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Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer

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(Note: The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 14 February 2012

Thank you for your patience with our evaluation of your manuscript on telomerase gene therapy in adult wild-type mice. I apologize for the considerable delay in its editorial and external review. Unfortunately, it was very difficult at this time of the year to assign three suitable experts and to receive their reports in a timely fashion; however, we have now finally received three sets of comments, and our Chief Editor and I have discussed them in depth in order to come to an editorial decision. Therefore, the specific problems and comments (especially regarding presentation, interpretation and statistics/reproducibility) raised by the referees may (and should) be addressed.

Given the potential translational relevance of your results, which set them apart from the recurrently mentioned work by DePinho and colleagues, we feel that the manuscript should be a promising and well-fitting candidate for EMBO Molecular Medicine. Should you be interested, the study would be treated as a revision of the current submission, and eventual acceptance would only depend on adequately addressing and clarifying the specific points raised in reports 1 and 3, but not on following up the deeper mechanistic questions, which we realize would greatly delay publication of this study and may thus be beyond the scope of the current manuscript.

Once again, I am sorry for this delayed evaluation.

Yours sincerely,

Editor
EMBO Molecular Medicine

REFEREE REPORTS:

Referee #1:

Late generation telomerase knockout mice with short telomeres age prematurely, while over expression of telomerase in cancer resistant mice extents animal lifespan. Similarly, defects in proteins involved in telomere maintenance often are associated with premature aging syndromes, both in humans and in telomerase knockout mice. Thus, it is likely that telomere shortening and dysfunction contributes to aging in populations with relatively short telomeres and that suppression of telomere erosion/dysfunction extents lifespan in these mice and potentially also in other mammals.
Bernandes de Jesus et al. have developed a novel technique to counteract telomere erosion/dysfunction in mice without increasing the incidence of cancer in these animals. The authors use non-integrating AAV vectors to drive over-expression of TERT and inject recombinant viruses into tail veins of C57BL6 mice. Animals of two age groups were injected, 1yr and 2yr. Telomerase activity and TERT levels were elevated in a variety of tissues, both at the mRNA and protein levels. Both groups displayed fewer aging associated changes such as increased BMD, increased thickness of subq. Fat, lower insulin levels, and increased IGF1 levels. Neuromuscular object recognition tests were also improved in TERT treated animals. Significantly, both age groups displayed a significant increase in median lifespan compared to control groups, or compared to animals over-expressing catalytically inactive TERT, demonstrating that TERT can extent lifespan without increasing cancer incidence when expressed using AAV vectors and that TERT catalytic activity is required for lifespan extension. The authors demonstrate a proof-of-principle of attenuating physiological aging in mice using telomerase based gene therapy.
This is an interesting and potentially important manuscript as it demonstrates, for the first time, that aging can be delayed using gene therapy. In addition, it confirms previous data by the same laboratory that telomerase expression can extent lifespan in mice, under conditions that also do not increase cancer incidence.

Comments:

1) In the abstract the sentence "Telomere loss is one of the best understood causes of aging in mammals" is not accurate. While we know that telomere loss is associated with aging in several mammals, similar to graying of hair, thinning of skin, etc. it is far from proven that this is a cause of aging. Clearly, late generation telomerase knockout mice age prematurely, and this again is associated with telomere shortening and dysfunction, but this only demonstrates that knockout of TERT or TR in short lived animals (mice) causes premature aging. It cannot be extended to other mammals, neither does it demonstrate that the associated telomere loss causes aging in mice.

2) Similarly, the last sentence in the abstract "these results constitute a proof-of-principle of reversal of physiological aging" is not accurate because the authors did not analyze or demonstrate reversal of any aging associated changes. They merely demonstrated a delay of these changes by injecting AAV tert virus.

