THE EMBO JOURNAL

SIRT5 impairs aggregation and activation of the signaling adaptor MAVS through catalyzing lysine desuccinylation

Xing Liu, Chunchun Zhu, Huangyuan Zha, Jinhua Tang, Fangjing Rong, Xiaoyun Chen, Sijia Fan, Chenxi Xu, Juan Du, Junji Zhu, Jing Wang, Gang Ouyang, Guangqing Yu, Xiaolian Cai, Zhu Chen, and Wuhan Xiao

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Editor: Karin Dumstrei

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.)

1st Editorial Decision 2 October 2019

Thank you for submitting your manuscript to The EMBO Journal. Your study has now been seen by three referees and their comments are provided below.

As you can see, the referees find the analysis interesting and suitable for consideration here. They raise a number of constructive and relevant concerns that I would like to ask you to addressed in a revised version. I should add that it is EMBO Journal policy to allow only a single major round of revision and that it is therefore important to address these concerns at this stage.

Let me know if we need to discuss anything specifically - happy to do so!

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1:

The RIG-I/MDA5 - MAVS pathway is critical for antiviral immunity to RNA viruses and is highly regulated, including by post-translational modifications. This manuscript adds to this growing list of regulatory mechanisms by identifying succinylation of MAVS. The authors further identify that this modification is counteracted by SIRT5, an enzyme known to remove succinate groups from proteins. Interestingly, loss of SIRT5 in vitro and in vivo results in increased type I interferon responses, while SIRT5 overexpression has the opposite effects. Mechanistically, MAVS and SIRT5 were found to interact. Lysine 7 in MAVS was identified to be succinylated, and removal of this modification by SIRT5 reduces aggregation of MAVS, a step required for signalling.

Overall, this manuscript is well written and was a pleasure to read. The data are mostly of high quality and well support the conclusions drawn. With some revisions as outlined below, this study would be a good candidate for publication in EMBO J (in particular, points 1 and 2 should be attempted to show specificity to MAVS). A number of wider questions, for example regarding the enzyme that adds a succinate to MAVS, how succinylation affects MAVS aggregation, and the link between metabolism and virus infection are in my view beyond the scope of this study.

Major points
1. Dose-titrations are lacking entirely. The authors should strengthen their conclusions by including titrations of different stimuli, at a minimum in key figures such as 1A/4A/5A (SeV), 1K (MDA5 = SIRT5-dependent), 1N, (IRF3 = SIRT5-independent).
2. It is important to exclude better that SIRT5 has pleiotropic effects on innate signalling. This should be done by stimulating the cytosolic DNA sensing pathway (for example with DNA transfection or DNA virus infection (e.g. HSV-1 or AdV) or cGAS/STING overexpression) in cells lacking and overexpressing SIRT5.
3. Is it possible to measure intercellular succinate levels? It would be informative to demonstrate increased succinate levels in RNA virus infected cells.
4. Fig 2A. Please provide a validation of MAVS and SIRT5 antibodies in immunofluorescence. Ideally, knockout cells should be stained and absence of signal demonstrated.
5. Fig 2D. Do the authors have access to MAVS knockout cells? An anti-MAVS immunoprecipitation in KO cells would further strengthen the specificity of the MAVS-SIRT5 interaction. Additionally or alternatively, do the authors have access to an anti-SIRT5 antibody that works in IP at endogenous levels?
6. Fig 4. Panel O. It appears the background in the GFP channel is higher in the KO cells. Have the same microscope settings been used for WT and KO cells? Panel Q. The difference between WT and KO cells appears marginal and repeats and statistics are missing from the bar graph. As such, the data in panels O and Q are not convincing. A VSV-GFP dose titration may be helpful.

Minor points
7. Is there any link between succinate and autoinflammatory disease?
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Referee #2:

The manuscript entitled "SIRT5 impairs aggregation and activation of the signaling adaptor MAVS through catalyzing lysine desuccinylation" described how the succinylation and desuccinylation of MAVS may control the aggregation and activation of MAVS. The authors also reported that SIRT 5 is the enzyme for MAVS desuccinylation. Overall the statements are supported by the experimental results. While this phenotype is intriguing, mechanistically how succinylation and desuccinylation of MAVS is regulated remained unclear. In previous studies, recombinant MAVS was sufficiently

able to form prion-like structure (F Hou et al, Science 2011). Whether the succinylation of MAVS is required for MAVS self-aggregation remain to be elucidated.

