EMBO reports

Functional Skewing of TRIM21-SIRT5 Interplay Dictates IL-1β Production in DSS-induced colitis

Pengbo Yao, Taiqi Chen, Peng Jiang, Li Li, and Wenjing Du
DOI: 10.15252/embr.202154391

Corresponding author(s): Wenjing Du (wenjingdu@ibms.pumc.edu.cn)

Review Timeline:

Submission Date:	25th Nov 21
Editorial Decision:	7th Feb 22
Revision Received:	20th Apr 22
Editorial Decision:	1st Jun 22
Revision Received:	1st Jun 22
Accepted:	8th Jun 22

Editor: Martina Rembold

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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Prof. Du

Thank you for the submission of your research manuscript to our journal. We have now received the full set of referee reports that is copied below.

As you will see, the referees acknowledge that the findings are potentially interesting. However, they also point out several technical concerns and have a number of suggestions for how the study should be strengthened, and I think that all of them should be addressed. Please also address minor comment 6 from referee 1 and provide data on the interaction of endogenous SIRT5 and TRIM21.
Please note that we also ask from the editorial side to specify the number of experiments/repeats and their nature (biological, technical repeats) in all figure legends.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the 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 or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (May 7, 2022). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 27,000 characters (including spaces but excluding materials & methods and references) and 5 main plus 5 expanded view figures. The results and discussion sections must further be combined, which will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. For a normal article there are no length limitations, but it should have more than 5 main figures and the results and discussion sections must be separate. In both cases, the entire materials and methods must be included in the main manuscript file.

IMPORTANT NOTE:
We perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:

1) A data availability section providing access to data deposited in public databases is missing. If you have not deposited any data, please add a sentence to the data availability section that explains that.

2) Your manuscript contains statistics and error bars based on n=2. Please use scatter blots in these cases. No statistics should be calculated if n=2.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

When submitting your revised manuscript, we will require:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).
Please download our Figure Preparation Guidelines (figure preparation pdf) from our Author Guidelines pages https://www.embopress.org/page/journal/14693178/authorguide for more info on how to prepare your figures.

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our

Author guidelines
()

6) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here:

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) Please note that a Data Availability section at the end of Materials and Methods is now mandatory. In case you have no data that requires deposition in a public database, please state so instead of refereeing to the database.
See also < https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

8) Figure legends and data quantification:
The following points must be specified in each figure legend:

- the name of the statistical test used to generate error bars and P values,

- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,

- the nature of the bars and error bars (s.d., s.e.m.)

- If the data are obtained from n {less than or equal to} 2, use scatter blots showing the individual data points.

Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.

See also the guidelines for figure legend preparation:
https://www.embopress.org/page/journal/14693178/authorguide#figureformat

- Please also include scale bars in all microscopy images.

9) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available .

10) 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 .

11) As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

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

Yours sincerely,

Martina Rembold, PhD
Senior Editor
EMBO reports

****

Referee #1:

In this manuscript the authors aim to identify novel interactors of SIRT5 and in the process identify the E3 ubiquitin ligase TRIM21 as a new SIRT5 binding protein. The authors demonstrate that TRIM21 induces formation of K48 linked polyubiquitin chains on SIRT5, leading to the degradation of SIRT5 in the proteasome. The authors also use Trim21-/- and Sirt5-/- mice in a DSS colitis model to demonstrate the role of the TRIM21-SIRT5 interaction in a disesase model. Although the authors convincingly demonstrate the interactions between TRIM21, SIRT5 and HAUSP (USP7), their manuscript still lacks some important information regarding technical details and statistical methods. Below I outline my main concerns I have with this manuscript.

Major issues
1. There are around 40 different known human E2 enzymes. The authors never mention which E2 enzyme they have used. Knowing the identity of the E2 is very important information. How did the authors select the E2 enzyme they used in their assays? Have the authors screened several E2 enzymes? What is the intracellular localization of the E2 they used in their assays?

2. In general, the descriptions of the statistical tests in the manuscript are inadequate and need to be improved. In Fig 7, the authors need to provide detailed information on the statistial tests, number of biological replicates etc.