3) It is surprising that animals with telomeres as long as 50kb age and die of aging related pathologies as a result of telomere erosion after just 2-3 years. Humans, on the other hand, live for ~80 years despite having telomeres that are dramatically shorter (~10kb at young age) compared to C57BL6 mice. If telomere erosion were a primary cause of aging, shouldn't mice live longer than humans? Along these lines, the authors count very short telomeres as less than 15kb (Fig S7E), a number that is still far greater than the longest telomeres in humans. Surely telomeres that are 15kb in length are not dysfunctional, the event that most likely causes the decline of cell and tissue function. How do the authors propose that it is telomere shortening that causes aging in these animals if the shortest telomeres are still very long? Is it non-detectable stochastic telomere attrition that causes cell senescence/apoptosis in mice? If yes, is there evidence for this? Does telomere shortening and dysfunction primarily affect stem cell compartments? If yes, why doesn't endogenous telomerase prevent telomere erosion/dysfunction in these compartments? Is it the non canonical functions of telomerase that promotes lifespan extension? A better explanation, and more experimental evidence, to resolve these questions would be appropriate.

4) The great majority of analyses use inconsistent and sometimes dramatically different numbers of animals. Why is this? How were animals selected? Why were some tissues from a given animal

omitted from the analysis? For example, Fig 1C: liver samples from 4 control animals are compared to 3 tert injected animals, while brain tissues from 6 control animals are compared to 5 tert injected animals. Since tissue appears to be collected from at least six control and six tert injected-animals, why don't the authors quantitate data for all tissues from all twelve animals? This is a consistent theme throughout the manuscript and even the coordination/balance tests are shown for a dramatically different number of control animals vs tert injected animals (Figure 2E shows data for 4 control animals and compares that to 21 tert injected animals). Other examples where the authors compared quite different numbers of control animals to tert injected animals are Figs 2A-F, S4D-J, S10, among others.

5) Fig 2F: why was tightrope success not analyzed in the 2 year old group?

6) Why is p16 up-regulated in the heart after TERT expression?

7) It makes sense that extension of median lifespan is greater in animals treated at 1 year of age, compared to the 2 year old group (24% vs 13%). However, why is the maximum lifespan extension smaller in group 1 year vs 2 year (13% vs 20%)?

8) Fig 5F: I am not convinced that expression of DN-TERT results in telomere erosion that is different for wt-TERT expression. More tissues need to be analyzed, and potentially also more than just two animals. This is an important control to demonstrate the different effects of wt and catalytic inactive tert on telomere lengths.

Referee #2:

De Jesus and co-workers investigate the consequences of AAV-induced TERT expression on lifespan of wild type mice. The authors describe a positive effect of TERT expression on the maintenance of bone and the function of various tissues including motor activity. On the molecular level the authors propose that TERT's function in telomere maintenance is required for these effects since overexpression of a mutant form of TERT (which can not maintain telomeres) does not exhibit lifespan prolonging effects. The beneficial effects of TERT expression do not associate with an increased risk of cancer formation.

My main concern is the lack of mechanistic insight on how AAV-mediated TERT expression can elongate the lifespan of wildtype mice. The author's conclusion on telomere length is not substantiated by any data on telomere dysfunction, DNA damage signaling, apoptosis, senescence, etc. Moreover, most of the beneficial effects of TERT expression are described in postmitotic cells (motoneurons) or slow dividing tissues (bone). In contrast work on telomerase mutant mice revealed that high-turnover organs are most strongly affected by premature aging as a consequence of telomere shortening (see work from DePinho and colleagues). Together, these findings argue against a telomere dependent effect in the current study. Thus, the mechanism of TERT-AAV on lifespan remain elusive. The authors show some evidence for Wnt/b-catenin activation in TERT-AAV treated tissues. Could this be involved? Maybe activation of Wnt could be a good approach to decipher the mechanism. Lacking such mechanistic inside the results remain too descriptive for a molecular journal. It may then be better to aim for publication in more medical oriented journals.

Referee #3:

This paper has findings that are potentially very important.

Major

"As expected, AAV9-mTERT-DN treatment increased CyclinD1 expression" needs a reference or other explanation as to why this was expected. Also need to explain why p16 was analyzed.

Figure 1D: RNAase makes no difference to the assay result for each of the eGFP constructs. Why is

this?

Figure 2B: "time of death" needs explanation in figure legend. How long after injection was the measurement made?

Figure 2C: legend needs to state timing of insulin levels (e.g. specify time after feeding).

Figure 4 (and other figures): % short telomeres is defined relative to mean, which is variable, so doesn't seem to me to have biological meaning. If the mean length is much shorter in one condition, then telomeres that are below 50% of the mean will be shorter than telomeres that are 50% below a larger mean. Can the data be recalculated in such a way so that "short telomeres" is defined relative to a common standard to allow comparison between treatments?

