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Loss of MT1-MMP causes cell senescence and nuclear defects which can be reversed by retinoic acid

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

Editor: Thomas Schwarz-Romond

1st Editorial Decision 18 December 2014

Thank you very much for submitting your study that reports novel phenotypes in MMP14-deficiency mice for publication in The EMBO Journal.

Two expert scientists commented on general advance and interest of your data. The comments enclosed below are very encouraging, but demand further experimentation as to substantiate the current conclusions (please refer to the requests from ref#1).
In line with ref#2's second point to significantly elaborate on the senescence phenotype, I strongly encourage you to attempt potential further molecular links/additional mechanistic insights into this MMP14-driven phenomenon. I am aware that this appears challenging. I am sure you have to agree however, that this is a direction to significantly boost general impact and confidence in your very novel and relevant results.

Please do not hesitate to get in touch regarding particularly this issue, its feasibility, experimental timeline, respective already available and further-reaching data you may want to include during necessary revisions of this work.

Conditioned on this, I am delighted to offer you the opportunity to submit a revised study and am

happy to engage in the timely development/eventual publication of your results at The EMBO Journal.

Please note, that I formally have to remind you that The EMBO Journal allows only one single round of major revisions.

I thank you very much for considering The EMBO Journal for presentation of your data and remain with my best regards.

REFEREE REPORTS:

Referee #1:

The authors have deeply studied tissues and cells from a MT1-MMP (MMP14) knockout mouse, which dies young and exhibits 'progeria like' defects at the tissue level, including heart, muscle, bone development. At the cell and molecular level, there are evident changes in lamin-A levels and the linker proteins to cytoskeleton, namely increases. Based on cited work of others (namely Swift et al 2013) that shows also lamin-A increases with matrix stiffness, the authors seem to suggest that MMP14 helps to degrade and soften matrices in ways that contribute to not just lamin-A levels but also to DNA repair and proliferation. The authors provide compelling evidence that digestion of ECM by MMP14 in vitro is essential to giving a wild-type cyto/nucleo-skeletal organization. Since Swift et al also showed all-trans retinoic acid (ATRA) could also (down)regulate lamin-A levels and influence cell fates (at least differentiation), the authors examined ATRA both in vitro and in vivo and demonstrated partial rescue of the MMP14-/- phenotypic defects. Overall, this manuscript provides insightful and timely findings regarding matrix remodeling by MMP14 and cyto/nucleo-skeletal structures as well as the potential utility of clinically relevant compounds such as ATRA. A number of corrections or clarifications are needed for this otherwise interesting paper:
1. In the Introduction, the sentence starting at the bottom of pg.4 needs to be re-written from "It is also remarkable ... " to the more accurate sentence "It is also remarkable the recent finding that ECM stiffness INCREASES the relative abundance of lamin A through a process modulated by the retinoid receptor signaling pathway (Swift et al, 2013)." The mechanosensing mechanism at the protein has been further elaborated in Buxboim et al, Current Biology 2014, which should be cited.
2. Images or image sets need scale bars.
3. Page 6, last paragraph: 'thickened muscular wall of the right ventricle and ...', should include an arrow in figure 1A to indicate this thickening.
4. Page 6, last paragraph: for a broader readership, is accumulation of type I collagen shown by picosirius red?
5. Page 7, first paragraph: indicate in more detail, what Sudan Black and SA-B-Gal show?
6. Page 11, gH2AX results: this seems extraneous, as it is not studied elsewhere or mechanistically. For example, is this occurring in vivo or is a standard 2D culture somehow causing DNA damage?
7. Page 12, last paragraph: vimentin immunostaining data seems insufficient for the authors to conclude that over-expression of wild-type MMP14 rescues cell integrity or cytoskeletal abnormalities. The lack of quantitative image analysis is also a problem (see 13,14 below).
8. The bottom of pg.13 refers to 'retinoic acid receptor beta' but the cited reference shows nuclear translocation of a different isoform in mesenchymal stem cells. Shin et al, PNAS 2013 added some generality by showing similar effects on CD34 hematopoietic cells, for which their cited references implicate different RAR isoforms (eg. RARA). This attention to RAR isoforms could be as pertinent to understanding the ATRA pathway as is the different isoforms of MMPs or lamins in the response.
9. Is it known whether any other MMPs change with ATRA?
10. Fig 1B: it seems that the 100x is a crop of the 40x image, but the actual 100x image should be provided.
11. Fig 1F: the figure legend indicates triplicates, and so error bars of the repeats should be included. Is this plot implying that it starts with 10 cells? Clearly state whether cells are passaged every "3 days".
12. Fig 2G: the figure legend indicates maximum projection for all of the images, why does the lamin-A/C staining look different between the various MMP14+/+ samples, especially for the

