Supplementary Material and Methods

Western blot analysis

Western blot analysis was done following standard procedures. Bone marrow cells were prepared using RIPA buffer or using nuclear extraction as described [1]. 15-25µg protein of the respective cell extracts were separated in a 5-20% gradient SDS polyacrylamid gels using electrophoresis. After transfer, nitrocellulose membranes were incubated with anti-TRF1 (Abcam, UK), anti-p53 (Santa Cruz, US), anti-SMC (Abcam, UK) or anti-Actin (Sigma, US) antibody. To detect the primary antibodybinding, a secondary antibody (Dako, Denmark) coupled to horseradish peroxidase using chemiluminescence was used (GE Healthcare, UK).

PCR

DNeasy kit (Qiagen, USA) was used for DNA isolation. PCR for genotyping of the isolated bone marrow was performed as described [1]. The following primer sequences were used: SA1: 5′-GCTTGCCAAATTGGGTTGG-3′, E1-F2: 5′-GATGCTCGACTTCCTCT-3′.

RT-PCR

Total RNA of FACS sorted bone marrow was prepared using RNeasy Micro kit (Qiagen, USA). For first-stranded cDNA synthesis was carried out following manufacturer's instruction (Ready-to-go you-prime-first, GE Healthcare, USA). Quantitative real-time PCR was performed using DNA Master SYBR Green I mix (Applied Biosystems, US). All values were performed as duplicates and repeated at least once. The following primers were used: p21 F-:5′-GTGGGTCTGA-CTCCAGCCC-3′, p21 R-:5′-CCTTCTCGTGAGACGCTTAC3′. Actin-F:5′-GGCACCACACCTTCTACAATG-3′,ActinR-5′-GTGGTGGTGAAGCTGTAG-3′.

Q-FISH, mmuno-Q-FISH and high throughput (HT) Q-FISH

Q-FISH on deparaffinized bone marrow tissue sections and image capturing were performed as described [2,3]. Briefly, one marrow sections were captured using a high-resolution Leica TCS-sp5 confocal icroscope (Leica, US). Multi-tracking mode was used to acquire images of DAPI and Cy3 staining and stacks were taken with a step size of 0.8 µm. Maximum projection of the images was done and Definiens XD 1.5 image analysis software (Definiens GmbH, Germany) was used for quantitative image analysis. Individual telomere signals were calculated after subtraction of the mean background value per detected nucleus.

Q-FISH on bone marrow metaphases was carried out as described [1,4] and images were captured with 100x magnification using a Leica DMRA fluorescence microscope (Leica, US). Telomere- γH2AX-immuno-Q-FISH for was performed as described[1,4]. Antiphospho histone H2AX (Millipore, USA) was used for immunofluorescence staining and images were captured using confocal microscope. For HT Q-FISH peripheral blood of jugular puncture was processed as described [5].

Telomere length was determined analyzing telomere spots (>5000 spots) and telomere length was calculated in kilobases as described5.

Immunohistochemistry, immunofluorescence and senescence specific β-galactosidase staining

Bone marrow samples were fixed in 10% buffered formalin, decalcified, dehydrated and embedded in paraffin. For further histopathological analysis, 4 µm sections were deparaffinated and stained with hematoxylin and eosin according to standard procedures. Immunohistochemistry (IHC) of p21 (Santa Cruz Biotechnology, US), phospho-Histon3 (Millipore, US) and phospho-CHK1 (Cell Signaling Technology, US) was performed as described using the strepatvidin-biotin-peroxidase method [4]. For