Figure 5. Is the DN construct known to act as a dominant negative in mouse tissues? In other words, does it decrease the activity of endogenous telomerase? Could this explain the result in panel 5F where the mutant TERT construct results in an increase of the short telomeres?

Figure S3 and elsewhere: shouldn't mean + SD be average {plus minus} range when n=2??

Figure S3 - D: can a better gel be supplied?

Figure S6-C: please explain in the legend what degenerative lesions/inflammatory pathologies were scored.

Figure S7B and elsewhere: "nucleous" should be "nuclei". Can statistical analyses be supplied comparing the histograms?

Figure S7F: statistics?

Figure S11: need to state at what age the mice were injected.

Minor

Abstract: "expressing telomerase" should be "expressing TERT"

"associated to" in multiple locations throughout the manuscript should be "associated with"

"owe to" in several locations in the MS should be "owing to"

vg presumably means "viral genomes" and should be defined

p.6: I recommend changing "overbearing" to "large"

p.6: "telomerase TRAP activity" should be "telomerase activity as measured by TRAP assay"

"Kaplan-Meyer" (several locations) should be "Kaplan-Meier"

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p.11: "reflect on" should be "reflect"

p. 16: "and a plasmid carrying the adenovirus helper functions (kindly provided by K.A. High, Children's Hospital of Philadelphia)" repeats the previous clause and should be deleted.

p. 17 "two consecutives cesium chloride gradients, dyalized" should be "two consecutive cesium chloride gradients, dialyzed"

p. 18: "Bone mineral density (BMD) indicates the density of minerals in mice bones" can be deleted.

1st Revision - Authors' Response 22 February 2012

Reviewer #1

Summary

REVIEWER: "Late generation telomerase knockout mice with short telomeres age prematurely, while over expression of telomerase in cancer resistant mice extents animal lifespan. Similarly, defects in proteins involved in telomere maintenance often are associated with premature aging syndromes, both in humans and in telomerase knockout mice. Thus, it is likely that telomere shortening and dysfunction contributes to aging in populations with relatively short telomeres and that suppression of telomere erosion/dysfunction extents lifespan in these mice and potentially also in other mammals.

Bernandes de Jesus et al. have developed a novel technique to counteract telomere erosion/dysfunction in mice without increasing the incidence of cancer in these animals. The authors use non-integrating AAV vectors to drive over-expression of TERT and inject recombinant viruses into tail veins of C57BL6 mice. Animals of two age groups were injected, 1yr and 2yr. Telomerase activity and TERT levels were elevated in a variety of tissues, both at the mRNA and protein levels. Both groups displayed fewer aging associated changes such as increased BMD, increased thickness of subq. Fat, lower insulin levels, and increased IGF1 levels. Neuromuscular object recognition tests were also improved in TERT treated animals. Significantly, both age groups displayed a significant increase in median lifespan compared to control groups, or compared to animals over-expressing catalytically inactive TERT, demonstrating that TERT can extent lifespan without increasing cancer incidence when expressed using AAV vectors and that TERT catalytic activity is required for lifespan extension. The authors demonstrate a proof-of-principle of attenuating physiological aging in mice using telomerase based gene therapy.

This is an interesting and potentially important manuscript as it demonstrates, for the first time, that aging can be delayed using gene therapy. In addition, it confirms previous data by the same laboratory that telomerase expression can extent lifespan in mice, under conditions that also do not increase cancer incidence."

ANSWER: We sincerely thank the reviewer for the detailed review of our manuscript and for considering that "this is an interesting and potentially important manuscript, as it demonstrates, for the first time, that aging can be delayed using gene therapy". As well as that we "confirm previous data by the same laboratory that telomerase expression can extent lifespan in mice, under conditions that also do not increase cancer incidence". The reviewer also has a number of questions and insightful suggestions for changes in the manuscript, which we have fully addressed in a revised manuscript. In particular:

Major concerns

REVIEWER: 1)" In the abstract the sentence "Telomere loss is one of the best understood causes of aging in mammals" is not accurate. While we know that telomere loss is associated with aging in several mammals, similar to greying of hair, thinning of skin, etc. it is far from proven that this is a cause of aging. Clearly, late generation telomerase knockout mice age prematurely, and this again is associated with telomere shortening and dysfunction, but this only demonstrates that knockout of TERT or TR in short lived animals (mice) causes premature aging. It cannot be extended to other mammals; neither does it demonstrate that the associated telomere loss causes aging in mice."

