EMBO reports

The L27 Domain of MPP7 enhances TAZ-YY1 Cooperation to Renew Muscle Stem Cells

Anwen Shao, Joseph Kissil, and Chen-Ming Fan

Corresponding author(s): Chen-Ming Fan (fan@carnegiescience.edu)

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Transaction Report:

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Dear Dr. Fan,

Thank you for the submission of your manuscript to EMBO reports. I have received the reports from two the three referees that were asked to evaluate your study, which can be found at the end of this email. A third referee agreed to evaluate the study, but never submitted a report, despite several reminders from our side. I thus decided to proceed with the two reports I have.

As you will see, the referees have several comments, concerns, and suggestions, indicating that a major revision of the manuscript is necessary to allow publication of the study in EMBO reports. As the reports are below, and all the concerns need to be addressed, I will not detail them further here.

Given the constructive referee comments, I would like to invite you to revise your manuscript with the understanding that the concerns of the referees must be addressed in the revised manuscript and in a detailed point-by-point response. Acceptance of your manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

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When submitting your revised manuscript, we will require:

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2) individual production quality figure files as .eps, .tif, .jpg (one file per figure), of main figures and EV figures. Please upload these as separate, individual files upon re-submission.

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4) a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14693178/authorguide). Please insert page numbers in the checklist to indicate where the requested information can be found in the manuscript. The completed author checklist will also be part of the RPF.

Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines:
http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

5) that primary datasets produced in this study (e.g. RNA-seq, ChIP-seq, structural and array data) are deposited in an appropriate public database. If no primary datasets have been deposited, please also state this in a dedicated section (e.g. 'No primary datasets have been generated and deposited'), see below.

See also: http://embor.embopress.org/authorguide#datadeposition

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability" section (placed after Materials & Methods) that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study. This section is mandatory. As indicated above, if no primary datasets have been deposited, please state this in this section

Data availability

The datasets produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

Moreover, I have these editorial requests:

6) We now request the publication of original source data with the aim of making primary data more accessible and transparent to the reader. Our source data coordinator will contact you to discuss which figure panels we would need source data for and will also provide you with helpful tips on how to upload and organize the files.

7) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at: http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

8) Regarding data quantification and statistics, please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also for EV figures and all those in an Appendix). Please also check that all the p-values are explained in the legend, and that these fit to those shown in the figure. Please provide statistical testing where applicable. Please avoid the phrase 'independent experiment', but clearly state if these were biological or technical replicates. Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant. In case n=2, please show the data as separate datapoints without error bars and statistics. See also: http://www.embopress.org/page/journal/14693178/authorguide#statisticalanalysis

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I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

Yours sincerely,

Achim Breiling
Senior Editor
EMBO Reports

Referee #1:

First, Shao and colleagues demonstrated that MuSC-specific Mpp7 conditional knockout (KO) exhibited impaired regeneration, reduced proliferative capacity, and a decreased number of self-renewed MuSCs. The authors identified the L27-domain of Mpp7 as involved in self-renewal of MuSCs through a rescue experiment using SM. Based on previous data showing the binding of Mpp7 and Amot, the authors focused on interaction between Mpp7 and Amot, finding that Mpp7 and Amot bind to each other via their respective PDZ and PDM domains. Furthermore, the authors showed a similar regenerative defect in Amot-cKO as in Mpp7-cKO.

Next, the authors investigated the genes commonly altered in Mpp7- and Amot-cKO MuSCs and identified Carm1, which methylates Pax7. Interestingly, the authors demonstrated that the forced expression of Carm1 was sufficient to rescue the defect of Mpp7 in MuSCs. During these analyses, the authors noted the presence of YY1 binding sites in the promoters of common target genes for Mpp7 and YAP/TAZ, in addition to TEAD binding sites. They found that: 1) the TEAD binding site is required for Carm1 expression, and Mpp7 and Amot significantly enhance its expression level, and 2) in the active form of Taz, the proportion of Pax7+ Myod+ cells could be rescued in the absence of Mpp7, but not self-renewal. Furthermore, using several mutated proteins, the authors demonstrated 1) the relationship between F-actin and AMOT localization, and 2) that MPP7, AMOT, TAZ, and YY1 form a robust transcription activator complex, essential for Cram1 expression.

