figure S1a,b).

Finally, we evaluated the status of lamin A in MEFs. Consistent with our previous results on human AKTIP (Burla, Carcuro, et al., 2016), wt cells displayed partial co-localization of lamin A with \(Ft1\) (Figure S1f). \(Ft1^{kof/kof}\) MEFs cells showed an altered lamin A distribution with a reduced concentration of lamin A at the nuclear rim (Figure 2j–l).

Altogether, our results indicate that \(Ft1^{kof}\) mutation cause DDR, telomere defects and abnormal lamin distribution, which are well-known hallmarks of aging. We thus asked whether \(Ft1^{kof}\) mutant mice exhibit signs of premature aging. In mouse models of progeroid disorders, premature aging alterations mostly affect body growth, fertility, bones, skin and heart. We therefore focused on these phenotypic traits in our analyses on \(Ft1^{kof}\) mutant mice.

### 2.2 \(Ft1^{kof}\) mice display growth defects, reduced lifespan and sterility

Macroscopic observation of \(Ft1^{kof/kof}\) mice revealed that mutant animals (\(n = 170\)) display a significant reduction in body weight compared to controls (Figure 3a–c); 21% of the animals showed a 30% reduction in body weight compared to controls; henceforth, we will...
FIGURE 2  DNA damage, telomere aberrations and lamin A alterations in Ft1<sup>kof/kof</sup> MEFs. (a–c) Staining for anti-53BP1 (red in merges) in MEFs (a) and quantification (b, c). (d–f) Staining with anti-γH2AX (red in merges) in MEFs (d) and quantification (e, f). Graphs (b, c, e, f) show mean ± SEM; **p < .01; ***p < .001 in Student’s t test. Scale bars 5 μm. (g) Partial DAPI-stained (red) metaphases from MEFs showing telomeric FISH signals (black and white; green in merges) including enlargement of single chromosomes with multiple telomeric signals (MTS). MTS are indicated by arrows. (h, i) MTS (h) and STA (i) frequencies in MEFs. Graphs show mean ± SEM; *p < .05; ***p < .001 in χ<sup>2</sup> test. (j) Immunostaining for lamin A in MEF nuclei. Scale bar 5 μm. (k) Quantification of lamin A signal at nuclear rim in MEFs. Graphs show mean ± SEM; *p < .05 in Student’s t test from two independent experiments on two MEF cultures (n = 100 cells/culture). (l) Z stack projections and quantification showing the altered distribution of lamin A in Ft1<sup>kof/kof</sup> MEF nuclei. See also Figure S1.

refer to these mice as severely affected Ft1<sup>kof/kof</sup> mice, abbreviated with SA Ft1<sup>kof/kof</sup> or SA mutant mice. By selecting a cohort of animals with a mild (non-SA) phenotype, we monitored body weight over a 100-week period and subdivided lifespan in three major intervals: young 3 < weeks < 20; juvenile 21 < weeks < 60; adult 61 < weeks < 100. We observed that the difference in body weight between wt and Ft1<sup>kof/kof</sup> animals significantly increases as mice age (Figure 3b,c). Ft1<sup>kof/kof</sup> mice had a reduced lifespan compared to wt. SA Ft1<sup>kof/kof</sup> animals died at day 12–14, while the remaining population displayed a median survival of 113 weeks (Figure 3d). We also observed reduced fertility in inbreeding; when Ft1<sup>kof/kof</sup> males were crossed with Ft1<sup>+/kof</sup> females, we did not observe any pregnancies (Figure 3e). Altogether, these observations show that Ft1 expression is critical for mouse growth, survival and fertility.

2.3  Ft1<sup>kof</sup> mice display skeletal alterations

Bone is altered in progeroid patients and mouse models for progeria syndromes (Bergo et al., 2002; Mounkes, Kozlov, Hernandez, Sullivan & Stewart, 2003; Osorio, Navarro, et al., 2011). Radiographic analyses of whole skeletons were collected at day 12 from eight SA Ft1<sup>kof/kof</sup> mice and three wt animals. X-ray images showed reduced skeleton size, craniofacial dysmorphism (Figure 4a), and kyphotic spine curvature (Figure 4a,b). Long bones in SA mutant mice were 70% of wt, consistent with the overall body size reduction in Ft1<sup>kof/kof</sup> mice (Figure 4c). This difference is evident in the magnification of femur and tibia (Figure 4c). A reduction in size was also observed in tail vertebrae (Figure 4d). Radiographic analysis further showed that femurs and tibiae from Ft1<sup>kof/kof</sup> mice are less reflective than wt bones. Quantification of pseudocolored images from X-ray images revealed a statistically significant difference between Ft1<sup>kof/kof</sup> and wt mice, suggesting osteopenic defects in mutant animals (Figure 4e,f).