3. The authors forget to reference some relevant articles, e.g. a previous report on the role of TRIM21 in DSS induced colitis (PMID: 34511601). The authors should therefore go through their reference list to verify that other relevant articles also have not been left out.

4. It is unclear how the authors score the histological sections in Fig 7H. This missing information is very important. The authors should mention the scoring system they have used and describe if the scoring was done in a blinded manner.

Minor issues
1. The siRNA sequences need to be disclosed.

2. The transfection protocol for the notoriously hard to transfect macrophages needs to be described. With low transfection efficiency, co-transfection (which is necessary for their assays in Fig 7A-D) is even more difficult. Could the authors disclose their transfection efficiency?

3. The authors need to clarify the number of repetitions and number of relicates for their experiments.

4. The color scheme to Fig 7F is very hard to read. The authors should improve the graphical clarity.

5. Are the results in Fig 7A-D and 7I based on biological replicates (e.g. 5 mice)? This must be clarified.

6. In Supplementary Fig 1A the authors aim to demonstrate that TRIM21 and SIRT5 "co-localize" to the cytosol by overexpressing SIRT5 and TRIM21 as fusion proteins to mCherry and FLAG. However, and in contrast to TRIM21, SIRT5 has mainly been described as a mitochondrial protein. Since the co-immunoprecipitation experiments are performed in a cell lysate where intracellular barriers are dissolved, this begs the question wether the interaction between TRIM21 with SIRT5 occurs in living cells. In addition, overexpression of tagged proteins is often is fraught with artifacts. The authors should at least discuss these issues in the article.

7. In the literature there are contradictory results on the LPS responsivness of Sirt5-/- BMDMs. In PMID: 30515162 the authors did not find that Sirt5-/- BMDMs responded differently to WT BMDMs after stimulation with LPS. The authors should comment on this.

Referee #2:

Yao et al present a mostly biochemical analysis of the mutual interaction between the deacetylase SIRT5 and the E3 ligase TRIM21. The authors convincingly prove the physical interaction between SIRT5 and TRIM21, the role of TRIM21 in ubquitinating and degrading SIRT5, and the role of SIRT5 in deacetylating TRIM21. Conversely, SIRT5 is deubuiquitinated and stabilized by HAUSP. Under the influence of LPS, the balance is shifted in favor of SIRT5 degradation and TRIM21-mediated cytokine induction. This is overall a well-documented story, in which the main conclusions are fully supported by the data. I only have a few minor remarks:

1) One aspect that is not clear from the manuscript is whether TRIM21 is upregulated by LPS? If so, is TRIM21 gene expression regulated by NF-kB?

2) p9. "Moreover, C-terminal of SIRT5 (a peptide containing residues 356-310) abrogated the formation of SIRT5 complex with HAUSP, confirming that N-terminal of SIRT5 is required for its binding to HAUSP (Supplementary Fig. 3C)." I don't understand this statement. If a C-terminal peptide prevents the complex formation, how can the N-terminal then be required for binding to HAUSP?

3) p13. "Surprisingly, the suppressive effect of wild-type SIRT5 on IFN-β and IL-1β could be totally reversed by enforced expression of Flag-TRIM21 (Fig. 7A, B), suggesting a critical role for SIRT5 in TRIM21-mediated pro-inflammatory cytokine generation." Wy is this surprising? Based on the previous sections, one would expect that TRIM21 ubiquitinates SIRT5 and mediates its degradation, hence leading to higher cytokine expression.

Response to Referee #1:

In this manuscript the authors aim to identify novel interactors of SIRT5 and in the process identify the E3 ubiquitin ligase TRIM21 as a new SIRT5 binding protein. The authors demonstrate that TRIM21 induces formation of K48 linked polyubiquitin chains on SIRT5, leading to the degradation of SIRT5 in the proteasome. The authors also use Trim21-/- and Sirt5-/- mice in a DSS colitis model to demonstrate the role of the TRIM21-SIRT5 interaction in a disease model. Although the authors convincingly demonstrate the interactions between TRIM21, SIRT5 and HAUSP (USP7), their manuscript still lacks some important information regarding technical details and statistical methods. Below I outline my main concerns I have with this manuscript.