vimentin samples compared to the others?
13. Fig 3A: is this done on ECM substrate as well? Please indicate in figure legend and in text.
14. Fig 3A: Are there cases where vimentin is totally absent from the nuclear region? Figure S3 shows traces of vimentin staining around the nucleus region for MMP14-/- sample, and so the authors should quantify the staining and decide a threshold to decide whether the nucleus contains fibre or not.
15. Fig 3B: As these are confocal imaging, actual or projection or reconstruction of the XZ images will be great to show the location of vimentin around the nucleus.
16. Fig 3C: Simple population count of the rescue will be very informative.
17. Fig 4 and 5: The aim of the data is to show the rescue by addition of ATRA, please provide statistical significance for the MMP14-/- with and without ATRA.
18. Fig 4A: How do the authors distinguish whether a nucleus is normal or abnormal? Please provide more explaination.
19. Fig 5B: Please provide error bars as these are from n=13, maybe shadings to show the error?
20. Fig 5D: Please provide arrows to show region of interest.
21. Fig. 5H: it seems that the 100x is a crop of the 40x image, will it possible to provide the actual 100x image?

Referee #2:

A new mouse model deficient in membrane-bound MT1-MMP (MMP14) was engineered. The molecular, biochemical and phenotypic pathologies of the model were studied in detail. As previous mouse models (Holmbeck et al, 1999; Zhou et al, 2000), these mice develop several skeletal abnormalities but in addition they show additional alterations in non-skeletal tissues. I am not an expert in Matrix metalloproteinase animal models, therefore I can't comment on the novelty and significance of the newly engineered mouse model. Meanwhile, this is a potentially interesting study because it shows that in vivo administration of Retinoid acid can improve lifespan and some abnormal phenotype in this mouse model by reversing a senescence program triggered by MMP14 deficiency. These observations underline a connection between the extracellular matrix and the nucleus shape that might regulate a senescence response.
There are, however, several methodological flaws, problems with data Analysis and presentation that weaken this manuscript.

1- If this knock out mouse represents an internal deletion of MMP14, why the authors show a complete absence of the protein by WB?
2- The senescence phenotype should be documented in deep, as this constitutes the key observation of the paper. A large scanning of images like those presented figure 1 A-C, should be performed with different animals from each genotype. WB p16 measurement should be performed with other controls than b-actin (a better control could be the genomic DNA) to make sure that the same number of cells was analysed.
3- It is not clear either what internal controls were used to establish the expression levels of the various mRNAs fig. 2 C and D. The WB quantitation should be done as before. Scale bars should be added to fig. 2G. Why only one cell is shown, where are the cell boundaries? This figure is not convincing at all and wider field should be shown together with cell shape.
4- P11, sup S2G what is the rational for testing integrin v, as this integrin is not involved in collagen binding?
5- Statement in P13 about results in Fig 3B, needs to be documented by images of the collagen ECM. Are there differences in structure and/or total amounts? Also there is no causal relationship that has been proven experimentally to state that deficient ECM remodelling is responsible for the cellar senescence phenotype.
6- There is no evidence in the paper to support the last sentence of the result section and therefore should be moderated. The same applies for the first sentence of the discussion section.