To further define bone tissue organization, we histologically analyzed sections from caudal vertebrae obtained from SA Ft1<sup>kof/kof</sup> mice. Hematoxylin and eosin (H&E) staining showed differences in the growth plate of Ft1<sup>kof/kof</sup> animals compared to wt (Figure 4g), while the marrow cavity appeared regularly formed in mutant mice. The analysis of femur sections showed that Ft1<sup>kof/kof</sup> mice exhibit

FIGURE 3  Growth, lifespan, and fertility of Ft1<sup>kof/kof</sup> mice. (a) Pictures of age-matched mice showing body size differences between Ft1<sup>kof/kof</sup> and wt. (b) Body weight analysis during growth of Ft1<sup>kof/kof</sup> and wt. Student’s t test ***p < .001. (c) ΔCt, in lifespan intervals showing that difference in body weight increases with mouse age; ***p < .001 in Student’s t test. (d) Kaplan–Meier survival curve of Ft1<sup>kof/kof</sup> and wt mice; ***p < .001—log-rank—Mantel–Cox test. (e) Pups generated by mice of different genotypes. Whiskers represent the minimum and the maximum values and the boxes the 25th to the 75th percentile; median values are shown as a line within the boxes. *p < .05; ***p < .001 in Student’s t test. See also Table S2.
regular columnar and conjugal cartilage, although slightly shorter than controls (Figure 4g). In mutant mice, newly formed bone trabeculae were also shorter, with a poorly defined osteoblastic rim, as compared to wt (Figure 4h). TRAP cytochemistry did not reveal significant differences in osteoclast numbers relative to bone surfaces between Ft1^kof/kof samples and controls, suggesting that the

FIGURE 4 Bone alterations in Ft1^kof/kof mice. (a) X-ray on total body of Ft1^kof/kof and wt mice at day 12. Arrowheads indicate spine defects and craniofacial dysmorphisms in Ft1^kof/kof animals. (b) Quantification of the angle formed by the cervical and thoracic vertebrae; ***p < .001 in Student's t test. (c) X-ray images of femurs and tibias from Ft1^kof/kof and wt at day 12. (d) X-ray of tail and magnification of caudal vertebrae (dotted white box) of Ft1^kof/kof and wt at day 12. (e, f) Pseudocolored femur images (e) and relative quantification (f) showing that X-ray absorption is lower in Ft1^kof/kof as compared to wt. Student's t test, *p < .05. (g) H&E-stained sections of caudal vertebrae (top) and femurs (bottom) of Ft1^kof/kof and wt at day 12. (h, i) TRAP (h) and relative quantification (i) on femur sections shows no significant differences in TRAP-positive cells between Ft1^kof/kof and matched wt (p = .67 Student's t test). See also Table S2
Osteopenic defects cannot be ascribed to increased osteoclastogenesis (Figure 4i).

Altogether, these results show that mutations in *Ft1* cause bone defects that partially phenocopy those observed in progeroid models caused by mutations in lamin coding genes or in genes involved in DNA metabolism (Bergo et al., 2002; Chen et al., 2012; Saeed et al., 2011).

### 2.4 | *Ft1* kof animals display skin and heart alterations

Several studies have shown that skin and heart are typically altered in premature aging disorders associated with impaired DNA metabolism, lamin, or telomere defects (Bergo et al., 2002; Cao & Hegele, 2003; Mounkes et al., 2003; Watson et al., 2013). We found that in SA *Ft1* kof/kof mice adipose tissue deposits are strongly reduced compared to age-matched controls (Figure 5a). The analysis on H&E-stained skin sections clearly showed the absence of subcutaneous fat layer in SA mutant animals, a defect similar to the skin defects described in mice carrying mutations in the lamin A coding gene (Mounkes et al., 2003) (Figure 5b).

The heart of SA *Ft1* kof/kof mice was smaller than in controls, with a reduction in size proportional to the overall body reduction (Figure 5c). In addition, analysis of H&E-stained hearts of *Ft1* kof/kof animals and wt mice showed a difference in tissue architecture.

### FIGURE 5 Lipodystrophy and heart defects in *Ft1* kof/kof mice. (a) Pictures of skinned *Ft1* kof/kof and wt at day 12, showing loss of body fat (yellow dot circles) in *Ft1* kof/kof animals. (b) H&E-stained skin sections of *Ft1* kof/kof and wt at day 12 showing reduction in fat layer. (c) H&E-stained hearts from wt and *Ft1* kof/kof mice (top) at day 12, and magnification of the heart tissue (bottom). (d) Quantification showing that *Ft1* kof/kof hearts have a higher number of nuclei per area compared to wt; **p < .01 in Student’s *t* test. (e) Images of γH2AX immunostaining of heart sections from wt and *Ft1* kof/kof mice at day 12. (f) Percentages of cells showing more than five γH2AX foci in the heart sections shown in e. Error bars indicate SEM; ***p < .001 in Student’s *t* test. (g) q-PCR quantification of the p21 senescence marker expression in wt and matched *Ft1* kof/kof mice at day 12 and at 6 months after birth. *p < .05 and ***p < .001 in Student’s *t* test. See also Table S2.
hearts of SA $Ft1^{ko/ko}$ animals, there was no apparent fibrotic tissue and the number of nuclei per area was higher than in wt hearts, suggesting an increase in the nuclear/cytoplasmic ratio (Figure 5c,d).

To gain additional insight into the origin of the defects detected in SA $Ft1^{ko/ko}$ hearts, we immunostained heart sections for γH2AX to reveal DNA damage foci. In mutant hearts, the frequency of cells with more than 5 foci was significantly higher than in controls, indicating DDR activation (Figure 5e,f). We also investigated whether mutant hearts express the p21 DDR and senescence marker to a higher extent than to control hearts. q-PCR analysis revealed that p21 is upregulated in heart extracts from both 12 days SA mutants and 6-month non-SA $Ft1^{ko/ko}$ mice compared to age-matched controls (Figure 5g).

Collectively, these results indicate that mutations in $Ft1$ affect the skin and heart structural organization, and activate DDR and senescence pathways.