Major points:
1. Importantly, what regulates the desuccinylation of MAVS by SIRT5. Does the expression, activity, and/or localization (to interact with MAVS) of SIRT5 change during viral infections? This really need to be determined before to state that SIRT5 has a role to regulate antiviral signaling pathways.
2. The succinylation levels of MAVS during mock or virus infections should be monitored to correlate with the signaling results (Fig. 5).
3. Based on previous reports of MAVS prion-like structures (Xu et al Elife. 2014 and F Hou et al, Science 2011), Lys7 of MAVS is far from the MAVS-MAVS interaction interface. How the additional sussinylation at this position affect the aggregation of MAVS is unclear and undiscussed.
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Liu et al. discovered that SIRT5 catalyzes desuccinylation of MAVS upon viral infection. The desuccinylation site on MAVS, lysine 7, was identified by Mass Spectrometry. Desuccinylated MAVS was insufficient to form prion-like aggregates and to activate downstream signaling transduction, including type I interferon production and anti-viral gene expression. In vivo study using SIRT5-/- mouse strain indicates the important role of SIRT5 in antiviral response. The study is within the scope of this journal and the manuscript is well prepared in terms of writing. Major and minor comments are as below:

Major Comments:

1. Control experiments. It would be necessary to provide a few control experiments for the specificity of SIRT5 desuccinylation in RLR signaling. For instance, reporter assays using DNA virus stimulation, include SIRT2 or other components of the RLR pathway that are not de-succinylated.
2. Antiviral aspect. This study conducted many experiments using overexpression of SIRT5, however, the impact of endogenous SIRT5 expression and function upon viral infection were not examined.
3. Viral titers. Please compare viral titers of VSV to quantify viral lytic replication. VSV-GFP infection results cannot support the claim that the viral lytic replication reduced in SIRT5 knockout MEF. It only shows SIRT5 knockout impact the expression of GFP from VSV infection, but not real productive infection.

Minor Comments:

Introduction
Please correct "RIG-1" to RIG-I throughout the paper.
It would be better to introduce PTMs in general, such as demethylation, deacetylation and deamidation, and their roles in the innate immunity.
Italic format of SIRT5 or not, upper case or lower case? Please unify the format. Apply to MAVS, RIG-I and a few other terms, IRF3....

Add article after "Due to lack of..."

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Authors wrote which level SIRT5 exerts, means which target of SIRT5?
Enzyme-deficient mutant of SIRT5 (SIRT5-H158Y) is generated from this study or previous studies? Please clarify.
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How about the interaction between SIRT5 and MAVS in the context of viral infection?
SIRT5 and mutant still have the interaction with MAVS-K7R suggesting the other function of SIRT5 on MAVS? Please explain.
In addition, the following studies all compare SIRT5+/+ and SIRT5-/-, but not desuccinylation-deficient SIRT5. Please consider testing desuccinylation-deficient SIRT5.

1st Revision - authors' response 13 January 2020

Referee #1:

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Overall, this manuscript is well written and was a pleasure to read. The data are mostly of high quality and well support the conclusions drawn. With some revisions as outlined below, this study would be a good candidate for publication in EMBO J (in particular, points 1 and 2 should be attempted to show specificity to MAVS). A number of wider questions, for example regarding the enzyme that adds a succinate to MAVS, how succinylation affects MAVS aggregation, and the link between metabolism and virus infection are in my view beyond the scope of this study.

Major points

1. Dose-titrations are lacking entirely. The authors should strengthen their conclusions by including titrations of different stimuli, at a minimum in key figures such as 1A/4A/5A (SeV), 1K (MDA5 = SIRT5-dependent), 1N, (IRF3 = SIRT5-independent).

Response: We really appreciate for the referee's suggestion. To strengthen the conclusion, in this revision, we performed a series of dose-titration experiments and made new figures (Fig EV2A-F; Fig EV7A-D; Fig EV9A). These figures marked as following: Fig EV2A corresponds to Fig 1A; Fig EV7A corresponds to Fig 4A; Fig EV9A corresponds to Fig 5A. Fig EV2B corresponds to Fig 1K; Fig 2C corresponds to Fig 1N.