879 quantitative analysis Axio-Vision (Carl Zeiss, Germany) and Scion Image (Scion Corp.,
880 US) software were used. Quantification of immunostaining was determined calculating
881 the area the number of positive cells in relation to all stained nuclei.
882 Immunofluorescence was carried out as described[1,4] using an anti-TRF1 antibody
883 recently generated in our institution 6. Images were captured using confocal
884 microscopy and image analysis was performed as described for Q-FISH. For analysis
885 of senescence, 5-10 $x10^5$ cells were plated into 100 mm dishes and senescence
886 specific β-galactosidase staining was performed following the manufacturer´s
887 instructions (Cell Signaling, US). For analysis, at least 500 cells per dish were counted.
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Supplementary References:

1. Martinez P, Thanasoula M, Munoz P, et al. Increased telomere fragility and fusions resulting from TRF1 deficiency lead to degenerative pathologies and increased cancer in mice. Genes Dev. 2009;23(17):2060-2075.

2. de Jesus BB, Schneeberger K, Vera E, Tejera A, Harley CB, Blasco MA. The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. Aging Cell;10(4):604-621.

3. Varela E, Schneider RP, Ortega S, Blasco MA. Different telomere-length dynamics at the inner cell mass versus established embryonic stem (ES) cells. Proc Natl Acad Sci U S A;108(37):15207-15212.

4. Tejera AM, Stagno d'Alcontres M, Thanasoula M, et al. TPP1 is required for TERT recruitment, telomere elongation during nuclear reprogramming, and normal skin development in mice. Dev Cell;18(5):775-789.

5. Canela A, Vera E, Klatt P, Blasco MA. High-throughput telomere length quantification by FISH and its application to human population studies. Proc Natl Acad Sci U S A. 2007;104(13):5300-5305.

6. Munoz P, Blanco R, de Carcer G, et al. TRF1 controls telomere length and mitotic fidelity in epithelial homeostasis. Mol Cell Biol. 2009;29(6):1608-1625.

Supplementary Figure Legends:

Supp. Figure 1: Overview of the different injections schemata:

(A) Overview of theacute injection schema: Cre induction every 2nd day

(B) Overview of intermediate injection scheme: Daily Cre induction for 7 days

(C) Overview of chronic/long-term Cre induction: Cre induction 3x per week (Monday, Wednesday, Friday) up to 13 weeks

Supp. Figure 2: Immunofluorescence of TRF1flox/floxMx1-Cre animals at day +18.

(A) Representative immunofluorescence of TRF1$^{flox/flox}$Mx1-wt and TRF1$^{flox/flox}$Mx1-Cre animals at day +18 (63x magnification and 2,5x optical zoom) (B) Quantification of TRF1 spot intensity minus background per nucleus is shown. Two-sided t-test was used for statistical comparison.

Supp. Figure 3: Intermediate 7 day Cre induction leads to a reliable and robust decrease of the bone marrow cellularity and TRF1 protein levels.

(A) Bone marrow cellularity calculated based on the cell number obtained from bone marrow isolation of the femura and tibiae. Two-sided t-test was used for statistical comparison.

(B) Western blot of TRF1 protein levels of nuclear protein extracts obtained from TRF1$^{flox/flox}$Mx1-Cre animals with and without intermediate Cre induction.

(C) Quantification of TRF1 protein levels in relation to SMC1 levels. Two-sided t-test was used for statistical comparison.

(D) ELISA analysis of serum G-CSF levels of TRF1$^{flox/flox}$Mx1-wt and TRF1$^{flox/flox}$Mx1-Cre mice with and without intermediate Cre induction. Two-sided t-test and student t-test was used for statistical comparison.

(E) Cell cycle profile of TRF1$^{flox/flox}$Mx1-wt and TRF1$^{flox/flox}$Mx1-Cre mice with and without intermediate Cre induction. Two-sided t-test and student t-test was used for statistical comparison.

(F) Representative histogram of the G1, S and G2-M phase using PI staining.

Supp. Figure 4: Metaphase analysis of TRF1$^{flox/flox}$Mx1-wt and TRF1$^{flox/flox}$Mx1-Cre mice and β-galactosidase staining

(A) Representative images of muti-telomeric signals (MTS) and chromatid fusion (CF) events (indicated by white arrow).