2.5 | $p53$ and $Ft1$ genically interact

p21 is a potent inhibitor of cyclin-dependent kinase (CDK) that mediates p53-dependent cell cycle arrest in response to DNA damage; it has been shown that p21 is activated by p53. We thus asked whether $p53$ contributes to the phenotypic traits observed in $Ft1^{ko/ko}$ mice. To test this possibility, we generated $Ft1;p53^{+/+}$ double mutant using $p53^{ko}$ male mice (Jacks et al., 1994). Double-mutant mice were examined for several phenotypic traits, particularly those affected in $Ft1^{ko/ko}$ single mutants.

We first analyzed fertility of mutant animals. In contrast with $Ft1^{ko/ko}$ male mice that were sterile, $Ft1^{ko/ko};p53^{+/-}$ and $Ft1^{ko/ko};p53^{ko/ko}$ male mice gave progeny when crossed to wt females, indicating that mutation in one or both $p53$ alleles rescues sterility (Figure 6a). We next examined body weight and survival; the body weight deficiency observed in $Ft1^{ko/ko}$ mice was rescued in $Ft1^{ko/ko};p53^{+/-}$ mutants at least until the 24th postnatal week. However, after the 31st week, the body weight of $Ft1^{ko/ko};p53^{+/-}$ mutants was reduced compared to controls, although to a lesser extent than in $Ft1^{ko/ko};p53^{+/-}$ (Figure 6b). Interestingly, $Ft1^{ko/ko};p53^{+/-}$ animals did not exhibit an improvement in their viability as compared to $Ft1^{ko/ko};p53^{+/-}$; rather, the double mutation resulted in additive lifespan reduction (Figure 6c).

Analysis of death causes revealed a further interplay between $Ft1$ and $p53$ (Figure 6d,e and Figure S3). Homozygosity for $Ft1^{ko}$ did not result in malignant tumors, and $Ft1^{ko}$ mutations were modestly cancer-protective in a $p53$ ko background (Table S1 and Figure S3). However, the simultaneous presence of mutations in $Ft1$ and of $p53$ loss induced miotogran lymphomas (Figure 6d, Figure S3b-e), which were not observed in $p53$ mutant animals that exhibit lymphomas in single organs (Figure 6d, Figure S3f and Table S1). In addition Mice with reduced levels of $Ft1$, both in the presence or absence of $p53$, appeared to be sensitive to other pathologies, including hepatitis, bone marrow aplasia, peritonitis, nephritis, and pneumonia (Figure 6e, and Table S1 and Figure S3h,i). Thus $p53$ deficiency in $Ft1^{ko/ko}$ mutant mice rescues the sterility and the reduced body weight phenotypes, but a concomitant deficiency of $p53$ and $Ft1$ affects lymphomagenesis.

2.6 | $Ft1$ mutant cells are sensitive to DNA damaging agents

The DDR foci observed in the MEFs and heart of $Ft1^{ko/ko}$ mice, and the telomeric aberrations found in $Ft1^{ko/ko}$ MEFs suggest that $Ft1$ mutant cells might be defective in DNA repair. To address this issue, we determined the sensitivity of $Ft1^{ko/ko}$ MEFs to DNA damaging agents. We exposed $Ft1^{ko/ko}$ MEFs to nonlethal doses of the radiomimetic compound bleomycin, which creates DNA double-strand breaks (DSBs). Cell density assessment at 10 days after treatment showed that $Ft1^{ko/ko}$ MEFs are significantly more sensitive to the drug compared to passage-matched wt MEFs (Figure S4a). Increased sensitivity of $Ft1^{ko/ko}$ MEFs to DNA damage was also observed after treatment with hydroxyurea, which depletes the cells of dNTPs, generating stalled replication forks that can collapse into DSBs (Figure S4b). Notably, the reduction in cell density observed in $Ft1^{ko/ko}$ MEFs after bleomycin or hydroxyurea treatment was rescued by the presence of a single $p53^{ko}$ mutant allele in the $Ft1^{ko/ko}$ background (Figure 6f,g). In line with these results, the Western blotting analysis showed that nonmutagenized $Ft1^{ko/ko}$ MEFs accumulate both p21 and $p53$ and that this accumulation was significantly reduced in $Ft1^{ko/ko};p53^{+/-}$ MEFs (Figure 6h and Figure S5a,b). Consistent with the finding that $p53$ and p21 accumulation is associated with cell senescence and reduced proliferation ( Ibrahim et al., 2013; Sharpless & Sherr, 2015), we observed a decrease in proliferation of $Ft1^{ko/ko}$ MEFs compared to wt MEFs. We also observed an excessive doublings of $Ft1^{ko/ko};p53^{+/-}$ MEFs with respect to $Ft1^{ko/ko};p53^{+/-}$ cells (Figure 6i). An increase in the proliferation rate of MEFs bearing mutation in $p53$ has been reported previously (Lang et al., 2004; Ma, Choudhury, Hua, Dai & Li, 2013).

Collectively, these results suggest that $Ft1$ deficiency renders cells more sensitive to DNA damaging agents, resulting in proliferation defects that are (over) rescued by the presence of a single $p53^{+/-}$ mutant allele.

3 | DISCUSSION

Human progeroid syndromes and their related animal models have been instrumental to identify factors involved in normal human aging. The cellular defects found in progeroid diseases that also characterize normal human aging include DNA damage and genome instability, telomere attrition, epigenetic alterations of histones, aberrations in the nuclear lamina, and cell senescence (de Boer et al., 2002; Liu et al., 2005; Osorio, Ugalde, et al., 2011; Varela et al., 2005).