2. It is important to exclude better that SIRT5 has pleiotropic effects on innate signalling. This should be done by stimulating the cytosolic DNA sensing pathway (for example with DNA transfection or DNA virus infection (e.g. HSV-1 or AdV) or cGAS/STING overexpression) in cells lacking and overexpressing SIRT5.

Response: Yes, we agree with the referee. In this revision, we performed experiments as

suggested and made new figures (Fig EV2G; Fig EV10G and H).

3. Is it possible to measure intercellular succinate levels? It would be informative to demonstrate increased succinate levels in RNA virus infected cells.
Response: According to the suggestion, in this revision, we measured intercellular succinate levels using a commercial assay kits (ab204718, Abcam). Surprisingly, VSV infection caused intercellular succinate level decreased instead of increased, which seemed to be inconsistent with an increasing of MAVS succinylation in response to VSV infection. This inconsistency suggests that the enhancement of MAVS succinylation in response to viral infection may be not resulted from changes of intercellular succinate levels after viral infection.

Actually, we did observe that SIRT5 binding to MAVS was reduced in response to viral infection. This might be one of reasons why MAVS succinylation was enhanced after viral infection. The consequence that VSV infection causes intercellular succinate level decreased might account for one of mechanisms beneficing virus escape.

4. Fig 2A. Please provide a validation of MAVS and SIRT5 antibodies in immunofluorescence. Ideally, knockout cells should be stained and absence of signal demonstrated.
Response: Yes, we performed validation experiments and made Fig EV4.

5. Fig 2D. Do the authors have access to MAVS knockout cells? An anti-MAVS immunoprecipitation in KO cells would further strengthen the specificity of the MAVS-SIRT5 interaction. Additionally or alternatively, do the authors have access to an anti-SIRT5 antibody that works in IP at endogenous levels?
Response: Yes, we performed experiments as suggested and made Fig 2E.

6. Fig 4. Panel O. It appears the background in the GFP channel is higher in the KO cells. Have the same microscope settings been used for WT and KO cells? Panel Q. The difference between WT and KO cells appears marginal and repeats and statistics are missing from the bar graph. As such, the data in panels O and Q are not convincing. A VSV-GFP dose titration may be helpful.
Response: Yes, we agree with the referee. As suggested by the referee, we repeated the experiments including VSV-GFP dose titration and performed statistical analysis. The revised and new figures are: Fig 4O and 4Q, Fig 5R, Fig EV7L and Fig EV9E.

Minor points
7. Is there any link between succinate and autoinflammatory disease?
Response: Yes, these are some references regarding the link between succinate and autoinflammatory disease. In this revision, we added some of these contents in Introduction (p.4).

8. Fig 5R. Repeats and statistics are missing from the bar graph.
Response: We revised accordingly (Fig 5R in this revision).

9. Fig EV4. Please provide a Western blot validating the SIRT5 KO in HCT116 cells.
Response: We revised accordingly (Fig EV8E in this revision).

10. Page 5. Fig 1J-L (not Fig 6)
Response: We corrected it.

11. Page 6. Fig 3F and G (not Fig 4)
Response: We corrected it.

Referee #2:

The manuscript entitled "SIRT5 impairs aggregation and activation of the signaling adaptor MAVS through catalyzing lysine desuccinylation" described how the succinylation and desuccinylation of MAVS may control the aggregation and activation of MAVS. The authors also reported that SIRT 5 is the enzyme for MAVS desuccinylation. Overall the statements are supported by the experimental results. While this phenotype is intriguing, mechanistically how succinylation and desuccinylation of MAVS is regulated remained unclear. In previous studies, recombinant MAVS was sufficiently able to form prion-like structure (F Hou et al, Science 2011). Whether the succinylation of MAVS is required for MAVS self-aggregation remain to be elucidated.

Major points:
1. Importantly, what regulates the desuccinylation of MAVS by SIRT5. Does the expression, activity, and/or localization (to interact with MAVS) of SIRT5 change during viral infections? This really need to be determined before to state that SIRT5 has a role to regulate antiviral signaling pathways.
Response: Yes, we totally agree with the referee. In this revision, we performed the experiments as suggested by the referee and made new figures (Fig EV6A-C in this revision).

2. The succinylation levels of MAVS during mock or virus infections should be monitored to correlate with the signaling results (Fig. 5).
Response: Yes, we agree with the referee. In this revision, we performed the experiments as suggested by the referee and made new figures (Fig EV 9E-F in this revision).