Overall, the experiments were well-conducted, and the data were convincing. Meanwhile, there is no conclusive evidence that the formation of this complex is necessary for the self-renewal of MuSCs. The data on self-renewal in vivo could be a secondary effect, as myofiber formation was impaired. The in vitro SM experiment was one experimental system to verify the self-renewal of MuSCs. However, the work is sufficiently novel and interesting as a regulatory mechanism for the expression of critical genes in MuSCs. Therefore, this reviewer recommends publishing this work in EMBO Reports.

Specific comments
1) Comment; During the muscle regeneration process, the proliferation of MuSCs has two phases. The first is the proliferation that peaks on the third day of regeneration, and the second is around the fifth day of regeneration. According to the work of

Olwin's group, self-renewal of MuSCs occurs around day 5. If L27 of Mpp7 has a limited role in the self-renewal of MuSCs, is the complex identified in this study characteristically present in Pax7-positive cells around day 5 of regeneration? As mentioned below, Carm1 is also expressed in differentiating muscle cells. It would therefore be useful for readers if the authors could explain how the complex discovered here functions in self-renewing MuSCs.

https://www.cell.com/iscience/fulltext/S2589-0042(22)00715-5

2) Fig. 2C
What is the possible mechanism by which ΔL27-Mpp7 restored the proportion of Pax7+MyoD+ cells? Does this indicate that the nuclear translocation of YAP shown in previous studies by the authors occurs normally with ΔL27-Mpp7? Could this be confirmed by staining for YAP/TAZ?

3) Fig. 3H
The following study showed that Carm1 was involved in myogenic differentiation. In contrast, current data demonstrated that Carm1 functions as an anti-myogenic factor, as the number of committed myogenic cells (MyoD+Pax7-, perhaps Myogenin+) was reduced. If possible, this reviewer recommends discussing the role of Carm1 in myogenic differentiation processes.

J Biol Chem. 2002 Feb 8;277(6):4324-33. doi: 10.1074/jbc.M109835200

4) Lines 225-229; 'Using TAZ as a representative (for TAZ and YAP), we found that Mpp7 co-expression indeed increased Taz's transcriptional activity on a TEAD-reporter36 (Fig. 4e). Amot alone inhibited Taz, but Amot, Mpp7, and Taz altogether best activated the reporter. Co-IP also revealed an enhanced interaction between MPP7 and TAZ by AMOT (Fig. 4f; Supplementary Fig. S4e).

Comment; The explanations for Fig 4e and Fig 4f are switched, so please correct them.

5)Fig. S5d
Comment; Is there really a significant difference between V5-Taz and V5-Tax S89A?

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Comment; There is a lack of explanation for the result indicating that S175A Amot does not affect self-renewal.

7)Lines 292-293; As such, AMOT is likely the F-actin-responsive conduit for nuclear MPP7 and YAZ/TAZ for MuSC renewal.
Comment; As the authors described, MuSC activation is accompanied by actin rearrangement. However, to the reviewer's knowledge, it is unclear whether the actin rearrangement is required for the self-renewal of MuSCs. If possible, the reviewer recommends adding evidence indicating the necessity of actin rearrangement for the self-renewal of adult stem cells.

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In this manuscript the authors describe a new function of MPP7, AMOT, and to some extent YAP/TAZ in the regeneration of muscle stem cells after injury. This follows the description, in 2017, of the MPP7-AMOT axis. In this manuscript, the authors advocate for the existence of a MPP7-AMOT-TAZ transcriptional complex, for which they provide evidence at the level of protein-protein interactions, cooperation on gene expression (RNAseq and luciferase assays), and similarity of in vivo phenotypes. This complex is not a general regulator of YAP/TAZ function, but only affects a subset of genes, likely because these genes are also regulated by the YY1 transcription factor, binding in the vicinity of TAD/TAZ complexes.

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2) the authors focused on TAZ, but TAZ (at difference with CARM1) GOF is very weak in the MUSC differentiation assay. This could be due to some degree of functional redundancy with YAP. The authors should try YAP in their functional assays (see point 1), also in light of the strong phenotype of YAP/TAZ DKO.

3) The axis with CARM1 is proposed as a main mediator of the end point muscle phenotype. If so, could the authors use the MUSC differentiation assay to probe whether CARM1 GOF also rescue, at least to some extent, YAP/TAZ KO MUSCS? This would e important not to validate/invalidate the model, but rather to gauge its overall strength and importance in the context of the wider biology.

Dear Editor,

We thank you and the reviewers for your time in processing our manuscript. We also very much appreciate the reviewers' comments. Please see our point-by-point response to reviewers' comments below. For easy identification of contents, reviewers' comments are *italicized*.