Here we analyzed the cellular, developmental, and physiological phenotypes of $Ft1$ mutant mice, focusing on traits related to the aging process. Importantly, our analysis of MEFs from $Ft1^{ko/ko}$ mice confirmed and extended our previous results obtained on the mouse
and human cells depleted of Ft1 or AKTIP (Burla et al., 2015; Burla, Carcuro, et al., 2016). Specifically, we showed that Ft1kof/kof mutant MEFs exhibit fragile telomeres and sister telomere associations, TIFs, DNA repair foci, increased sensitivity to bleomycin and hydroxyurea, and reduced cell proliferation. In addition, we confirmed that in Ft1 mutant MEFs there is an alteration in lamin A, resulting in a strong

**FIGURE 6** 
Ft1kof/kof mouse phenotype is p53 sensitive. (a) Pups generated by animals bearing mutations in Ft1 and/or p53; ***p < .001 in Student’s t test. Whiskers represent the minimum and the maximum values observed for each mating and the boxes the 25th to the 75th percentile. (b) Body weight in Ft1kof/kof animals in the presence or absence of a null mutation in p53; note that loss of a single p53 allele dominantly rescues the Ft1-dependent body weight reduction; *p < .05; ***p < .001 in Student’s t test. (c) Survival of Ft1kof/kof;p53+/− mice is decreased compared to that of Ft1+/−p53+/− and Ft1−/−p53−/− animals (p < .001—log-rank—Mantel-Cox test). (d, e) Case analysis on wt mice and mice bearing mutations in p53 (p53−/−) and Ft1, showing that Ft1 mutation impacts on lymphomagenesis and inflammatory conditions. See also Figure S3 and Table S1. (f, g) Cell survival response in MEFs from Ft1+/+;p53+/+, Ft1kof/kof;p53+/+, and Ft1kof/kof;p53+/- mice upon increasing doses of bleomycin (f) or hydroxyurea (HU) (g) showing that cells homozygous for mutations in Ft1 and bearing a null mutation in p53 (p53−/−) are less sensitive to DNA damage than Ft1 mutant cells bearing two wt copies of p53. Graphs show mean ± SEM; **p < .01 in Student’s t test. (h) Western blotting analysis of p21 and p53 expression in Ft1+/+;p53+/+, Ft1kof/kof;p53+/+, and Ft1kof/kof;p53+/- mice showing that Ft1kof/kof MEFs have a reduced pd compared to Ft1+/+ cells; this phenotype is rescued by a p53−/− mutation. Each dot represents the mean ± SEM of the cumulative pd at the indicated day; *p < .05, ***p < .001 in Student’s t test. See also Figures S4 and S5
reduction in the lamin nuclear rim. Thus, Ft1 mutant MEFs display many traits that have been previously observed in progeroid syndromes and progeroid animal models, as well as in normal human aging.

Consistent with the results on mutant MEFs, our analysis of \( \text{Ft1}^{\text{ko}}/\text{ko} \) mutant animals detected progeroid phenotypes. \( \text{Ft1}^{\text{ko}}/\text{ko} \) mice displayed multiple traits that have been previously observed in several progeroid models (Table S2). We found that Ft1 mutant mice have reduced body weight, fertility defects, and reduced lifespan, as previously observed in models of laminopathies (Bergo et al., 2002; Osorio, Navarro, et al., 2011) and telomeropathies (Martinez et al., 2009). In addition, the growth defects of \( \text{Ft1}^{\text{ko}}/\text{ko} \) mice were exacerbated with aging, suggesting that the effects of Ft1 mutations intercept the normal aging-induced degeneration pathways. \( \text{Ft1}^{\text{ko}}/\text{ko} \) mice also displayed skin and bone defects, which were previously observed in lamin mutant mice (Bergo et al., 2002; Mounees et al., 2003; Osorio, Navarro, et al., 2011), in Tert ko animals (Rudolph et al., 1999), and in mice with reduced Trf1 expression (Martinez et al., 2009). Skeletal alterations and lipodystrophy have been imputed to failures in the proliferation of mesenchymal stem cell progenitors, which are sensitive to lamin mutations and senescence (Scaffidi & Misteli, 2008). Mutant hearts were smaller than those of wt animals and showed a higher nuclear density compared to wt, with an increase in the nuclear/cytoplasmic ratio. In addition, we found that mutant hearts display DNA damage and activation of the DDR, and up-regulation of p21 expression. The relationships between increased DNA damage and a change in nuclear density in the mutant hearts are unclear. A possible explanation is that DNA damage and the related inflammation process induce local cell reprogramming. This explanation is consistent with the observation that cellular reprogramming in vivo occurs following tissue injury (Yanger et al., 2013).