3. Based on previous reports of MAVS prion-like structures (Xu et al Elife. 2014 and F Hou et al, Science 2011), Lys7 of MAVS is far from the MAVS-MAVS interaction interface. How the additional sussinylation at this position affect the aggregation of MAVS is unclear and undiscussed.
Response: Yes, we agree with the referee. In this revision, to clarify this issue, we discussed more about this concern (p.12 in this revision).

"Notably, compared to other modifications, succinylation causes a relatively large

increase in mass (100.02 Daltons) in addition to a protein charge flip from positive to negative (Alleyn et al, 2018; Yang & Gibson, 2019; Zhang et al, 2011). This may cause it to have a large impact on the structure as well as function of the proteins that it modifies, particularly on dramatic conformational alternations of its targeted substrates (Alleyn et al, 2018). These notions may provide a possible explanation for the effect of succinylation or desuccinylation of Lysine 7 on MAVS aggregation even though Lysine 7 of MAVS is far from the MAVS-MAVS interaction interface (Hou et al, 2011; Xu et al, 2014; Yang & Gibson, 2019)."

4. A number of PTMs on MAVS that can regulated the signaling activities of MAVS has been reported. Whether desuccinylation promotes other modifications (such as polyubiquitination) that enhances MAVS singaling need to be assessed.
Response: In this revision, we performed ubiquitination assays and found that SIRT5 impaired k63-linked polyubiquitination of MAVS (Fig. EV6E-G in this revision).

Minor points:
1. Fig. 4, M-N, the protein expression levels of HA-MAVS and HA-MAVS-K7R need to be shown by immunoblotting to conclude the reporter results.
Response: We revised accordingly and made Fig EV6J-K**.**

2. Fig. 4L, SDD-AGE can show that protein aggregates. Whether the molecular weight shift is merely self-aggregation or heteroligomerization with other proteins could not be distinguished by SDD-AGE. It is overstated that the MW shift of MAVS on SDD-AGE is due to the prion-like structure formation of MAVS.
Response: Yes, we agree with the referee. In this revision, we changed this kind of statement throughout the manuscript.

Referee #3:

Liu et al. discovered that SIRT5 catalyzes desuccinylation of MAVS upon viral infection. The desuccinylation site on MAVS, lysine 7, was identified by Mass Spectrometry. Desuccinylated MAVS was insufficient to form prion-like aggregates and to activate downstream signaling transduction, including type I interferon production and anti-viral gene expression. In vivo study using SIRT5-/- mouse strain indicates the important role of SIRT5 in antiviral response. The study is within the scope of this journal and the manuscript is well prepared in terms of writing. Major and minor comments are as below:

Major Comments:
1. Control experiments. It would be necessary to provide a few control experiments for the specificity of SIRT5 desuccinylation in RLR signaling. For instance, reporter assays using DNA virus stimulation, include SIRT2 or other components of the RLR pathway that are not de-succinylated.
Response: Yes, we agree with referee. In this revision, we performed more control experiments as suggested by the referee and made new figures (Fig EV2G-L; Fig EV10G-H).

2. Antiviral aspect. This study conducted many experiments using overexpression of SIRT5, however, the impact of endogenous SIRT5 expression and function upon viral infection were not examined.
Response: As suggested by the referee, in this revision, we performed additional experiments and made new figures (such as Fig EV6A-C).

3. Viral titers. Please compare viral titers of VSV to quantify viral lytic replication. VSV-GFP infection results cannot support the claim that the viral lytic replication reduced in SIRT5 knockout MEF. It only shows SIRT5 knockout impact the expression of GFP from VSV infection, but not real productive infection.
Response: Yes, we agree with the referee. In this revision, we compared the viral titer in WT and Sirt5 KO MEF (Fig EV 9D).

Minor Comments:
Introduction
Please correct "RIG-1" to RIG-I throughout the paper.
Response: We revised accordingly.

It would be better to introduce PTMs in general, such as demethylation, deacetylation and deamidation, and their roles in the innate immunity.
Response: Yes, we added these contents in Introduction (p.1 in this revision)

Italic format of SIRT5 or not, upper case or lower case? Please unify the format. Apply to MAVS, RIG-I and a few other terms, IRF3....
Response: In this revision, we have tried our best to unify the format throughout the manuscript. The general rules we used are as following: human gene, upper case and italic format; human protein, upper case; mouse gene, upper case for the first letter and italic format; mouse protein, upper case for the first letter.