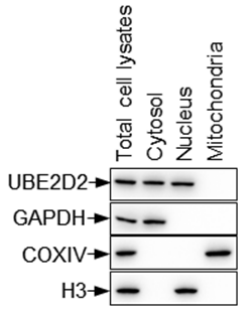
We very much appreciate the referee's positive comments on our work. We are also grateful for the referee's insightful and constructive critiques. As detailed below, we have performed new experiments or made the modification to address these comments, which we believe greatly improves the manuscript.

Major issues

1. There are around 40 different known human E2 enzymes. The authors never mention which E2 enzyme they have used. Knowing the identity of the E2 is very important information. How did the authors select the E2 enzyme they used in their assays? Have the authors screened several E2 enzymes? What is the intracellular localization of the E2 they used in their assays?

We thank the referee for this insightful comment. We agree that identification of E2 is very important information. We did screen several E2 enzymes and went through the relevant references to identify the UbcH5b/UBE2D2 E2 enzyme for use in our assays (Pan et al., 2016; Wada and Kamitani, 2006; Zhu et al., 2022).

To detect the intracellular localization of the E2, we performed the subcellular fraction analysis and found that the E2 (UbcH5b/UBE2D2) used in our assays localizes in the cytoplasmic and nucleic fractions (Please see Response Figure 1 below).



Response Figure 1. BMDM cells were fractionated and cytoplasmic, mitochondria and nuclear protein extracts were analyzed by western blot using indicated antibodies. GAPDH, COXIV and total H3 were used as fraction and loading controls respectively.

2. In general, the descriptions of the statistical tests in the manuscript are inadequate and need to be improved. In Fig 7, the authors need to provide detailed information on the statistical tests, number of biological replicates etc.

We thank the referee for this suggestion. We have improved the descriptions of the statistical tests in our revised manuscript (Please see the revised manuscript Method Section "**Statistical analysis**").

As the referee suggested, we have provided detailed information on the statistical tests and the number of biological replicates in our revised legends of Figure 7.

3. The authors forget to reference some relevant articles, e.g. a previous report on the role of TRIM21 in DSS induced colitis (PMID: 34511601). The authors should therefore go through their reference list to verify that other relevant articles also have not been left out.

We thank the referee for this insightful suggestion. As suggested, we have now added this literature *(PMID: 34511601)* and additional references in the revised manuscript.

4. It is unclear how the authors score the histological sections in Fig 7H. This missing information is very important. The authors should mention the scoring system they have used and describe if the scoring was done in a blinded manner.

We thank the referee for this constructive suggestion. We apologize that we didn't provide the detailed information in our previous version of manuscript. Now we have included this information in our revised manuscript (Please see the revised manuscript Method Section "**Histological analysis**").

We score the histological sections as described(Ou et al., 2022). Briefly, the histological scores of H&E stained sections were used to assess the colon pathology, based on assigned scores of tissue damage (0 =normal; 1 = irregular crypts; 2 = mild to moderate crypt loss (10–50%); 3 =severe crypt loss (50–90%); 4 = complete crypt loss) and inflammation (0 =absent, 1 = mucosal, 2 = submucosal, 3 = transmural extending into muscularis and serosa and 4 = diffuse). More than six different locations on each sample were used to count the score for each mouse, and the scoring was done in a blinded manner.

Minor issues

1. The siRNA sequences need to be disclosed.

We apologize that we forgot to add the siRNA sequences in our previous version of manuscript. We have now disclosed the siRNA sequences in the revised manuscript.

2. The transfection protocol for the notoriously hard to transfect macrophages needs to be described. With low transfection efficiency, co-transfection (which is necessary

for their assays in Fig 7A-D) is even more difficult. Could the authors disclose their transfection efficiency?