The fact that the organismal phenotypes observed in Ft1 mutant animals have also been found in models specifically defective in lamin structure and/or expression, or bearing mutations in genes required for telomere maintenance or DNA repair, poses an interesting question. Which of the cellular phenotypes observed in Ft1 mutant MEFs (defective lamin behavior, telomere dysfunction, DNA damage) is responsible for the organismal progeroid phenotypes? Answering this question is difficult because the traits that characterize Ft1 mutants at the cellular level are deeply interconnected. For example, alterations in lamin function affect DNA replication and repair, epigenetic modification of chromatin and transcription (Gonzalo & Kreienkamp, 2015). Moreover, multiple interactions link telomeres to the lamin network, including the association of telomeres with the nuclear envelope (Burla, La Torre & Saggio, 2016). Finally, telomeres recruit and interact with many DNA repair factors, which play crucial functions in telomere maintenance (Doksani & de Lange, 2014). Thus, current information does not allow identification of the specific cellular phenotype that leads to progeroid traits observed in Ft1 mutant mice. The most likely hypothesis is that all cellular defects observed in Ft1 mutant MEFs contribute to the organismal phenotype of mutant animals. It is indeed quite possible that these defects lead to senescence in most if not all tissues, causing developmental defects and infertility.

### 3.1 Relationships between Ft1, p53, and cancer

We have shown that p53 deficiency in Ft1 mutant MEFs induces cell over proliferation and rescues the sensitivity to both bleomycin and hydroxyurea. Consistent with these findings, in \( \text{Ft1}^{\text{ko}}/\text{ko} \) mutant mice, mutations in p53 rescue the body weight and sterility phenotypes but do not improve survival. Impairment of the p53 function also ameliorates the progeroid phenotypes in BRCA1-deficient mice (Cao, Li, Kim, Brodie & Deng, 2003) and in HGPS mouse models (Varela et al., 2005). However, p53 deficiency worsens the progeroid phenotype in telomere dysfunctional mice (Begus-Nahrmann et al., 2009). An explanation for this discrepancy is that p53 deficiency allows beneficial propagation of damaged cells rescuing certain progeroid traits. However, when cellular damage is extensive and the regenerative capacity of tissues is severely limited, p53 deficiency would become deleterious and accelerate aging (Lopez-Otín, Blasco, Partridge, Serrano & Kroemer, 2013). Our results are consistent with this model; they indicate that mutations in Ft1 result in a relatively mild genomic damage that triggers DDR-related checkpoints, which are abolished by mutations in p53 allowing resumption of cell proliferation.

The relationships between mutations in progeria-related genes and cancer are also complex. Progeroid models have been used to study the interplay between aging and cancer, given that age is a major risk factor for cancer developing. It has been shown that some forms of progeria can exert a protective role against tumor development (de la Rosa et al., 2013). On the other hand, mutations in the WRN helicase causing a segmental progeroid syndrome have been associated with an elevated cancer risk (Blander et al., 2000). We found that \( \text{Ft1}^{\text{ko}} \) mutation does not induce cancer and that \( \text{p53} \) \( \text{Ft1}^{\text{ko}}/\text{ko} \) double-mutant mice do not exhibit an increase in the overall frequency of malignancies. However, \( \text{p53} \) \( \text{ko} \) combined with \( \text{Ft1} \) deficiency induced an increase in the diffusion of lymphomas as compared to the restricted localization of this type of tumor in \( \text{p53} \) \( \text{ko} \) mice. It has been reported that T-cell lymphomas in \( \text{p53} \) \( \text{ko} \) mice are oligoclonal and generated by a characterized sequence of mutational events (Dudgen et al., 2014). We therefore hypothesize that in \( \text{p53} \) \( \text{Ft1}^{\text{ko}}/\text{ko} \) double mutants, this sequence is altered causing multicolonality and/or histotype change of lymphomas.

In conclusion, we have shown that mutations in Ft1 affect lamin, telomeres, DNA repair, and cell senescence. At the organismal level, these mutations result in a number of phenotypes that have been previously observed in several progeria mouse models. Thus, we believe that Ft1 is a new player in both the normal and accelerated aging processes, and that Ft1 mutant mice will be instrumental to analyze the interactions between Ft1 and other mouse progeria genes.
4 | EXPERIMENTAL PROCEDURES

4.1 | Mice

ES (HEPD0589_6_H06) from C57Bl/6 animals carrying the knockout first mutations in the Ft1 gene (referred as Ft1 kof) were generated by the International Knock-out mouse consortium. Injections into C57Bl/6 blastocyst were performed in EMBL (Monterotondo, Italy). Chimeras were crossed with C57Bl/6 and heterozygous animals backcrossed with C57Bl/6 and/or intercrossed. Ft1+/kof were crossed with p53−/− (Jacks et al., 1994) animals to obtain double mutants. Offspring were weaned at 3 weeks, and tail biopsies were geno-typed and transgene expression analyzed. When needed, mice were anesthetized by intramuscular Zoletil 20 (Virbac S.A., France), or euthanized by asphyxiation with carbon dioxide or cervical dislocation.

4.2 | Cells

MEFs were isolated and cultured as described in Rinaldo et al. (2012). Population doubling (pd) was calculated with the formula Log (n_f/n_0) × 3.33. For Bleomycin and Hydroxyurea sensitivity assay, cells plated 24 hr in advance were treated with Bleomycin (Sanofi Aventis) or Hydroxyurea (Sigma) for 7 hr and replaced with medium w/o drugs. Cell density was calculated 10 days after treatment by staining with crystal violet (5% in methanol, Sigma) for 10 min and analyzed by IMAGEJ.