Referee #1:

First, Shao and colleagues demonstrated that MuSC-specific Mpp7 conditional knockout (KO) exhibited impaired regeneration, reduced proliferative capacity, and a decreased number of self-renewed MuSCs. The authors identified the L27-domain of Mpp7 as involved in self-renewal of MuSCs through a rescue experiment using SM. Based on previous data showing the binding of Mpp7 and Amot, the authors focused on interaction between Mpp7 and Amot, finding that Mpp7 and Amot bind to each other via their respective PDZ and PDM domains. Furthermore, the authors showed a similar regenerative defect in Amot-cKO as in Mpp7-cKO.
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Overall, the experiments were well-conducted, and the data were convincing. Meanwhile, there is no conclusive evidence that the formation of this complex is necessary for the self-renewal of MuSCs. The data on self-renewal in vivo could be a secondary effect, as myofiber formation was impaired. The in vitro SM experiment was one experimental system to verify the self-renewal of MuSCs. However, the work is sufficiently novel and interesting as a regulatory mechanism for the expression of critical genes in MuSCs. Therefore, this reviewer recommends publishing this work in EMBO Reports.

We thank reviewer 1 for the positive recommendation for publishing our manuscript.

Specific comments
1) Comment; During the muscle regeneration process, the proliferation of MuSCs has two phases. The first is the proliferation that peaks on the third day of regeneration, and the second is around the fifth day of regeneration. According to the work of Olwin's group, self-renewal of MuSCs occurs around day 5. If L27 of Mpp7 has a limited role in

the self-renewal of MuSCs, is the complex identified in this study characteristically present in Pax7-positive cells around day 5 of regeneration? As mentioned below, Carm1 is also expressed in differentiating muscle cells. It would therefore be useful for readers if the authors could explain how the complex discovered here functions in self-renewing MuSCs.

https://www.cell.com/iscience/fulltext/S2589-0042(22)00715-5

Thank you for this comment. We have now included additional comments on the timing of renewed quiescent MuSC relevant to our work (ln 123-127); the above reference is also added:
"The majority of renewed quiescent MuSCs are documented to be derived from cell divisions at d 5 and onwards after injury (Cutler et al., 2022). Our data therefore suggest either that the early proliferation defect (1-5 dpi) of *Mpp7* cKO leads to the reduction of renewed quiescent SCs at 21 dpi or that *Mpp7* also acts in later renewal divisions prior to quiescence, and possibly both."

Briefly, the work by Cutler et al. (2022) provided data that after injury, MuSCs that divide (assessed by EdU incorporation) in the time window of d 5-7 contributed to the largest fraction of MuSCs that retained EdU and returned to quiescence at d14. MuSCs divided in d 0-4 contributed to a smaller fraction (~1/2 of those in d 5-7) of SCs returned to quiescence. This was based on MuSCs incorporated EdU at select time windows and still retained detectable level of EdU at d 14. Our understanding of their main conclusion is that more 'return-to-quiescence' MuSCs undergo their last round(s) of division from d 5 and onwards and retain sufficient EdU for detection. This does not exclude the possibility that many MuSCs divide at d2-4 continue to divide and dilute EdU to below detection later in quiescence. An additional complication is that we do not know, in each select time window, the fraction of MuSCs that undergo parental-DNA-strand-retention-division and are not labeled by EdU. In our view, all regenerative MuSCs are derived from the original pool of Pax7+ MuSCs prior to injury. Therefore, defects in earlier divisions likely lead to a smaller pool of SCs that can later divide and return to quiescence. Our data do show that an earlier defect in EdU incorporation and Pax7 cell number at d 5 (of Mpp7 cKO) and the reduction of return-to-quiescence Pax7+ MuSCs at d 21. Given that all components in our proposed protein complex are pleiotropically expressed, they are likely ready for action at all times unless prevented by other regulatory mechanisms (e.g., F-actin state/cytoplasmic retention).

Importantly, we consider that renewal divisions do not have to be those just prior to returning to quiescence, but also include earlier divisions to maintain the pool of SCs that later continue to divide and return to quiescence. The original work on Carm1's role in MuSC renewal also focused on symmetric vs asymmetric renewal divisions (instead of returning to quiescence). Regarding Carm1's role in myogenic differentiation, please see our response to comment 3 below.

2) Fig. 2C
What is the possible mechanism by which ΔL27-Mpp7 restored the proportion of

Pax7+MyoD+ cells? Does this indicate that the nuclear translocation of YAP shown in previous studies by the authors occurs normally with ΔL27-Mpp7? Could this be confirmed by staining for YAP/TAZ?