We thank the referee for the suggestion. We agree that the transfection efficiency in macrophages is somehow not high. We tested several transfection reagents and found that Lipofectamine 3000 (L3000150, Thermo Fisher Scientific) regent works well for transfecting both siRNA and plasmids according to the manufacturer's instructions. The transfection efficiency of siRNA is about 40-50% in our transfection assay and the transfection efficiency of plasmid is over 50%. We have incorporated this protocol in the Methods section (Please see the revised manuscript Method Section "**BMDMs isolation and transfection**").

3. The authors need to clarify the number of repetitions and number of replicates for their experiments.

We thank the referee for this suggestion. The number of repetitions and number of replicates for our experiments are at least three times and we have now added this information into our revised figure legends and the Method Section "**Statistical analysis**".

4. The color scheme to Fig 7F is very hard to read. The authors should improve the graphical clarity.

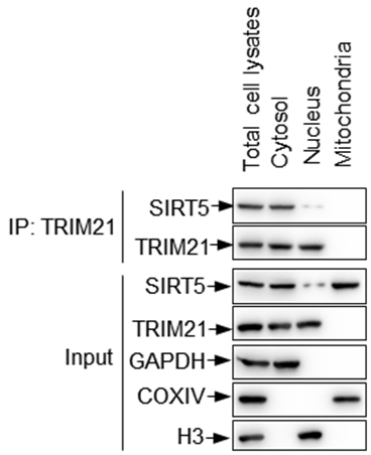
We apologize that the original Fig 7F is hard to read. Now, we have changed the size of the graphic markers to distinguish between groups. Please see the revised Figure 7F.

5. Are the results in Fig 7A-D and 7I based on biological replicates (e.g. 5 mice)? This must be clarified.

We apologize that we didn't provide the detailed numbers of biological replicates. Data in Figure 7A-D, n=3 biological replicates, in 7I, n=5 biological replicates (5 mice). We have now added this information into the revised Figure legends.

6. In Supplementary Fig 1A the authors aim to demonstrate that TRIM21 and SIRT5 "co-localize" to the cytosol by overexpressing SIRT5 and TRIM21 as fusion proteins to mCherry and FLAG. However, and in contrast to TRIM21, SIRT5 has mainly been described as a mitochondrial protein. Since the co-immunoprecipitation experiments are performed in a cell lysate where intracellular barriers are dissolved, this begs the question whether the interaction between TRIM21 with SIRT5 occurs in living cells. In addition, overexpression of tagged proteins is often is fraught with artifacts. The authors should at least discuss these issues in the article.

We thank the referee for this comment. To address this comment, we have performed IP assay using subcellular fractionation from BMDMs. Consistent with our immunofluorescence assay (revised Figure EV1A), fractionation studies showed that a large amount of SIRT5 was present in cytosolic, mitochondria and a very little amount of SIRT5 was in the nuclear fraction, which is also consistent with the previous study by Park et al (Park et al., 2013). Moreover, the IP assay using subcellular fractionation showed that endogenous SIRT5 interacts with TRIM21 mainly in the cytoplasm (Response Figure 2). We have added this figure into our revised manuscript (Please see revised Figure EV1B).



Response Figure 2. BMDM cells were fractionated. Total cell lysates from BMDM cells, and cytoplasmic, mitochondria and nuclear protein extracts were used for IP assay with anti-TRIM21 antibody and analyzed by western blot using indicated antibodies. GAPDH, COXIV and total H3 were used as fraction and loading controls respectively.

7. In the literature there are contradictory results on the LPS responsiveness of Sirt5-/- BMDMs. In PMID: 30515162 the authors did not find that Sirt5-/- BMDMs responded differently to WT BMDMs after stimulation with LPS. The authors should comment on this.