4.3 | Genotyping

Tail biopsies were digested overnight at 56°C with a proteinase K/SDS solution; genomic DNA (gDNA) was extracted using the NucleoSpin® Tissue columns kit (Macherey-Nagel, Duren, Germany) following manufacturer’s instructions. Mice were PCR genotyped using the following primers:

\[
\text{Ft1 E6 F: 5'}-\text{TTGCCAACCGCTTITCCACA;  \\
\text{mGAPDH F: GGGGAAAGTGGGATTTGCC;  \\
mGAPDH R: TGTGCCGTTAATTGCCTG;  \\
p21 F: 5'-TGTCGAGCCGCGTCGAAAGAT-3';  \\
p21 R: 5'-CTTGCGCTTTGAGTATAGAA-3'}
\]

4.4 | q-PCR

RNA was extracted using the TRizol reagent (Invitrogen) according to manufacturer, after DNasel treatment (Invitrogen) was reverse transcribed into cDNA with oligo d(T) primer and OMNISCRPT RT KIT (Qiagen). q-PCRs were performed as described (Burla et al., 2015) using following primers:

\[
\text{Ft1 E3 F: AACCAGTCTCCACAGAGTAGC;  \\
\text{Ft1 E3 R: TAGGGCTTGTCATTGTGGTAGAGCA;  \\
\text{Ft1 E6 F: CCGTCTTTCCACCCACTTTGAT;  \\
}\]

4.5 | Western blotting

Western blotting was carried out as described in Burla et al. (2015). Filters were incubated with rabbit monoclonal anti-Ft1 (Sigma HPA 046300), rabbit anti-actin HRP-conjugated (C-11; Santa Cruz—sc1615), rabbit anti-p21 (C-19; Santa Cruz—sc397), and rabbit anti-pS3 antibodies produced by S. Soddu as described in Cecchinelli et al. (2006). Filters were incubated with appropriate HRP-conju-gated secondary antibodies (Santa Cruz).

4.6 | Immunostaining, FISH, and cytology

For immunostaining, cells were fixed with 3.7% formaldehyde for 10 min at 4°C and permeabilized with 0.25% Triton X-100 in PBS for 5 min. Where indicated, cells were prepermeabilized according to (Burla et al., 2015). Cells were then incubated with the following anti-bodies in the presence of 3% BSA: anti-53BP1 (Novus Biologicals NB100-304), anti-γH2AX (05-636 clone JBW301 Upstate Biotechnol-ogy), anti-Trf1 (Abcam 1423), anti-Ft1 (Sigma, HPA 046300), and anti-Lamin A (H102, Santa Cruz Biotechnology sc 20680). Primary antibodies were detected with the pertinent secondary antibodies: anti-rabbit-ALEXA 555 (Invitrogen A21430) or anti-goat-FITC (Jackson ImmunoResearch 705-095-003). FISH was carried out according to Burla et al. (2015). Cytological preparations were examined with a Carl Zeiss (Thornwood, NY) Axioplan fluorescence microscope equipped with an HBO100W mercury lamp and a cooled charged-coupled device (CCD camera; Photometrics CoolSnap HQ). Optical sections were captured at 0.3 μm Z steps using a Prior Priscan stepping motor with an EM-CCD camera (Cascade II, Photometrics) connected to a spinning-disk confocal head (Carvill, Beckton Dickinson) mounted on an inverted microscope (Eclipse TE2000S, Nikon). Each image is a maximum-intensity projection of all sections. Images were recorded using METAMORPH software package (Universal Imaging) and processed using IMAGEJ (http://imagej.nih.gov) and Adobe Photoshop.

4.7 | Histology, immunohistochemistry, and TRAP

Skin, bone, and heart were fixed in 4% formaldehyde. Tissues were cleared with ascendant alcohol concentration, embedded in paraffin, and sectioned at 3.5 μm. Sections were hydrated with descendant alcohol concentration, stained with Hematoxylin (Carlo Erba) and Eosin (Sigma), cleared, and mounted with DPX Mountant for Histology (Sigma). For γH2AX analysis on paraffin, embedded heart sec-tions were treated as previously described (Martinez, Ferrara-Romeo, Flores & Blasco, 2014). Tissues were incubated overnight with an anti-γH2AX (Abcam 2893) diluted in BSA 3%, Triton X-100 0.1%, and the day after incubated for 1 hr at room temperature with the
pertinent secondary antibody (anti-rabbit-ALEXA 555, Invitrogen A21430). Slides were counterstained with Mayer hematoxylin (Carlo Erba) and mounted with DPX mounting solution for microscopic evaluation (Sigma). Pictures were taken with ZEISS-Axio Phot (Zeiss) microscope connected to Progress-CS JENO-PTIK camera with the software PROGRESS MAC (Capture PRO). TRAP staining was performed according to manufacturer’s instructions (Sigma 387A).

4.8  | X-ray and bone density analysis

Total body X-ray images were taken using Faxitron MX-20 (Faxitron X-ray Corp.) at 24 kV for 6 s; images captured with Medical Imaging Film HM Plus (Ferrania). Cervical-thoracic vertebrae angle quantification was measured with Photoshop CS6 plugin. Femurs were imaged using a Faxitron MX20 operating at 24 kV for 4 s. Image density was determined as previously described (Bassett, van der Spek, Gogakos & Williams, 2012).

4.9  | Statistics

Kaplan–Meier curves were analyzed using the log-rank (Mantel-Cox) test. Inheritance of kof allele was analyzed using the Mendelian ratio for heterozygous mating, and $\chi^2$ test was applied. The Kolmogorov–Smirnov test was used to compare gray-level cumulative frequency distributions in X-ray image quantification. Independent data sets were compared with the Student’s $t$ test (unpaired, two-tailed).

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CONFLICT OF INTEREST

We have no conflict of interest.