1st Revision - authors' response 02 March 2015

Referee #1:

We thank this reviewer for his/her encouraging comments on our work. His/her criticisms have been addressed as follows:

1. In the Introduction, the sentence starting at the bottom of pg.4 needs to be re-written from "It is also remarkable ... " to the more accurate sentence "It is also remarkable the recent finding that ECM stiffness INCREASES the relative abundance of lamin A through a process modulated by the retinoid receptor signaling pathway (Swift et al, 2013)." The mechanosensing mechanism at the protein has been further elaborated in Buxboim et al, Current Biology 2014, which should be cited.

We agree with the reviewer's comment, and following his/her indication the paragraph has been re-written and the indicated reference has been introduced.

2. Images or image sets need scale bars.

Scale bars have been introduced in the images.

3. Page 6, last paragraph: 'thickened muscular wall of the right ventricle and ....', should include an arrow in figure 1A to indicate this thickening.

As suggested by the reviewer, we have introduced arrows on Figure 1A to indicate the thickening of the ventricle wall.

4. Page 6, last paragraph: for a broader readership, is accumulation of type I collagen shown by picosirius red?

We apologize for not being more specific about this issue and a sentence to describe the function of picrosirus red staining has been introduced in page 7 of the revised manuscript.

5. Page 7, first paragraph: indicate in more detail, what Sudan Black and SA-B-Gal show?

We have now included in the text a brief explanation regarding the specificity of both techniques to make more understandable the observed effect.

6. Page 11, gH2AX results: this seems extraneous, as it is not studied elsewhere or mechanistically. For example, is this occurring in vivo or is a standard 2D culture somehow causing DNA damage?

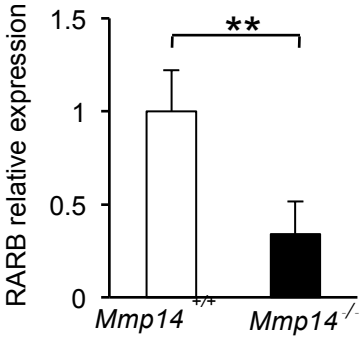
As shown in Figure 1, during our initial characterization of the senescent phenotype of *Mmp14*-deficient animals, we analyzed different independent markers of senescence, including the presence of DNA damage, measured by a standard technique such as the accumulation of histone H2Aγ foci, to demonstrate the presence of this previously uncharacterized process in *Mmp14*-deficient mice. Once the senescent phenotype was confirmed by different independent techniques, for subsequent experiments we did not use all these initial techniques, but only a subset of them. Regarding the specific question on whether this was done *in vivo* or in culture, the performance of this antibody on fresh or fixed tissue is very poor, and therefore, for this particular experiment, we used cultured fibroblasts on a collagen matrix.

7. Page 12, last paragraph: vimentin immunostaining data seems insufficient for the authors to conclude that over-expression of wild-type MMP14 rescues cell integrity or cytoskeletal abnormalities. The lack of quantitative image analysis is also a problem (see 13,14 below).

We had performed a quantitative analysis of the percentage of fiber-containing nuclei as shown in Figure 3A, right panel, in which it is shown that over-expression of wild type *MMP14* construct in *Mmp14*$^{-/-}$ fibroblasts, partially rescued the cytoskeleton structure (60% vs 25%). In addition, we had also shown that the proteolytic activity of MT1-MMP was required for this process, as over-expression of a mutant construct (E217Q) failed to significatively rescue the described phenotype. Nevertheless, and following the reviewer's comments, we have modified the sentence in the revised text (pg. 13) stating that exogenous MT1-MMP expression "partially rescues the cytoskeletal abnormalities", that it is more accurate according to the results presented.

8. The bottom of pg.13 refers to 'retinoic acid receptor beta' but the cited reference shows nuclear translocation of a different isoform in mesenchymal stem cells. Shin et al, PNAS 2013 added some generality by showing similar effects on CD34 hematopoietic cells, for which their cited references implicate different RAR isoforms (eg. RARA). This attention to RAR isoforms could be as pertinent to understanding the ATRA pathway as is the different isoforms of MMPs or lamins in the response.