Add article after "Due to lack of..."
Response: We added the references accordingly**.**

Results
Authors wrote which level SIRT5 exerts, means which target of SIRT5?
Response: We actually mean which target of SIRT5. In this revision, we changed this sentence.

Enzyme-deficient mutant of SIRT5 (SIRT5-H158Y) is generated from this study or previous studies? Please clarify.
Response: Actually, it is based on previous studies. In this revision, we provide the references.

Colocalization images have a few concerns:

1- Could authors provide a few more staining results?
2- It would be much helpful to also show the merged images of only SIRT5 or MAVS
3- To me, not all the MAVS are colocalized with SIRT5, any explanation?

Response: In this revision, according to the referee's suggestions, we provide more staining results and show the merged images of only SIRT5 or MAVS (Fig EV4).

Yes, we agree with the referee that not all the MAVS are colocalized with SIRT5. We think that a couple of causes might explain this phenomenon. Firstly, SIRT5 may interact with MAVS dynamically in cells, which responds to some stimulations or some specific physiologic conditions. Secondly, SIRT5 may have targets other that MAVS and MAVS may be modified by enzymes other than SIRT5 in cells. Actually, the observations that not all the MAVS are colocalized with its modification enzymes have been also noticed by other investigators (Liu et al. The ubiquitin E3 ligase TRIM31 promotes aggregation and activation of the signaling adaptor MAVS through Lys63-linked polyubiquitination. Nature Immunology, **18:** 214-224; He et al. RNF34 functions in immunity and selective mitophagy by targeting MAVS for autophagic degradation. EMBO Journal, 38(14)).

How about the interaction between SIRT5 and MAVS in the context of viral infection?

Response: In this revision, we provide a new figure (Fig 6C), which shows that SIRT5 binding to MAVS is diminished in the context of viral infection**.**

SIRT5 and mutant still have the interaction with MAVS-K7R suggesting the other function of SIRT5 on MAVS? Please explain.

Response: Actually, based on our domain mapping, SIRT5 mainly binds to the TM region of the C-terminus in MAVS. So, it is not surprised that SIRT5 still interact with MAVS-K7R. Binding to the C-terminus of MAVS may facilitate SIRT5 to catalyze K7 of MAVS, which is possibly prerequired for SIRT5 to desuccinylate MAVS.

SIRT5 H158Y can still interact with MAVS, indicating that one amino acid mutation is not enough to disrupt the binding ability of SIRT5 to MAVS. In addition, it may also suggest that deficiency of SIRT5 H158Y on desuccinylation of MAVS is not resulted from loss of binding ability of SIRT5 H158Y to MAVS, but due to loss of enzymatic activity of SIRT5 H158Y.

In addition, the following studies all compare SIRT5+/+ and SIRT5-/-, but not desuccinylation-deficient SIRT5. Please consider testing desuccinylation-deficient SIRT5.

Response: Actually, in the previous version, Fig.4J-K have provided some information about the role of desuccinylation-deficient SIRT5.

In this revision, we performed more experiment by testing desuccinylation-deficient SIRT5 and made new figures (Fig EV 7H, I and L).

2nd Editorial Decision 12 February 2020

Thank you for submitting your revised manuscript to The EMBO Journal. Your study has now been seen by the three referees and their comments are provided below.

As you can see the referees appreciate the added data and support publication here. They raise a few editorial points that are easy enough to sort out. Congratulations on a nice study!!

When you submit your revised version please also address the following points:

- There is a PDF with 2 suppl. tables labeled "Table EV1" etc. and 12 suppl. figures and legends labeled "Figure EV1". Please make this into an appendix file with a table of content and the nomenclature "Appendix Table S1" etc. and " Appendix Figure S1" etc. You can choose to have 5 of the supplementary figures as EV figures if you want to. If so please upload them as individual figure files and add their legends added to the manuscript, following the main figure legends. See also https://www.embopress.org/page/journal/14602075/authorguide#expandedview

- Please make sure that all images have size bars. The one in figure 7G is very hard to see. OK to leave the size indicators on the images, but maybe nicer to add them to the figure legends.