We have now addressed this question with new data. As expected, ΔL27-Mpp7 restored the level of TAZ/YAP, explaining why it restores the progenitor fate (as TAZ or YAP does alone in this context). The data is now included as Fig.S4h with accompanying text (ln 265-270) and legend.

We note that the text flow does not allow us to place the data immediately following the Mpp7 domain deletion/renewal data (Fig. 2), as the TAZ/YAP connection is introduced later (Fig. 4). As such, we placed this data in Fig.S4h, albeit slightly meandering in the main text.

3) Fig. 3H
The following study showed that Carm1 was involved in myogenic differentiation. In contrast, current data demonstrated that Carm1 functions as an anti-myogenic factor, as the number of committed myogenic cells (MyoD+Pax7-, perhaps Myogenin+) was reduced. If possible, this reviewer recommends discussing the role of Carm1 in myogenic differentiation processes.
J Biol Chem. 2002 Feb 8;277(6):4324-33. doi: 10.1074/jbc.M109835200

Thank you for bringing this to our attention. We agree and have now included a statement (and the reference) for Carm1's role in myogenic differentiation in the discussion (ln 385-385):
"Worth noting is that CARM1 has also been documented to potentiate myogenic differentiation via distinct interacting proteins."

4) Lines 225-229; 'Using TAZ as a representative (for TAZ and YAP), we found that Mpp7 co-expression indeed increased Taz's transcriptional activity on a TEAD-reporter36 (Fig. 4e). Amot alone inhibited Taz, but Amot, Mpp7, and Taz altogether best activated the reporter. Co-IP also revealed an enhanced interaction between MPP7 and TAZ by AMOT (Fig. 4f; Supplementary Fig. S4e).
Comment; The explanations for Fig 4e and Fig 4f are switched, so please correct them.

Thank you for pointing this out. We have corrected the error by re-phrasing those sentences (now ln. 230-232):
"…we found an enhanced interaction between MPP7 and TAZ by AMOT using co-IP assay (Fig. 4e; Supplementary Fig. S4i). Furthermore, Mpp7 co-expression indeed increased Taz's transcriptional activity on a TEAD-reporter (Fig. 4f)."

5)Fig. S5d
Comment; Is there really a significant difference between V5-Taz and V5-Tax S89A?

Apologies, we accidentally put the wrong chart in the previous subfigure (Fig. S5d), which was a duplicate of Fig. 4h. It should have been the quantification for Carm1 levels by expressing L27-Taz vs Taz, and this has been corrected (currently S5e). Regarding Fig. 4h, although it does not look like there is a difference, the p-value does indicate a significant difference between Taz and Taz S89A.

6)In Fig. 6e
Comment; There is a lack of explanation for the result indicating that S175A Amot does not affect self-renewal.

We have now added an explanation (ln 304-305):
"S175A Amot has been shown to elevate nuclear YAP, which helps explain the rescue of progenitor fate."

7)Lines 292-293; As such, AMOT is likely the F-actin-responsive conduit for nuclear MPP7 and YAZ/TAZ for MuSC renewal.

Comment: As the authors described, MuSC activation is accompanied by actin rearrangement. However, to the reviewer's knowledge, it is unclear whether the actin rearrangement is required for the self-renewal of MuSCs. If possible, the reviewer recommends adding evidence indicating the necessity of actin rearrangement for the self-renewal of adult stem cells.

Thank you for pointing out the lack of direct evidence to support actin re-arrangement being a requirement for renewal to date. We used 'likely' for a speculative proposal but understand the reviewer's concern. We have now deleted this sentence, as the prior sentence interprets/summarizes the data without the need to speculate further.

Referee #2:

In this manuscript the authors describe a new function of MPP7, AMOT, and to some extent YAP/TAZ in the regeneration of muscle stem cells after injury. This follows the description, in 2017, of the MPP7-AMOT axis. In this manuscript, the authors advocate for the existence of a MPP7-AMOT-TAZ transcriptional complex, for which they provide evidence at the level of protein-protein interactions, cooperation on gene expression (RNAseq and luciferase assays), and similarity of in vivo phenotypes. This complex is not a general regulator of YAP/TAZ function, but only affects a subset of genes, likely because these genes are also regulated by the YY1 transcription factor, binding in the vicinity of TAD/TAZ complexes.