ANSWER: The reviewer has a good point and we have removed this inaccurate sentence from the revised Abstract.

REVIEWER: 2) "Similarly, the last sentence in the abstract "these results constitute a proof-of-principle of reversal of physiological aging" is not accurate because the authors did not analyse or demonstrate reversal of any aging associated changes. They merely demonstrated a delay of these changes by injecting AAV Tert virus."

ANSWER: The reviewer is right and we have re-phrased this in the revised Abstract.

REVIEWER: 3) "It is surprising that animal with telomeres as long as 50kb age and die of aging related pathologies as a result of telomere erosion after just 2-3 years. Humans, on the other hand, live for ~80 years despite having telomeres that are dramatically shorter (~10kb at young age) compared to C57BL6 mice. If telomere erosion were a primary cause of aging, shouldn't mice live longer than humans? Along these lines, the authors count very short telomeres as less than 15kb (Fig S7E), a number that is still far greater than the longest telomeres in humans. Surely telomeres that are 15kb in length are not dysfunctional, the event that most likely causes the decline of cell and tissue function. How do the authors propose that it is telomere shortening that causes aging in these animals if the shortest telomeres are still very long? Is it non-detectable stochastic telomere attrition that causes cell senescence/apoptosis in mice? If yes, is there evidence for this? Does telomere shortening and dysfunction primarily affect stem cell compartments? If yes, why doesn't endogenous telomerase prevent telomere erosion/dysfunction in these compartments? Is it the non-canonical functions of telomerase that promotes lifespan extension? A better explanation, and more experimental evidence, to resolve these questions would be appropriate."

ANSWER: We understand this concern by the reviewer. It is true that mice have much longer telomeres than humans, but we have evidence that telomere length is rate limiting for mouse aging. On the one hand, the first generation of telomerase deficient mice in a C57BL6 background already show a decreased median and maximum longevity, which is anticipated with increasing generations (this was published by Garcia-Cao et al, *EMBO Reports*, 2006; we cite the paper in the manuscript). On the other hand, increasing TERT expression in cancer resistant mice, can delay mouse aging and extend normal mouse longevity (Tomás-Loba et al., *Cell*, 2008). Furthermore, we have previously reported that even though mouse telomeres are very long at birth, they suffer a dramatic telomere shortening in very old mice (Flores et al., *Genes & Dev*, 2008). We have included a sentence in the revised manuscript Discussion referring to these papers (page 13, lines 10-18). Finally, for the reviewer´s information, we have unpublished evidence by performing the first longitudinal telomere length analysis in mice that shows that mouse telomeres shorten at a much faster rate than in humans (Vera et al., Submitted).

Regarding a cut of telomeres <15 Kb to quantify short telomeres, we arbitrarily chose this cut owe to the fact that it better reflected differences between the different interventions.

REVIEWER: 4) "The great majority of analyses use inconsistent and sometimes dramatically different numbers of animals. Why is this? How were animals selected? Why were some tissues from a given animal omitted from the analysis? For example, Fig 1C: liver samples from 4 control animals are compared to 3 tert injected animals, while brain tissues from 6 control animals are compared to 5 tert injected animals. Since tissue appears to be collected from at least six control and six tert injected-animals, why don't the authors quantitate data for all tissues from all twelve animals? This is a consistent theme throughout the manuscript and even the coordination/balance tests are shown for a dramatically different number of control animals vs tert injected animals (Figure 2E shows data for 4 control animals and compares that to 21 tert injected animals). Other examples where the authors compared quite different numbers of control animals to tert injected animals are Figs 2A-F, S4D-J, S10, among others."

ANSWER: We always used the maximum number of mice possible per experiment. This is indicated in the figure legends. The variability in numbers of mice between different assays is due to both the nature of the assays used (in vivo [invasive or non-invasive] or ex vivo) and to the time-course of the experiments and/or natural death occurring in the cohorts. In the case of the Western Blots and qPCR assays, they were performed at different time-points and with independent mice, which explains the variations in mice numbers.