We have analyzed the expression of different RAR isoforms, finding that RARB expression is altered. This result suggests that alteration of the functional structure of the nuclear envelope by MT1-MMP could lead to changes in the RARs gene regulatory pathway affecting the expression of different genes implicated in the normal function of the cell. If the reviewer or the editor considers that it is necessary to include these data in the revised manuscript, we would be glad to do it.



9. Is it known whether any other MMPs change with ATRA?

There are a number of evidences in the literature that ATRA can directly modulate the expression of MMPs in different cell types, either suppressing or activating their expression (i.e. Jimenez et al., J. Cell Biol 2001; Dutta et al., Cell Adh. Migr. 2010; Zhang & Ross, J Cell Biochem 2012; Rankin et al., Br J Pharmacol 2013). We have included these selected references in the revised manuscript.

10. Fig 1B: it seems that the 100x is a crop of the 40x image, but the actual 100x image should be provided.

Due to the small size of the panels, and in order for the reader to appreciate the staining on individual cells, we originally included a zoomed version of the selected area shown on the 40x panel (indicated by the dashed white square). We have now re-labeled this panel “Detailed view” instead of 100X to avoid confusion.

11. Fig 1F: the figure legend indicates triplicates, and so error bars of the repeats should be included. Is this plot implying that it starts with 10 cells? Clearly state whether cells are passaged every "3 days".

We thank the reviewer for pointing out this question, as we have realized that although the graph was correct, the labeling of the y axis was not appropriate and gave rise to misinterpretation. The experiment started with one million cells, which were passaged every 3 days. The plot is expressed as the base 2 logarithm of the number of million cells, which means that the scale mainly reflects the number of divisions. We have now corrected this error in the revised Figure 1H. We have also mentioned both in the figure legend and in the main text, the number of days between passages.

12. Fig 2G: the figure legend indicates maximum projection for all of the images, why does the lamin-A/C staining look different between the various MMP14+/+ samples, especially for the vimentin samples compared to the others?

The different staining observed for lamin A/C in this panel derives from the fact that we had to use two different lamin A/C antibodies (goat anti-lamin A/C sc-6215 and rabbit anti-lamin A/C sc-20681) due to incompatibilities with the hosts of the other antibodies used for the immunofluorescence experiments. As the main purpose of this experiment was to show the differences between wild type and *Mmp14*-deficient cells, the comparison should be made between both genotypes, as in both cases the same set of antibodies were used.

13. Fig 3A: is this done on ECM substrate as well? Please indicate in figure legend and in text.

The fibroblasts shown on Figure 3A had been obtained by culturing on a collagen matrix. We have now included a sentence in the main text as well as in the figure legend to clarify this important point.

14. Fig 3A: Are there cases where vimentin is totally absent from the nuclear region? Figure S3 shows traces of vimentin staining around the nucleus region for MMP14-/- sample, and so the authors should quantify the staining and decide a threshold to decide whether the nucleus contains fibre or not.

We agree with the reviewer that in some cases there are traces of vimentin staining in the *Mmp14*-deficient fibroblasts. In this regard, we must emphasize that this is an "*in vitro*" model and many cells may contain minor alterations in the cytoskeleton, increasing the variability between samples. Although the establishment of a threshold could be somehow arbitrary, we decided to set a cutoff which could represent the most dramatic change in phenotype, counting only those cells that had short actin fragments around the nucleus, disturbed or collapsed perinuclear vimentin network organization or the absence of fibers over the nuclei (*Lombardi et al, J Biol Chem, 286(30): 26743*). Based on our observations, it is very likely that by setting a different cutoff, we would have reached similar conclusions as those shown in this manuscript. We have now included in the revised figure legend and material and methods section an explanation to clarify this question.

15. Fig 3B: As these are confocal imaging, actual or projection or reconstruction of the XZ images will be great to show the location of vimentin around the nucleus.