AUTHOR CONTRIBUTIONS

MLT, CM, RB, GZ, SDG, MC, IV, EB, AB, IM, and GRV performed the design of the experiments and to the writing of the manuscript. IS designed the experiments and wrote the manuscript.

REFERENCES

Bachrati, C. Z., & Hickson, I. D. (2003). RecQ helicases: Suppressors of tumorigenesis and premature aging. Biochemical Journal, 374, 577–606. https://doi.org/10.1042/bj20030491
Barrowman, J., Wiley, P. A., Hudlon-Miller, S. E., Hrycyna, C. A., & Michaelis, S. (2012). Human ZMPSTE24 disease mutations: Residual proteolytic activity correlates with disease severity. Human Molecular Genetics, 21, 4084–4093. https://doi.org/10.1093/hmg/ddc233
Bassett, J. H., van der Spek, A., Gogakos, A., & Williams, G. R. (2012). Quantitative X-ray imaging of rodent bone by Faxitron. Methods in Molecular Biology, 816, 499–506. https://doi.org/10.1007/978-1-61779-415-5
Begus-Nahrmann, Y., Lechel, A., Obenauf, A. C., Nalapareddy, K., Peit, E., Hoffmann, E. … Rudolph, K. L. (2009). p53 deletion impairs clearance of chromosomal-instable stem cells in aging telomere-dysfunctional mice. Nature Genetics, 41, 1138–1143. https://doi.org/10.1038/ng.426
Bergo, M. O., Gavino, B., Ross, J., Schmidt, W. K., Hong, C., Kendall, L. V., … Young, S. G. (2002). Zmpste24 deficiency in mice causes spontaneous bone fractures, muscle weakness, and a prelamin A processing defect. Proceedings of the National Academy of Sciences USA, 99, 13049–13054. https://doi.org/10.1073/pnas.192460799
Blander, G., Zalle, N., Leal, J. F., Bar-Or, R. L., Yu, C. E., & Oren, M. (2000). The Werner syndrome protein contributes to induction of p53 by DNA damage. FASEB Journal, 14, 2138–2140.
Burla, R., Carcuro, M., La Torre, M., Fratini, F., Crescenzi, M., D’Apice, M., … Saggio, I. (2016). The telomeric protein AKTIP interacts with A- and B-type lamins and is involved in regulation of cellular senescence. Open Biology, 6, 160103. https://doi.org/10.1098/rsob. 160103
Burla, R., Carcuro, M., Raffa, G. D., Galati, A., Raimondo, D., Rizzo, A., … Saggio, I. (2015). AKTIP/P1f1, a new shelterin-interacting factor required for telomere maintenance. PLoS Genetics, 11, e1005167. https://doi.org/10.1371/journal.pgen.1005167
Cao, H., & Hegele, R. A. (2003). LMNA is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). Journal of Human Genetics, 48, 271–274. https://doi.org/10.1007/s10038-003-0025-3
Cao, L., Li, W., Kim, S., Brodie, S. G., & Deng, C. X. (2003). Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Brc1a1 full-length isoform. Genes & Development, 17, 201–213. https://doi.org/10.1101/gad.1050003
Cecchinelli, B., Porrello, A., Lazzari, C., Gradi, A., Bossi, G., D’Angelo, M., … Soddu, S. (2006). Ser58 of mouse p53 is the homologue of human Ser46 and is phosphorylated by HIPK2 in apoptosis. Cell Death and Differentiation, 13, 1994–1997. https://doi.org/10.1038/sj.cdd.4401933
Cenci, G., Ciapponi, L., Marzullo, M., Raffa, G. D., Morciano, P., Rai- mond, D., … Gatti, M. (2015). The analysis of pendolino (peo) mutants reveals differences in the fusigenic potential among Drosophila telomeres. PLoS Genetics, 11, e1005260. https://doi.org/10.1371/journal.pgen.1005260
Chen, C. Y., Chi, Y. H., Mutilaf, R. A., Starost, M. F., Myers, T. G., Anderson, S. A., … Jeang, K. T. (2012). Accumulation of the inner nuclear envelope protein Sun1 is pathogenic in progeric and dystrophic laminopathies. Cell, 149, 565–577. https://doi.org/10.1016/j.cell.2012.01.059
de la Rosa, J., Freije, J. M., Cabanillas, R., Osorio, F. G., Fraga, M. F., Fernandez-Garcia, M. S., … Lopez, C. T. (2013). Prelamin A causes progeria through cell-extrinsic mechanisms and prevents cancer invasion. Nature Communications, 4, 2268.
De Sandre-Giovannoli, A., Bernard, R., Cau, P., Amiel, J., Boccaccio, L., … Luca, P. (2014). The role of double-strand break repair and p53 in prevention of anchorage-independent stem cells in aging telomere-dysfunctional mice. Nature, 510, 480–486.
Doksani, Y., & de Lange, T. (2014). The evolution of thymic lymphomas in p53 knockout mice. Genes & Development, 28, 2613–2620. https://doi.org/10.1101/gad.252148.114
Gonzalo, S., & Kreienkamp, R. (2015). DNA repair defects and genome instability in Hutchinson-Gilford Progeria Syndrome. Current Opinion in Cell Biology, 34, 75–83. https://doi.org/10.1016/j.ceb.2015.05.007

Hennekam, R. C. (2006). Hutchinson-Gilford progeria syndrome: Review of the phenotype. American Journal of Medical Genetics. Part A, 140, 2603–2624. https://doi.org/10.1002/ajmg.1552-4833