REVIEWER: 5) "Fig 2F: why was tightrope success not analysed in the 2 year old group?"

ANSWER: This was due to the fact that the 2-year old group suffered more deaths and we had lower "n" values at the time of carrying the tests.

REVIEWER: 6) "Why is p16 up-regulated in the heart after TERT expression?"

ANSWER: We decided to include the p16 analysis in order to mechanistically explain the physiological effects observed after TERT treatment as p16 is a *bona fide* marker of cellular senescence (Collado et al. *Cell*, 2007), which has been described to be highly up-regulated associated to mouse aging (Krishnamurthy et al, *Nature*, 2006; Molofsky et al, *Nature*, 2006). We have now included a sentence in the revised manuscript describing this (page 11, lines 22-27). We

are currently studying in more depth the effects of TERT treatment in different signalling pathways in the heart but still have no explanation for the p16 increase that we reproducibly observed.

REVIEWER: 7) "It makes sense that extension of median lifespan is greater in animals treated at 1 year of age, compared to the 2 year old group (24% vs 13%). However, why is the maximum lifespan extension smaller in group 1 year vs 2 year (13% vs 20%)?"

ANSWER: We agree with the reviewer that the higher benefit of TERT treatment in the 1-year old group regarding median life span is expected as 2-year old tissues maybe already too damaged. Regarding maximum lifespan, we would like to highlight that the maximal lifespan observed in the 2 yr old group is basically due to a single mouse, reflecting on the fact that maximum longevity is always determined by a single out-layer mouse, which could explain the observations.

REVIEWER: 8) "Fig 5F: I am not convinced that expression of DN-TERT results in telomere erosion that is different for wt-TERT expression. More tissues need to be analysed, and potentially also more than just two animals. This is an important control to demonstrate the different effects of wt and catalytic inactive tert on telomere lengths."

ANSWER: We agree with the reviewer and, in the revised manuscript, we have included the analysis of more tissues in the TERT-DN treated mice, namely lung and muscle (see new Fig 5F and new Fig. S11). The new data supports that the percentage of short telomeres is rescued with the TERT-WT but not with the TERT-DN.

Reviewer #2

Summary

REVIEWER: "De Jesus and co-workers investigate the consequences of AAV-induced TERT expression on lifespan of wild type mice. The authors describe a positive effect of TERT expression on the maintenance of bone and the function of various tissues including motor activity. On the molecular level the authors propose that TERT's function in telomere maintenance is required for these effects since overexpression of a mutant form of TERT (which cannot maintain telomeres) does not exhibit lifespan prolonging effects. The beneficial effects of TERT expression do not associate with an increased risk of cancer formation.

My main concern is the lack of mechanistic insight on how AAV-mediated TERT expression can elongate the lifespan of wild type mice. The author's conclusion on telomere length is not substantiated by any data on telomere dysfunction, DNA damage signalling, apoptosis, senescence, etc. Moreover, most of the beneficial effects of TERT expression are described in postmitotic cells (motoneurons) or slow dividing tissues (bone). In contrast work on telomerase mutant mice revealed that high-turnover organs are most strongly affected by premature aging as a consequence of telomere shortening (see work from DePinho and colleagues). Together, these findings argue against a telomere dependent effect in the current study. Thus, the mechanism of TERT-AAV on lifespan remains elusive. The authors show some evidence for Wnt/b-catenin activation in TERT-AAV treated tissues. Could this be involved? Maybe activation of Wnt could be a good approach to decipher the mechanism. Lacking such mechanistic inside the results remain too descriptive for a molecular journal. It may then be better to aim for publication in more medical oriented journals."

ANSWER: We are surprised by the commentaries of this reviewer, which seems to have missed Figure 5. In this Figure, and as requested by the reviewer, we clearly show that TERT treatment extents mouse longevity through its effects on the canonical telomere-elongation pathway rather than on its telomere-independent effects on Wnt target genes. In particular, treatment with a catalytically dead DN-TERT does not rescue short telomeres, and does not increase mouse longevity even though it is still able to upregulate Wnt target genes (see Fig. 5). This is the first time that is ever demonstrated that TERT over-expression but not the over-expression of a catalytically dead TERT is able to extent mouse longevity. These results are unprecedented and demonstrate that the effect of TERT in delaying aging and extending longevity are dependent on the canonical telomere elongation pathway by telomerase and cannot be achieved by the telomere-independent effect of TERT on the Wnt pathway.