(B) Quantification of muti-telomeric signals and chromatid fusion events in TRF1$^{flox/flox}$Mx1-wt and TRF1$^{flox/flox}$Mx1-Cre mice undergoing intermediate Cre induction. Two-sided ttest was used for statistical comparison.

(C) Quantification of β-galactosidase staining of TRF1$^{flox/flox}$Mx1-wt and TRF1$^{flox/flox}$Mx1-Cre mice after 7 days of pI-pC injections and 3 days of pause before euthanizing the mice.

Supp. Figure 5: Long-term Cre induction results in compensatory higher cell proliferation and replicative stress

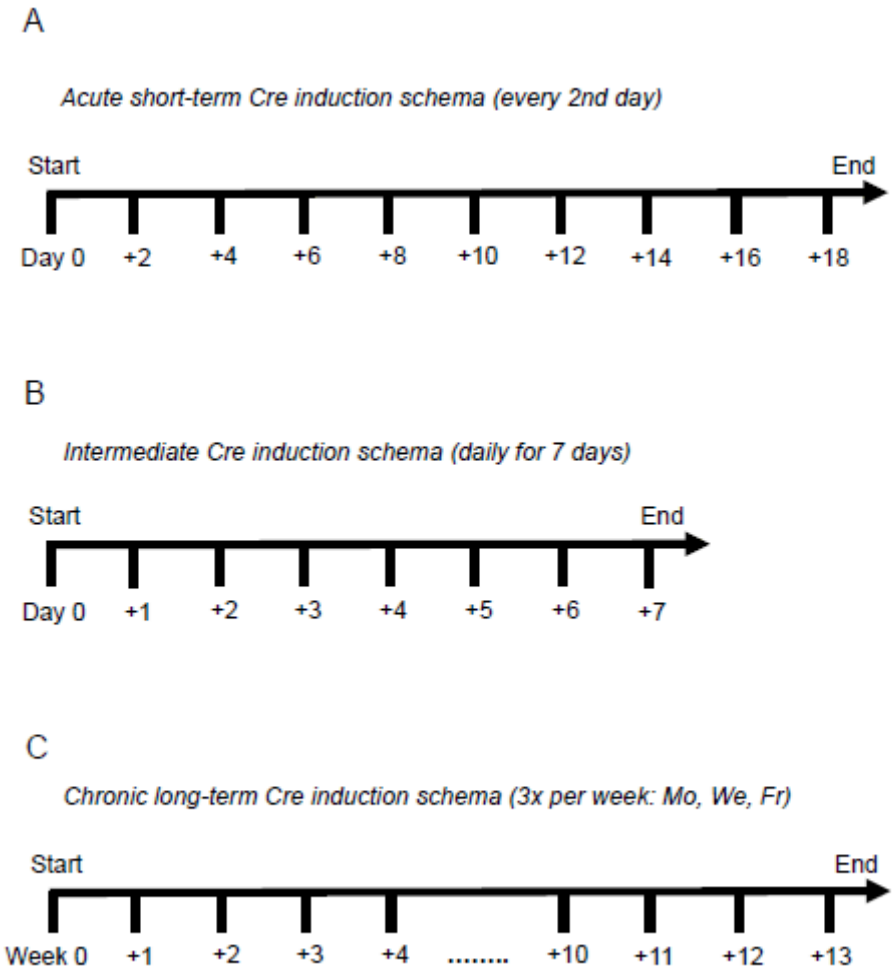
(A) Representative images of phospho-histon3 IHC staining of TRF1$^{flox/flox}$Mx1-wt and TRF1$^{flox/flox}$Mx1-Cre mice at time point 0 and after 8 weeks of Cre induction. Image was captured with 20x magnification (blue bar represents 100 µm), small image shows 120x magnification.