We thank the referee for this comment. We have carefully read the literature referred by the referee (*PMID: 30515162*) (Heinonen et al., 2018). We speculate that the contradictory results of the responsiveness of *SIRT5*$^{-/-}$ BMDMs to LPS may be due to differences in the concentration and duration of LPS treatment. In this paper (*PMID: 30515162*), the authors treated *SIRT5*$^{-/-}$BMDMs and WT BMDMs with 10 ng/mL LPS for 1h to detect the TNF mRNA level, and for 4h to detect IL-6 mRNA level respectively (*page 6, Fig. 2D and 2E*). However, we treated BMDMs with 100 ng/mL LPS for 4 hours, and found that the mRNA levels of IFN-β and IL-1β were significantly increased in *SIRT5*$^{-/-}$ BMDMs compared to WT BMDMs (revised Figure EV 5E and 5F). In addition, enforced overexpressing SIRT5 in *SIRT5*$^{-/-}$ BMDMs reduced IFN-β and IL-1β mRNA expression (revised Figure 7A and 7B). Furthermore, we established DSS-induced colitis in vivo model. Upon DSS treatment, the expression of IL-1β was significantly higher in colons of *SIRT5*$^{-/-}$ mice than in colons

of WT mice (revised Figure 7I). Together, our findings suggest SIRT5 has an inhibitory effect on IL-1β production in LPS-activated macrophages, which is consistently with previous work by Wang et al published in Cell reports, in which the BMDMs were treated with 100 ng/mL LPS for 6 hours (Wang et al., 2017). We have now added additional discussion on these observations in the revised manuscript.

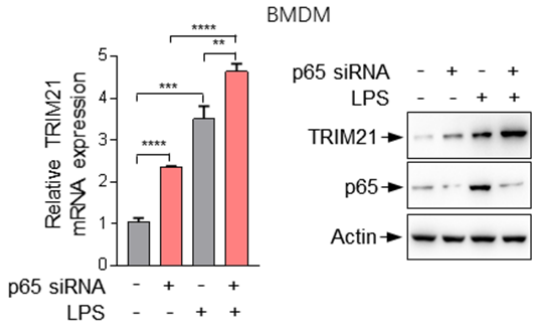
Response to Referee #2:

Yao et al present a mostly biochemical analysis of the mutual interaction between the deacetylase SIRT5 and the E3 ligase TRIM21. The authors convincingly prove the physical interaction between SIRT5 and TRIM21, the role of TRIM21 in ubiquitinating and degrading SIRT5, and the role of SIRT5 in deacetylating TRIM21. Conversely, SIRT5 is deubiquitinated and stabilized by HAUSP. Under the influence of LPS, the balance is shifted in favor of SIRT5 degradation and TRIM21-mediated cytokine induction. This is overall a well-documented story, in which the main conclusions are fully supported by the data. I only have a few minor remarks:

We are very grateful to the referee for all the positive comments.

1) One aspect that is not clear from the manuscript is whether TRIM21 is upregulated by LPS? If so, is TRIM21 gene expression regulated by NF-kB?

We thank the referee for this constructive comment. To address the comment, we knocked down NF-κB using siRNA and then treated BMDMs with LPS for 4h. LPS treatment resulted in an upregulation of TRIM21 expression in control and NF-κB knockdown BMDMs. Moreover, knockdown of NF-κB led to increased TRIM21 expression even in the presence of LPS treatment (Response Figure 3). These data suggest that LPS can upregulate TRIM21 expression. Additionally, TRIM21 gene expression is also regulated by NF-κB.



Response Figure 3. BMDM cells were treated with control or p65 (NF-κB) siRNA as indicated for 48h, and then treated cells with 100ng/mL LPS for 4h. TRIM21 mRNA levels were detected by qRT-PCR (left panel). Protein expression was analyzed by western blot (right panel). Data are mean ± SD. P values were determined by unpaired two-tailed Student's t-tests. **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

2) p9. "Moreover, C-terminal of SIRT5 (a peptide containing residues 256-310) abrogated the formation of SIRT5 complex with HAUSP, confirming that N-terminal of SIRT5 is required for its binding to HAUSP (Supplementary Fig. 3C)." I don't understand this statement. If a C-terminal peptide prevents the complex formation, how can the N-terminal then be required for binding to HAUSP?