A new image with the location of the vimentin staining around the nucleus in control conditions has been introduced in the revised Figure 3B.

16. Fig 3C: Simple population count of the rescue will be very informative.

To provide more information about the effect of the ECM on the cytoskeleton rescue, we have now included an inset in Figure 3D showing the percentage of cells rescued in this experiment.

17. Fig 4 and 5: The aim of the data is to show the rescue by addition of ATRA, please provide statistical significance for the MMP14-/- with and without ATRA.

Figure 4D showed a quantification of the number of nuclei containing fibers without and after ATRA treatment, reflecting that ATRA treatment partially rescued the cytoskeleton alterations observed in *Mmp14*-deficient fibroblasts (27% *vs.* 65%), including also the statistical significance. Figure 5 showed the effect of ATRA on other different aspects, such as cell survival. The statistical significance had only been included in the Figure legend, but now, we have also included this parameter in the revised Figure 5A.

18. Fig 4A: How do the authors distinguish whether a nucleus is normal or abnormal? Please provide more explanation.

In the case of *Mmp14*-deficient animals, we had observed that the cell nucleus was different from that of wild type animals. Most of the anomalies observed in these animals included alterations previously described in other physiological or pathological contexts, including ageing, progeroid syndromes, or alterations of nuclear lamina components. These abnormalities included irregular shape nuclei, more ellipsoid and presence of alterations including the formation of structures called nuclear blebs, defined as protrusions from the nuclear surface, as well as nuclear herniations (Shimi et al., Genes Dev. 2008; Ostlund et al., J Cell Sci. 2009; revised in Gordon et al., Cell 2014). Therefore, we specifically looked for those alterations and a certain nucleus was classified as abnormal if these aberrations were present at significant levels. We have followed these criteria in all previous studies from our laboratory with different accelerated aging syndromes (Varela et al., Nature 2005; Varela et al., Nature Med 2008; Espada et al., J Cell Biol 2008; Puente et al., Am J Hum Genet 2011; Osorio et al., Genes & Dev 2012; de la Rosa et al., Nature Commun 2013).

19. Fig 5B: Please provide error bars as these are from n=13, maybe shadings to show the error?

The error bars have been indicated in the Figure.

20. Fig 5D: Please provide arrows to show region of interest.

The arrows have been included in the corresponding figures to point out the presence of cartilage (blue) in *Mmp14*-deficient mice, indicating a delay in the bone formation process, whereas this cartilage has been replaced by mineralized tissue (red) in control mice.

21. Fig. 5H: it seems that the 100x is a crop of the 40x image, will it possible to provide the actual 100x image?

Similar to point 10, this image corresponds to a zoomed view of the selected area of the 40x micrograph (indicated by the dashed white square). We have now renamed that panel to avoid this confusion.

Referee #2:

We also thank this reviewer for his/her encouraging comments on our work. His/her criticisms have been addressed as follows:

1- If this knock out mouse represents an internal deletion of MMP14, why the authors show a complete absence of the protein by WB?

We have performed Southern blot analysis to demonstrate the expected deletion of exons 4 and 5 of *Mmp14*, and Western blot to show that no MT1-MMP protein was produced in homozygous *Mmp14*$^{-/-}$ animals or cells. Regarding the question raised by the referee about the possibility of an in frame splicing between exons 3 and 6 when exons 4 and 5 are deleted, we had selected those two exons because they encode the catalytic domain of MT1-MMP, and because exons 3 and 6 are not in the same reading frame (see scheme below). In addition, no MT1-MMP can be detected in KO tissues when using an antibody against the C-terminal domain, and the phenotype of mutant mice is very similar to other *Mmp14*-deficient mice previously generated by deletion of additional exons. Collectively, these data are clearly indicating that this targeting strategy eliminates any active MT1-MMP. To further clarify this question, we include below a scheme showing the reading frame of the 3'-end of exon 3, as well as the 5'-end of exon 6. As discussed above, even if splicing between both exons was generated by the deletion of exons 4 and 5, the reading frame for exons 3 and 6 is different, preventing the production of a functional MT1-MMP protein, as we have demonstrated by Western blot analysis.