(B) Quantification of the phospho-histon3 IHC staining at the time points 0,+4 and +8 weeks after starting Cre induction. Two-sided t-test was used for statistical comparison.

(C) Representative images of phospho-CHK1 IHC staining of TRF1$^{flox/flox}$Mx1-wt and TRF1$^{flox/flox}$Mx1-Cre mice at time point 0 and after 8 weeks of Cre induction. Image was captured with 20x magnification (blue bar represents 100 µm), small image shows 120x magnification.

(D) Quantification of the phospho-CHK1 IHC staining at the time points 0,+4 and +8 weeks after starting Cre induction. Two-sided t-test was used for statistical comparison.

965 Supplementary Figure 1



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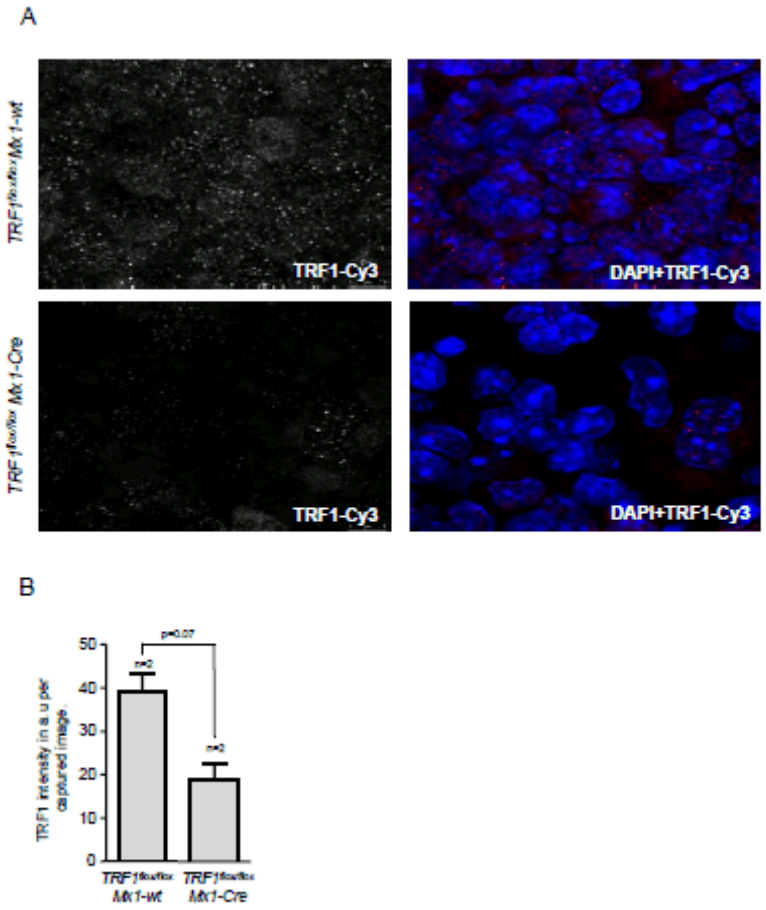
968 Supplementary Figure 2