We apologize for the uncleared statement for this figure. Supplementary Fig. 3C (revised Figure EV 3C) showed that N-terminal (residues 1-141) and middle portion (residues 142-255) of SIRT5 can bind to HAUSP, while the C-terminal (residues 256-310) cannot. Thus, this data suggests that the N-terminal (1-255aa) of SIRT5 is required for its binding to HAUSP. We have now amended this statement in our revised manuscript.

3) p13. "Surprisingly, the suppressive effect of wild-type SIRT5 on IFN-β and IL-1β could be totally reversed by enforced expression of Flag-TRIM21 (Fig. 7A, B), suggesting a critical role for SIRT5 in TRIM21-mediated pro-inflammatory cytokine generation." Why is this surprising? Based on the previous sections, one would expect that TRIM21 ubiquitinates SIRT5 and mediates its degradation, hence leading to higher cytokine expression.

We apologize for the inappropriate tone used in our original manuscript. We have amended this in the revised manuscript. We are very grateful to the referee for the insightful comment.

References

Heinonen, T., Ciarlo, E., Theroude, C., Pelekanou, A., Herderschee, J., Le Roy, D., and Roger, T. (2018). Sirtuin 5 Deficiency Does Not Compromise Innate Immune Responses to Bacterial Infections. Front Immunol *9*, 2675.

Ou, J., Guan, X., Wang, J., Wang, T., Zhang, B., Li, R., Xu, H., Hu, X., and Guo, X.K. (2022). Epithelial NELF guards intestinal barrier function to ameliorate colitis by maintaining junctional integrity. Mucosal Immunol *15*, 279-288.

Pan, J.A., Sun, Y., Jiang, Y.P., Bott, A.J., Jaber, N., Dou, Z., Yang, B., Chen, J.S., Catanzaro, J.M., Du, C.*, et al.* (2016). TRIM21 Ubiquitylates SQSTM1/p62 and Suppresses Protein Sequestration to Regulate Redox Homeostasis. Mol Cell *61*, 720-733.

Park, J., Chen, Y., Tishkoff, D.X., Peng, C., Tan, M., Dai, L., Xie, Z., Zhang, Y., Zwaans, B.M.,

Skinner, M.E.*, et al.* (2013). SIRT5-mediated lysine desuccinylation impacts diverse metabolic pathways. Mol Cell *50*, 919-930.

Wada, K., and Kamitani, T. (2006). Autoantigen Ro52 is an E3 ubiquitin ligase. Biochem Biophys Res Commun *339*, 415-421.

Wang, F., Wang, K., Xu, W., Zhao, S., Ye, D., Wang, Y., Xu, Y., Zhou, L., Chu, Y., Zhang, C.*, et al.* (2017). SIRT5 Desuccinylates and Activates Pyruvate Kinase M2 to Block Macrophage IL-1beta Production and to Prevent DSS-Induced Colitis in Mice. Cell Rep *19*, 2331-2344.

Zhu, X., Xue, J., Jiang, X., Gong, Y., Gao, C., Cao, T., Li, Q., Bai, L., Li, Y., Xu, G.*, et al.* (2022). TRIM21 suppresses CHK1 activation by preferentially targeting CLASPIN for K63-linked ubiquitination. Nucleic Acids Res *50*, 1517-1530.

1st Revision - Editorial Decision **1st Jun 2022**

Dear Prof. Du

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the enclosed report on it. As you will see, former referee 2 is satisfied with the revision and supports publication of your study.

Browsing through the manuscript myself, I noticed a few editorial things that we need before we can proceed with the official acceptance of your study:

- Please update the 'Conflict of interest' paragraph to our new 'Disclosure and competing interests statement'. For more information see
https://www.embopress.org/page/journal/14693178/authorguide#conflictsofinterest

- Please update the references to the alphabetical Harvard style. The abbreviation 'et al' should be used if more than 10 authors. You can download the respective EndNote file from our Guide to Authors
https://endnote.com/style_download/embo-reports/

- Data availability paragraph: This paragraph is meant to refer readers to data deposited in external databases. If such data were not generated, please state this instead of the current text, e.g. "No data were generated that require deposition in a public database".