Exon 3 **Exon 6**

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...ATC ACT TTC TGgtg.....cagGA AGC AAG TCA...
   I   T   F                 G   S   K
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2- The senescence phenotype should be documented in deep, as this constitutes the key observation of the paper.

We agree with the reviewer that the key observation of this study is the existence of a senescent phenotype generated by deletion of the extracellular matrix protease MT1-MMP. By this reason, we had originally performed different experiments commonly used in the literature to prove the existence of a senescence phenotype both in tissues as well as in isolated cells (Muñoz-Espin et al., Cell 2013; Storer et al., Cell 2013). These different approaches included analysis of senescence markers p16, p21, DNA damage as revealed by the presence of H2AX foci, SAP phenotype (IL-6 and CXCL-2), alterations in the somatotroph axis and SA-B-gal activity. These markers are widely associated with senescent phenotypes observed in other animal models, and they reflect both cell-intrinsic and cell-extrinsic processes usually altered by senescence. In all cases, the alterations observed in *Mmp14*-deficient animals were in full agreement with this senescence phenotype. Nevertheless, to follow the reviewer's recommendation, we have performed additional experiments aimed at supporting this claim. These experiments include BrdU incorporation assays to demonstrate the lack of proliferation in *Mmp14*-deficient cells besides the increase in SA-B-gal activity. We have also performed immunofluorescence assays of HP1g, a heterochromatin-associated protein that it is enriched in the foci of senescence cells. The results obtained from these new experiments have been now introduced in the revised Figures 1D and 1E, and in both cases the results are consistent with our proposal that lack of MT1-MMP causes a senescence phenotype. We have also included and discussed these data on page 7 of the revised manuscript.

A large scanning of images like those presented figure 1 A-C, should be performed with different animals from each genotype.

The images presented on Figures 1A-C constitute representative micrographs of the phenotype observed in animals from each genotype. It is specified in the main text that we have performed these experiments with three animals per genotype for both SA-B-gal staining and Sudan Black. However, the inclusion of additional figures for different animals would dramatically increase the density of this multipanel figure that we already consider to be very dense. So, due to space restrictions, we have kept only a representative image in the revised version of this

manuscript. Nevertheless, if the reviewer or the editor consider that it is necessary to include those additional figures either as part of Figure 1 or as supplementary figures, we would be glad to include them.

WB p16 measurement should be performed with other controls than b-actin (a better control could be the genomic DNA) to make sure that the same number of cells was analysed.

We thank the reviewer for this comment. Regarding the internal control used in the case of Western-blot analysis of p16, we first quantified the total amount of protein by using the colorimetric assay BCA (Bicinchoninic acid) to ensure that the same amount of protein has been loaded in all samples. For the figure, we also performed an internal control using b-actin to normalize the expression of p16. A densitometry to normalize the activity is also provided in Figure 1D.

3- It is not clear either what internal controls were used to establish the expression levels of the various mRNAs fig. 2 C and D.

We apologize for not making this point sufficiently clear to the reviewer. To normalize the expression of mRNA, a GAPDH probe was used, and this information was included in the Material and Methods section. We have now included also this important information in the revised figure legend.

The WB quantitation should be done as before. Scale bars should be added to fig. 2G.

The quantitation for this Western-blot was performed as before and now the scale bars have been introduced in the revised Figures 2G.

Why only one cell is shown, where are the cell boundaries? This figure is not convincing at all and wider field should be shown together with cell shape.