Ibrahim, M. X., Sayin, V. I., Akula, M. K., Liu, M., Fong, L. G., Young, S. G., & Bergo, M. O. (2013). Targeting isoprenylcysteine methylation ameliorates disease in a mouse model of progeria. Science, 340, 1330–1333. https://doi.org/10.1126/science.1238880

Jacks, T., Remington, L., Williams, B. O., Schmitt, E. M., Halachmi, S., Bronson, R. T., & Weinberg, R. A. (1994). Tumor spectrum analysis in p53-mutant mice. Current Biology, 4, 1–7. https://doi.org/10.1016/S0960-9822(00)00002-6

Lang, G. A., Iwakuma, T., Suh, Y. A., Liu, G., Rao, V. A., Parant, J. M., … Lopez-Otin, C. (2011). Cell autonomous and systemic factors in the phenotype. Aging Cell, 10, 90–103. https://doi.org/10.1111/j.1474-9726.2009.00621

Marti, M., Choudhary, S. N., Hua, X., Dai, Z., & Li, Y. (2013). Interaction of the oncogenic miR-21 microRNA and the p53 tumor suppressor pathway. Carcinogenesis, 34, 1216–1223. https://doi.org/10.1093/carcin/bgt044

Martinez, P., Ferrara-Romeo, I., Flores, J. M., & Blasco, M. A. (2014). Essential role for the TRF2 telomere protein in adult skin homeostasis. Aging Cell, 13, 656–668. https://doi.org/10.1111/acel.12221

Mourique, L. C., Kozlov, S., Hernandez, L., Sullivan, T., & Stewart, C. L. (2003). A progeroid syndrome in mice is caused by defects in A-type lamins. Nature, 423, 298–301. https://doi.org/10.1038/nature01631

Osorio F. G., Navarro C. L., Cadinanos J., Lopez-Mejia I. C., Quiros P. M., Bartoli C., … Lopez-Otín C. (2011) Splicing-directed therapy in a new mouse model of human accelerated aging. Science Translational Medicine 3, 106ra107.

Osorio, F. G., Ugalde, A. P., Marino, G., Puente, X. S., Freije, J. M., & Lopez-Otín, C. (2011). Cell autonomous and systemic factors in progeria development. Biochemical Society Transactions, 39, 1710–1714. https://doi.org/10.1042/BST20110677

Rinaldo, C., Moncada, A., Gradi, A., Ciuffini, L., D’Eliseo, D., Siepi, F., … Soddu, S. (2012). HIKP2 controls cytokinesis and prevents tetraploidization by phosphorylating histone H2B at the midbody. Molecular Cell, 47, 87–98. https://doi.org/10.1016/j.molcel.2012.04.029

Rudolph, K. L., Chang, S., Lee, H. W., Blasco, M., Gottlieb, G. J., Greider, C., & DePinho, R. A. (1999). Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell, 96, 701–712. https://doi.org/10.1016/S0092-8674(00)80580-2

Saeed, H., Abdallah, B. M., Ditzel, N., Catala-Lehnen, P., Qiu, W., Amling, M., & Kassem, M. (2011). Telomerase-deficient mice exhibit bone loss owing to defects in osteoblasts and increased osteoclastogenesis by inflammatory microenvironment. Journal of Bone and Mineral Research, 26, 1494–1505. https://doi.org/10.1002/jbmr.349

Scaffidi, P., & Misteli, T. (2008). Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. Nature Cell Biology, 10, 452–459. https://doi.org/10.1038/ncb1708

Sfeir, A., Kosiyatrakul, S. T., Hockemeyer, D., MacRae, S. L., Karlseeder, J., Schildkraut, C. L., & de Lange, T. (2009). Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. Cell, 138, 90–103. https://doi.org/10.1016/j.cell.2009.06.021

Sharpless, N. E., & Sherr, C. J. (2015). Forging a signature of in vivo senescence. Nature Reviews Cancer, 15, 397–408. https://doi.org/10.1038/nrc3960

Testa, G., Schaft, J., van der Hoeven, F., Glaser, S., Anastassiadis, K., Zhang, Y., … Stewart, A. F. (2004). A reliable lacZ expression reporter cassette for multipurpose, knockout-first alleles. Genesis, 38, 151–158. https://doi.org/10.1002/GENS.1526-968X

Varela, I., Cadinanos, J., Pendas, A. M., Gutierrez-Fernandez, A., Folgueras, A. R., Sanchez, L. M., … Lopez-Otin, C. (2005). Accelerated ageing in mice deficient in Zmpste24 protease is linked to p53 signalling activation. Nature, 437, 564–568. https://doi.org/10.1038/nature04019

Watson, L. A., Solomon, L. A., Li, J. R., Jiang, Y., Edwards, M., Shin-ya, K., … Berube, N. G. (2013). Atrx deficiency induces telomere dysfunction, endocrine defects, and reduced life span. Journal of Clinical Investigation, 123, 2049–2063. https://doi.org/10.1172/JCI65634

Yanger, K., Zong, Y., Maggs, L. R., Shapira, S. N., Maddipati, R., Aiello, N. M., … Stanger, B. Z. (2013). Robust cellular reprogramming occurs spontaneously during liver regeneration. Genes & Development, 27, 719–724. https://doi.org/10.1101/gad.207803.112

SUPPORTING INFORMATION

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