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the MPP7-TAZ complex for it to work efficiently. If so, a WW-mutant of TAZ should be functionally inactive, both on luciferase assays and on MUSC differentiation assays. This is important because in the past many claimed that the WW domain mediates important functions of YAP and/or TAZ based on biochemistry data (protein-protein interaction), without otherwise providing more important functional data.

Following the reviewer's comment, we have now included:
1) By luc assay, Taz-WWm (has its own TAD) could activate the TEAD-reporter, but not to the same level as wt Taz (new Fig. S5c). This new figure is placed next to the co-IP data showing a lack of interaction between Mpp7 and Taz-WWm. The reduced activity is likely due to its inability to interact with endogenous Amot and Mpp7 for higher transcriptional activity as wt Taz in 293T cells.
2) By SM assay (new Fig 4i), Taz-WWm is also not able to rescue Mpp7 cKO renewal. Due to the text flow and presenting the data next to each other for comparison, we merge this data with that of wt Taz. As such, we refer to it after the Luc data. It is a bit unconventional, but the best option.
The accompanying description in the main text is in ln 260-262.

2) the authors focused on TAZ, but TAZ (at difference with CARM1) GOF is very weak in the MUSC differentiation assay. This could be due to some degree of functional redundancy with YAP. The authors should try YAP in their functional assays (see point 1), also in light of the strong phenotype of YAP/TAZ DKO.

We see the reviewer's point to provide a key functional data regarding Yap being similar to Taz (hence Taz as an example/representative). We have now provided new functional data in Fig. S4e: Forced expression of neither wt Yap nor constitutively active Yap (S127A) in *Mpp7* cKO also could not rescue the renewal fraction, similar to that by forced expression of constitutively active Taz.
The accompanying description in the main text is in ln 243-250.

3) The axis with CARM1 is proposed as a main mediator of the end point muscle phenotype. If so, could the authors use the MUSC differentiation assay to probe whether CARM1 GOF also rescue, at least to some extent, YAP/TAZ KO MUSCS? This would be important not to validate/invalidate the model, but rather to gauge its overall strength and importance in the context of the wider biology.

We have performed this requested experiment and included the data in Fig.S4f. Carm1 overexpression in Yap/Taz dKO MuSCs partially rescues the progenitor fraction, but not the renewal fraction. We described this result and suggested an explanation in the result section (ln 247-252) and elaborated further in the discussion (ln 376-385).

Dear Dr. Fan,

Thank you for the submission of your revised manuscript to our editorial offices. I have now received the report from the referee that was asked to re-evaluate the study, you will find below. As you will see, referee #2 now fully supports the publication of the study in EMBO reports. Going through your p-b-p-response, I also consider the points of referee #1 as adequately addressed.

Before I can proceed with formal acceptance, I have these editorial requests I ask you to address in a final revised manuscript:

- Please shorten the abstract to not more than 175 words.

- Please provide individual production quality figure files as .eps, .tif, .jpg (one file per figure), of the main figures. Please upload these as separate, individual files upon re-submission, without their legends. Please add the legends to the main manuscript text file (for the order see below).

- We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy https://www.embopress.org/competing-interests and update your competing interests if necessary. Please name this section 'Disclosure and Competing Interests Statement' and put it after the Acknowledgements section.

- We now use CRediT to specify the contributions of each author in the journal submission system. CRediT replaces the author contribution section. Please use the free text box to provide more detailed descriptions and do NOT provide your final manuscript text file with an author contributions section. See also our guide to authors:
https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

- Please add up to 5 keywords and order the manuscript sections like this, using these names:
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- Please provide one pdf file with Supplementary data named 'Appendix'. The Appendix should have page numbers and needs to include a table of content on the first page (with page numbers) and legends for all content. Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text (for the callouts), and also label the figures and tables in the Appendix file according to this nomenclature.

- Supplementary Table S1 is a s dataset. Please upload this as dataset file (named 'Dataset EV1') with its legend on the first TAB of the excel file. Please also update the call-out for the table accordingly.

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Chen-Ming Fan
Carnegie Institution for Science
United States

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The data shown in figures should satisfy the following conditions:
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Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
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Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

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Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Not Applicable	

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Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	**Yes**	All phenotypic characterizations were randomly chosen among mice of different genotypes.
Include a statement about **blinding** even if no blinding was done.	**Yes**	The researchers were not blinded in experiments performance and outcome assessment. Blinding was impossible for the researchers who were aware of the experimental groups and treatment outcome. However, key experiments were repeated by at least two independent researchers.
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	**Yes**	Inclusion/exclusion criteria was similar with studies in this field
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