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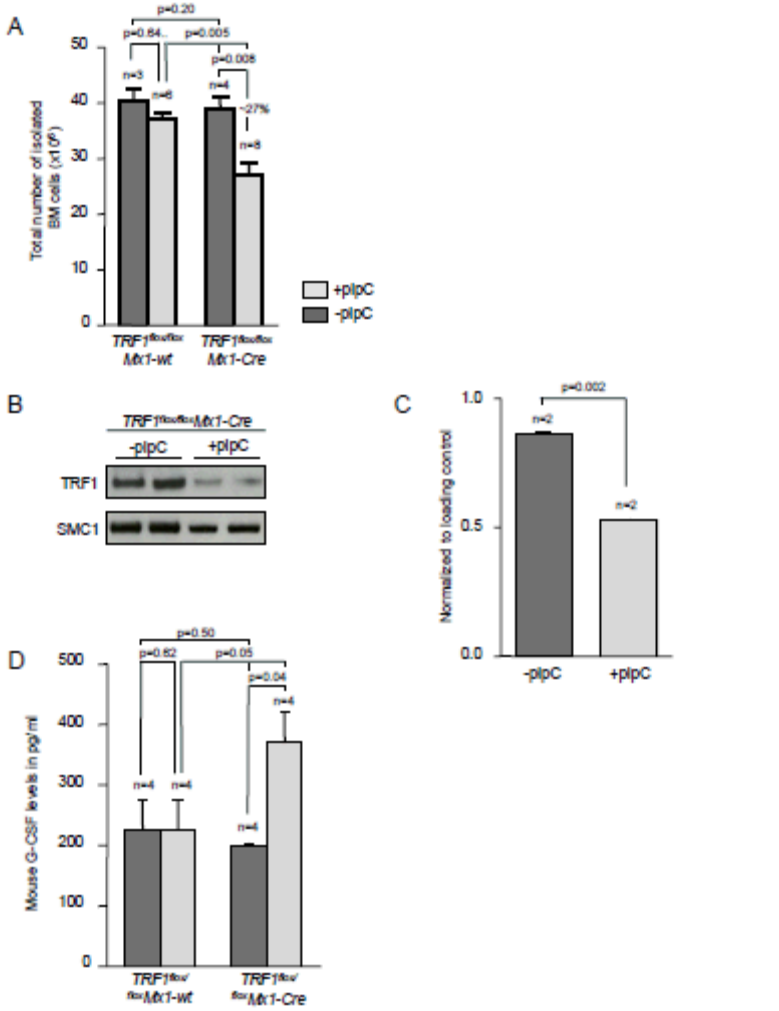

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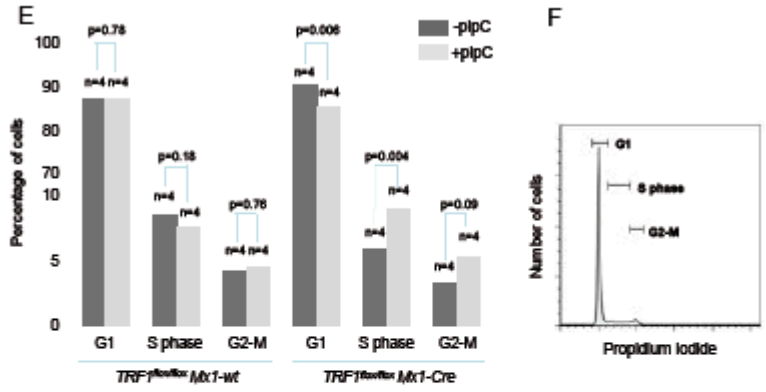

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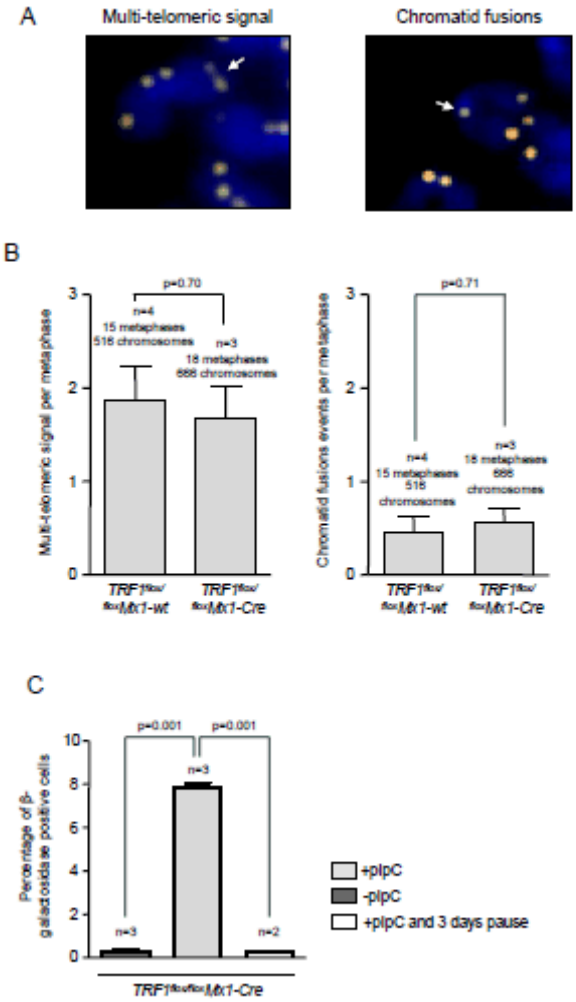