Reviewer #3

Summary

REVIEWER: "This paper has findings that are potentially very important."

ANSWER: We sincerely thank the reviewer for the detailed review of our manuscript and for considering that "this paper has findings that are potentially very important". The reviewer also has a number of questions and insightful suggestions for changes in the manuscript, which we have fully addressed in a revised manuscript. In particular:

Major concerns

REVIEWER: 1) "As expected, AAV9-mTERT-DN treatment increased CyclinD1 expression" needs a reference or other explanation as to why this was expected. Also need to explain why p16 was analyzed."

ANSWER: As suggested by the reviewer, we have now included the corresponding reference showing that TERT-DN increases the expression of the CyclinD1 Wnt target gene (Park et al, *Nature*, 2009) (page 12, line 21).

The p16 levels were determined to understand the effects of TERT treatment on this bona fide senescence marker (Collado et al. *Cell*, 2007), which has been described to be highly up-regulated associated to mouse aging (Krishnamurthy et al, *Nature*, 2006; Molofsky et al, *Nature*, 2006). We have now included a sentence in the revised manuscript (page 11, lines 22-27).

REVIEWER: 2) "Figure 1D: RNAase makes no difference to the assay result for each of the eGFP constructs. Why is this?"

ANSWER: The RNase treatment is a negative control for the specificity of the TRAP assay, as telomerase activity is dependent on the telomerase RNA component. In mice treated with AAV9-eGFP, there is no detectable TRAP activity in the lungs from 2-year old mice, therefore, treatment with RNase does not change the negative result. In contrast, when mice are treated with AAV9-mTERT, there is a TRAP-positive signal and RNase treatment leads to a disappearance of the signal demonstrating the specificity of the signal.

REVIEWER: 3) "Figure 2B: "time of death" needs explanation in figure legend. How long after injection was the measurement made?"

ANSWER: Time of death means that we measure that parameter immediately post-mortem (Fig. 2B). This information is now included in the revised Figure legend (page 37, line 19, 20).

REVIEWER: 4) "Figure 2C: legend needs to state timing of insulin levels (e.g. specify time after feeding)."

ANSWER: We have included this information in the figure legend (page 37, line 24).

REVIEWER: 5) "Figure 4 (and other figures): % short telomeres is defined relative to mean, which is variable, so doesn't seem to me to have biological meaning. If the mean length is much shorter in one condition, then telomeres that are below 50% of the mean will be shorter than telomeres that are 50% below a larger mean. Can the data be recalculated in such a way so that "short telomeres" is defined relative to a common standard to allow comparison between treatments?"

ANSWER: The telomere length was acquired and calculated for each organ separately and the percentage of short telomeres was determined as the % of telomeres below 50 % of the mean intensity of the corresponding control (which are 1 year old mice treated with AAV9-eGFP; this information has been added at page 38, line 19 and page 39, line 25). In this way (using a constant threshold within each experimental set), we can compare between treatments, as requested by the referee. In the revised figure we have re-calculated and updated the percentage of short telomeres following strictly this threshold, as suggested by the referee.

REVIEWER: 6) "Figure 5. Is the DN construct known to act as a dominant negative in mouse tissues? In other words, does it decrease the activity of endogenous telomerase? Could this explain the result in panel 5F where the mutant TERT constructs results in an increase of the short telomeres?"

ANSWER: This is the first time that this construct is expressed *in vivo*. It was previously described to have a dominant negative effect on telomerase activity *in vitro* (Sachsinger, Cancer Research, 2001), in a murine kidney tumour cell line (RenCa). However, while RenCa cells are positive for telomerase, this is not the case for the majority of adult mouse tissues, therefore a dominant negative

effect should not be very predominant. In the revised manuscript, we have recalculate and updated the short telomere data in Figure 5 (see answer to point 5), following the suggestion by the reviewer. In spite of a trend to have higher % of short telomeres in the mice treated with AAV9-mTERT-DN, this did not reach statistical significance compared to the controls. We now describe the TERT-DN construct in the revised manuscript (page 12, lines 19,20).