- Please add Headings 'Figure legends' and 'Expanded View Figure legends'.

- Please add the information that you used UBE2D2 as E2 enzyme to the manuscript. Currently, this information is only present in the response to referee 1 (point 1).

- Regarding the Author Contributions, 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. See also guide to authors https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines.

- I attach to this email a related manuscript file with comments by our data editors. Please address all comments and upload a revised file with tracked changes with your final manuscript submission.

We look forward to seeing a final version of your manuscript as soon as possible.

Kind regards,

Martina Rembold, PhD
Senior Editor
EMBO reports

****

Referee #2:

The authors sufficiently responded to my remarks

Institute of Basic Medical Sciences,
Chinese Academy of Medical Sciences

Wenjing Du, Ph.D.

School of Basic Medicine, Peking Union Medical College

Professor, Department of Cell Biology
5 Dongdansantiao Street, Dongcheng district,Beijing, China, 100005

Dear Dr. Martina Rembold,

Thank you very much for handling our manuscript. We greatly appreciate your and the referees' comments and suggestions, which are valuable and very helpful for improving our manuscript.

We have now formatted our revised manuscript follow the instructions, including:

1) updated the 'Conflict of interest' paragraph to 'Disclosure and competing interests statement'
2) updated the references to the alphabetical Harvard style
3) updated Data availability paragraph
4) added Headings 'Figure legends' and 'Expanded View Figure legends'
5) added the information of E2 (UBE2D2) to the manuscript
6) used CRediT to specify the contributions of each author in the journal submission system
7) revised the manuscript according to the data editor's comments
8) uploaded a revised file with tracked changes

We deeply appreciate all your time and kind help. Please feel free to contact me if you have any queries.

Best wishes, and looking forwards to hearing back from you.

Wenjing Du, Ph.D. (Corresponding author)
E-mail: wenjingdu@ibms.pumc.edu.cn

Prof. Wenjing Du
Peking Union Medical College
No.5 Dongdansantiao, Dongcheng District
Beijing, Beijing 100005
China

Dear Prof. Du,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Martina Rembold, PhD
Senior Editor
EMBO reports

****

THINGS TO DO NOW:

Please note that you will be contacted by Wiley Author Services to complete licensing and payment information. The required 'Page Charges Authorization Form' is available here: https://www.embopress.org/pb-assets/embo-site/er_apc.pdf

You will receive proofs by e-mail approximately 2-3 weeks after all relevant files have been sent to our Production Office; you should return your corrections within 2 days of receiving the proofs.

Please inform us if there is likely to be any difficulty in reaching you at the above address at that time. Failure to meet our deadlines may result in a delay of publication, or publication without your corrections.

All further communications concerning your paper should quote reference number EMBOR-2021-54391V3 and be addressed to emboreports@wiley.com.

Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.

EMBO Press Author Checklist

Corresponding Author Name: Dr. Wenjing Du	
Journal Submitted to: EMBO Reports	
Manuscript Number: EMBOR-2021-54391V1	

USEFUL LINKS FOR COMPLETING THIS FORM

The EMBO Journal - Author Guidelines
EMBO Reports - Author Guidelines
Molecular Systems Biology - Author Guidelines
EMBO Molecular Medicine - Author Guidelines

Reporting Checklist for Life Science Articles (updated January 2022)

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots 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. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship 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 χ2 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.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

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	

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Materials and Methods

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Materials and Methods

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Materials and Methods
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Materials and Methods
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Materials and Methods

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Yes	Materials and Methods
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Yes	Materials and Methods
Please detail **housing and husbandry conditions**.	Yes	Materials and Methods

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgements

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Not Applicable	
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	Materials and Methods
Include a statement about **blinding** even if no blinding was done.	Not Applicable	
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.	Not Applicable	
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Figures

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Figures
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figures

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving **human participants**: 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.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Yes	Materials and Methods
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Yes	Materials and Methods

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Not Applicable	
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.	Not Applicable	
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.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Not Applicable	
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	