- The graphs in Fig 5L and Fig EV10C look very similar. Can you please double check that they are what they are supposed to be,

- We encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. It would be great if you could provide me with a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labeled with the appropriate figure/panel number, and should have molecular weight markers; further annotation could be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files.

- We include a synopsis of the paper (see http://emboj.embopress.org/). Please provide me with a general summary statement and 3-5 bullet points that capture the key findings of the paper.

- We also need a summary figure for the synopsis. The size should be 550 wide by 400 high (pixels). You can also use something from the figures if that is easier.

I have asked our publisher to do their pre-publication checks on the paper. They will send me the file within the next few days and I will pass it on to you as soon as I receive it.

That should be it!! Please don't hesitate to contact me if you have any further questions

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REFEREE REPORTS

Referee #1:

This reviewer congratulates the authors on their revisions; all points raised have been well addressed. The manuscript should now be published in EMBO J.

Referee #2:

All the concerns have been addressed.

Referee #3:

I believe that the manuscript is greatly improved and my questions are well addressed.

One minor suggestion here, please go over the figures and manuscript to keep the terminology consistent: For example, in the figures, MAVS and Mavs are used, the same with RIG-I, Rig-i, SIRT5 vs Sirt5, IRF3 vs Irf3. Please be careful when genes and proteins are used, different names are used accordingly.

2nd Revision - authors' response 29 February 2020

Referee #3:

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One minor suggestion here, please go over the figures and manuscript to keep the terminology consistent: For example, in the figures, MAVS and Mavs are used, the same with RIG-I, Rig-i, SIRT5 vs Sirt5, IRF3 vs Irf3. Please be careful when genes and proteins are used, different names are used accordingly.

Response: Yes, we fully understand the reviewer's concern. In this revision, we re-checked the terminology used in the manuscript. Even though they look confused, actually, we used them following the general rules: human gene, upper case and italic format; human protein, upper case; mouse gene, upper case for the first letter and italic format; mouse protein, upper case for the first letter.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Wuhan Xiao
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2019-103285R

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- ➔ the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ➔ figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- ➔ graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- ➔ if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be iustified
- ➔ Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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.
- ➔ an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- ➔ the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- ➔ a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- ➔ a statement of how many times the experiment shown was independently replicated in the laboratory.
- ➔ definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For gene expression and protein modification assays, we chose three littermates with the same sex (each group). For ELASA assay, we chose four littermates with the same sex (each group). For survival curve determination, we chose seven littermates with the same sex (each group). We repeated each experiment two to three times.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For experiment using mice, we chose three littermates with the same sex (each group) for gene expression and protein modification assays; we chose four littermates with the same sex (each group)n for ELASA assay; we chose seven littermates with the same sex (each group) for survival curve determination.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	We did not exclude mice from analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Yes. We used randomization procedure for the assays by using mice.
For animal studies, include a statement about randomization even if no randomization was used.	Littermates of the same sex were randomly assigned to experimental groups.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Littermates of the same sex were randomly assigned to experimental groups. All the analyses were performed blindly.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Yes. All the analyses for mice were performed blindly.
5. For every figure, are statistical tests justified as appropriate?	Yes.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes.GraphPad Prism 6 software (GraphPad Software, San Diego, CA) was used for all statistical analysis. Differences between experimental and control groups were determined by unpaired two-tailed Student's t test (where two groups of data were compared) or two-way ANOVA analysis (where more than two groups of data were compared). P values less than 0.05 were considered statistically significant. For animal survival analysis, the Kaplan–Meier method was adopted to generate graphs, and the survival curves were analyzed by log-rank analysis.
Is there an estimate of variation within each group of data?	Yes.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repor

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Yes.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Yes. EV table 1
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	We identified the source of cell lines by STR and confirmed that they were not contaminated by mycoplasm.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Sirt5 knockout (KO) mice (B6; 129 background) were purchased from the Jackson Laboratory (https://www.jax.org/strain/012757). The Sirt5-deficient mice were backcrossed 7 generations onto a C57BL/6J background before conducting this study. Mice were housed (12 h light/dark cycle, 22℃) and given unrestricted access to standard diet and tap water under specific pathogen-free conditions in Animal Research Center of Wuhan University. Littermates of the same sex were randomly assigned to experimental groups. All the analyses were performed blindly.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal procedures were approved by Institutional Animal Care and Use Committee (IACUC) at Institute of Hydrobiology, Chinese Academy of Sciences.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Yes.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A