REVIEWER: 7) "Figure S3 and elsewhere: shouldn't mean + SD be average {plus minus} range when n=2?"

ANSWER: The errors bars have been added to the referred graphs.

REVIEWER: 8) "Figure S3 - D: can a better gel be supplied?"

ANSWER: We don´t have a clearer gel for the requested result. However, although the gel presents some background, we can clearly identify the positive signal in TERT infected tissues, in comparison to the negative controls injected with the AAV9-eGFP vectors.

REVIEWER: 9) "Figure S6-C: please explain in the legend what degenerative lesions/inflammatory pathologies were scored."

ANSWER: The degenerative lesions and inflammatory pathologies were scored by our pathologist using the same criteria as that previously used in Tomás-Loba, Cell, 2008):
"Senil lesions / Infections:
severe infections:
GI tract: enteritis, gastritis, peritonitis
Skin: dermatitis
degenerative lesions of the GI tract: muscular atrophy and associated lesions (peritonitis, enteritis).
other degenerative pathologies related to normal aging: benign neoplasias (adenoma, hemangioma, lipoma) or degenerative lesions in the intestine (atrophy of the small and large intestine), kidney (glomerulonephritis, tubular degeneration), spleen (atrophy, hemosiderosis, myeloid and lymphoid hyperplasia), liver (congestion, vacuolar degeneration, microgranuloma), testis (atrophy, ectasis of seminal vesicles), ovary (atrophy), uterus (cystic endometrial hyperplasia), skin (hyperplasias, inflammatory processes), lung (trombosis, congestion, fibrosis), heart (congestion, cardiomyopathy) or brain (calcification)."

In the revised Figure legend (SOM page 3, line 20), we now direct to the revised Materials and Methods section (page 20, 21, line 23-5).

REVIEWER: 10) "Figure S7B and elsewhere: "nucleous" should be "nuclei". Can statistical analyses be supplied comparing the histograms?"

ANSWER: We have corrected nucleous to nuclei in the Figures. Statistical analysis has been also included in the revised Figure.

REVIEWER: 11) "Figure S7F: statistics?"

ANSWER: Statistical analysis has been included.

REVIEWER: 12) "Figure S11: need to state at what age the mice were injected."

ANSWER: We use in these experiments 1 yr old mice. This information has been included in the figure legend (SOM page 6, line 1).

Minor concerns

REVIEWER: "Abstract: "expressing telomerase" should be "expressing TERT".

"Associated to" in multiple locations throughout the manuscript should be "associated with". "Owe to" in several locations in the MS should be "owing to". Vg presumably means "viral genomes" and should be defined.

p.6: I recommend changing "overbearing" to "large"

p.6: "telomerase TRAP activity" should be "telomerase activity as measured by TRAP assay"

"Kaplan-Meyer" (several locations) should be "Kaplan-Meier"

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p.11: "reflect on" should be "reflect"

p. 16: "and a plasmid carrying the adenovirus helper functions (kindly provided by K.A. High, Children's Hospital of Philadelphia)" repeats the previous clause and should be deleted.

p. 17 "two consecutives cesium chloride gradients, dyalized" should be "two consecutive cesium chloride gradients, dialyzed"

p. 18: "Bone mineral density (BMD) indicates the density of minerals in mice bones" can be deleted."

ANSWER: All these minor points have been corrected in the revised manuscript.

2nd Editorial Decision 19 March 2012

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed report from the referee who re-assessed it. As you will see the reviewer is now supportive for publication and I am pleased to inform you that we will be able to accept your manuscript pending the following editorial final amendments:

- I am afraid that at 67,000 characters, the manuscript greatly exceeds our limit (60,000 characters including spaces) and I would appreciate if you could try to shorten it before we can proceed. You may choose to take advantage of the fact that we allow the presentation of any peripheral data and materials and methods in the form of Supplementary Information, to be published online alongside the article (materials and methods essential to the repetition of experiments described in the main body of the manuscript may not be presented in this way).

- Please provide up to 5 keywords

We would appreciate if you could submit your revised manuscript within two weeks.

I look forward to reading a new revised version of your article as soon as possible.

Yours sincerely,

Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System):

The authors have adequately addressed my initial questions and concerns. I recommend this important and novel study for publication in EMBO Molecular Medicine