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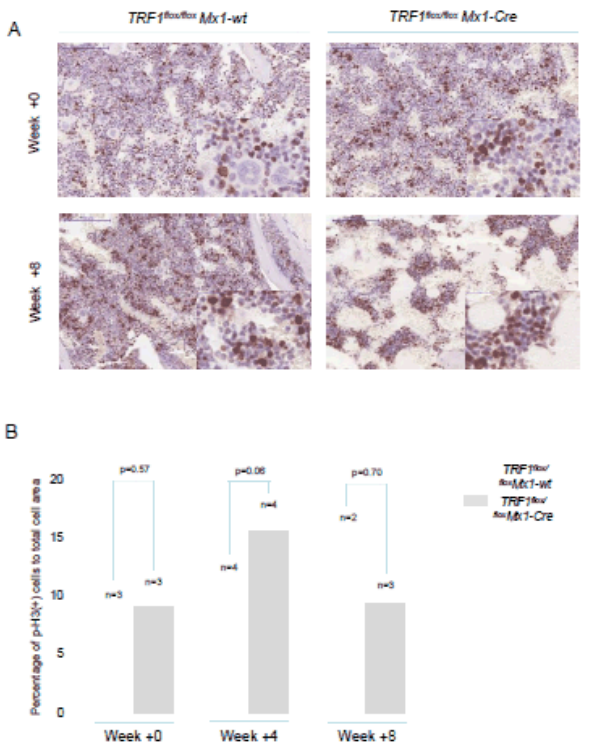

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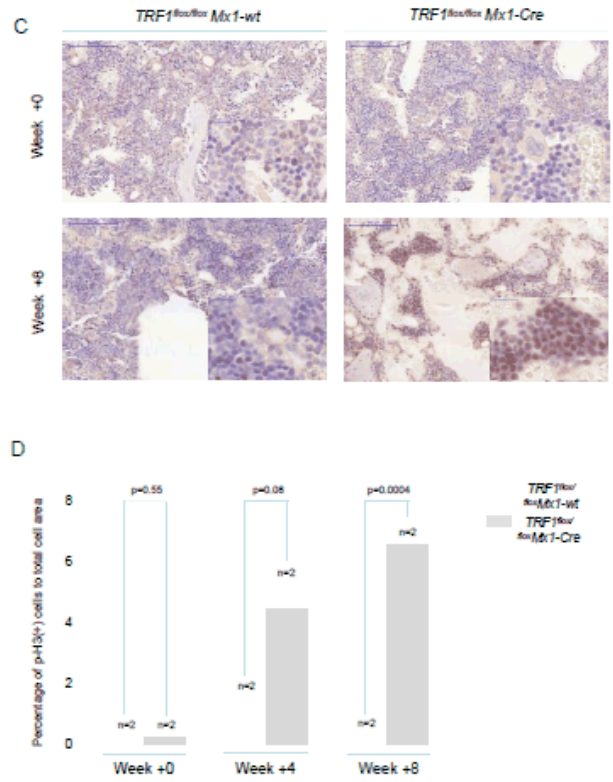

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