In relation to this comment, our aim was to show, with maximum detail, the alterations in the cytoskeleton structure that we described in the text. For this purpose, we captured images at 100x magnification, where only 1 or 2 cells can be seen. We have analyzed more than 100 cells from different random fields to show in Figure 2G a representative image. Moreover, in Figures 3D and 4E, as well as in Supplemental Figure S3, we show a wider field with more cells. However, we understand the reviewer´s concern about showing only one cell in Figure 2G. We have performed additional experiments to capture images at lower magnification, where a wider field with more cells is shown, together with a 100x magnification with a single cell to clearly appreciate the altered structure. These new images are included in Figure 2G.

4- P11, sup S2G what is the rational for testing integrin αv, as this integrin is not involved in collagen binding?

We performed a broad analysis of different integrins to find out whether these receptors were involved in the described phenotype; with the finding that integrin αv exhibited an altered expression pattern in our system. Although the result is interesting, we agree with the reviewer that due to the fact that this integrin is not involved in collagen binding, there is no clear rationale to introduce these data and we have decided to remove this supplemental Figure from the revised manuscript.

5- Statement in P13 about results in Fig 3B, needs to be documented by images of the collagen ECM. Are there differences in structure and/or total amounts? Also there is no causal relationship that has been proven experimentally to state that deficient ECM remodelling is responsible for the cellar senescence phenotype.

We have addressed this important question raised by the referee as described in supplemental Figure 1G and by using two different approaches. First, we have performed an *in vitro* degradation assay using a collagen matrix with *Mmp14*$^{+/+}$ and *Mmp14*$^{-/-}$ fibroblasts observing that there is an important reduction in the ability of mutant fibroblasts to degrade the collagen matrix. Second, we have also performed a Sircoll assay (right panel), a dye-binding method that is suitable for monitoring collagen production during *in vitro* cell culture and *in vitro* extracellular matrix formation, with the finding that *Mmp14*-deficient cells grown on collagen matrix have an impaired collagenolytic activity.

6- There is no evidence in the paper to support the last sentence of the result section and therefore should be moderated. The same applies for the first sentence of the discussion section.

Following the reviewer's recommendation, we have now modified these sentences to tone them down.

2nd Editorial Decision 24 March 2015

Thank you very much for your revised study that has been re-evaluated by one of the original referees

I am please to inform you that we are in principle ready to accept for publication. Before formal acceptance, I kindly ask you to provide the following items:

- F1A-C+E, 2A+F, 3A, 4A+B, 5D+E seem to miss scale bars; please add (the same applies to some of the supplementary figures, but I leave this to your discretion)

- the referee requests inclusion of further images for different animals from each genotype: please add either as supplement OR as one of the source data files (either way will be acceptable to us); we may run this for consistency briefly with ref#2 for approval.

- the text file needs an author contribution statement

Please also note that The EMBO Journal encourages the publication of SOURCE DATA, particularly for electrophoretic gels/blots, with the aim to make primary data more accessible and transparent to the reader. This entails presentation of un-cropped/unprocessed scans for KEY data of published work. We encourage you to participate in this initiative. Please provide one PDF-file per figure with this information.

-Please also send us a 2 up to 4 'bullet point' synopsis, that highlights major novelty/advance/significance of your study.

-An integrating figure/graphical abstract in the format 550 x 150 (max 400) pixel would facilitate featuring your study in eToC's and our homepage.

I am very much looking forward to receive the relevant files/ultimate amendments to your study before timely presentation in The EMBO Journal.

REFEREE REPORT:

Referee #2:

The current version is significantly improved and the authors have addressed satisfactorily all but one of my original concerns:

1) The authors should have included the images requested in my previous review in a supplemental figure that I can appreciate them before publication.

2nd Revision - authors' response 15 April 2015

Please find enclosed the final revised version of our manuscript entitled ìLoss of MT1-MMP causes cell senescence and nuclear defects which can be reversed by retinoic acidî by Gutierrez-Fernandez et al. (Manuscript number EMBOJ-2014-90594R - Decision). Following your indications, we have

now provided the scale bar to the indicated figures, including the Supplementary Material.
Regarding the referee¥s request, we have included further images of Figure 1 A-C of two more mice for each genotype. This new information has been included as Supplementary Figure 2 in the revised manuscript.