EMBO reports

Parp1 Hyperactivity Couples DNA Breaks to Aberrant Neuronal Calcium Signalling and Lethal Seizures

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1st Editorial Decision 11th Nov 2020

Dear Keith,

Thank you for the submission of your manuscript to EMBO reports. We have now received the full set of referee reports that is pasted below.

As you will see, all referees acknowledge that the findings are interesting. However, they also suggest some more experiments to strengthen the study. I think that all points raised are interesting and should be addressed, but please let me know in case you disagree and we can discuss the revisions further, also per video chat, if this is easier for you.

I would thus like to invite you to revise your manuscript with the understanding that the referee concerns 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 major 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.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss this further.

Regarding data quantification, please specify the number "n" for how many independent experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. This information must be provided in the figure legends. Please also include scale bars in all microscopy images.

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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.

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).
See https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf for more info on how to prepare your figures.

3) 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.

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- 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.

4) 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.

5) a complete author checklist, which you can download from our author guidelines <https://www.embopress.org/page/journal/14693178/authorguide>. Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

6) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines <https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>

7) Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database (see https://www.embopress.org/page/journal/14693178/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 & Method (see also https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please note that the Data Availability Section is restricted to new primary data that are part of this study. * Note - All links should resolve to a page where the data can be accessed. *
If your study has not produced novel datasets, please mention this fact in the Data Availability Section.

8) 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 at <https://www.embopress.org/page/journal/14693178/authorguide#sourcedata>.

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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 https://www.embopress.org/page/journal/14693178/authorguide#referencesformat

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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.

Best wishes,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

The manuscript by Komulainen et al is an investigation of the role of Parp1 in promoting or alleviating the symptoms of conditional Xrcc1 deletion (Xrcc1Nes-Cre). The authors report that death is likely caused by lethal seizures leading to a dramatically shortened lifespan. They use electrophysiological and optical approaches to demonstrate that increased Parp1 activity (caused by Xrcc1 deficiency) triggers seizure-like activity in vivo, in slices and in isolated hippocampal neurons in vitro. Under these conditions, Parp1 inhibition and/or deletion in conditional Xrcc1 knockout mice suppresses seizures, restores electrophysiological activity and lengthens lifespan. The authors speculate as to how PARP inhibition might serve as a therapeutic approach to treatment of XRCC1-dependent neurological disease.

I found this to be an absolutely lovely paper. The model is well chosen, the problems addressed are significant and the experimental approaches are well thought out and well controlled. I was particularly impressed by the sophisticated activity measurements used - the MEAs and the modified GCaMP6 optical imaging. I literally have no changes to request (and I don't think I have said that in one my reviews in many years).

My only regret is that the authors focused all of their energies on the hippocampus and to a lesser extent on the cortex. This is logical and in the context of the large PARP1 increases in these areas, perfectly appropriate. But the human condition has a prominent cerebellar phenotype and it would

have been of considerable interest to repeat some key experiments, particularly the MEA recordings, on slice preparations from this region to determine if there were lesser but still significant network changes there. Any answer would be interesting, even a negative one. For EMBO Reports, however, these additional observations are not required, and their absence does not diminish my strong enthusiasm for seeing this paper published in close to its current form.

Referee #2:

In this manuscript, Komulainen et al. show that lethal seizures and shortened lifespan due to loss of Xrcc1 in the nervous system can be rescued by deleting PARP1. The authors further show that deleting PARP1 can correct defects in presynaptic calcium signaling in Xrcc1-deficient neurons, and also decrease seizure like activity in Xrcc1-deficient hippocampal slices. The experiments are well designed and executed. Importantly, the reduction of seizure-like activity in Xrcc1Nes-Cre animals by PARP1 deletion is quite interesting and new. Addressing the comments below would further strengthen the points made in the manuscript:

1. Figure 1 shows that loss of PARP1 can cause a reduction in ADP-Ribose levels in Xrcc1 KO mice. Taken together with the reduced PARP1 staining, the authors conclude that PARP1 is hyperactive in Xrcc1 KO brains. However, the figure does not directly show that the remaining PARP1 protein in these brains is actually hyperactive. The authors say throughout the text and figures that PARP1 is aberrant/hyperactive in Xrcc1 KO brains, but this is never directly demonstrated biochemically.
2. Related to the point above, the quantification in Figure 1 shows that the loss of PARP1 causes a general reduction in ADP-Ribose levels unrelated to the loss of Xrcc1 (blue vs gray bars). This could account for the lack of detectable ADP-ribose staining instead of a specific PARP1 hyperactivation in Xrcc1 KO brains. Both points could be addressed by assessing the biochemical activity of PARP1 from these tissues.
3. Several correlations described by the authors need further clarification. For instance, in Figure 2, the observation is made that the loss of a single PARP1 copy is protective. However, in Figure 1, poly-ADP ribose levels are significantly elevated under these conditions. On the other hand, whereas the loss of PARP1 alone does not seem to affect mortality, its loss in the context of Xrcc1 seems toxic. Based on these results, the correlation between PARP1 levels, PARP1 activity, and mortality seem unclear. Finally, the loss of a single copy of PARP1 is sufficient to reduce seizures even though this PARP1 would seem to be hyperactive based on the authors interpretations of Figure 1.
4. The results in Figure 4 showing the reduction of seizures following the loss of PARP1 in Xrcc1 KO mice is indeed interesting. Does the loss of PARP1 also affect the ability to induce seizures in a WT background? The same comment extends to the results shown in Figure 6. Does the loss of PARP1 affect calcium signaling in a WT background?
5. The results in Figure 5 further suggest the importance of directly assessing PARP1 activity in WT and Xrcc1 KO neurons and tissues.

Referee #3:

Komulainen et al. investigated the mechanism linking defects in DNA SSBR with neurological dysfunction. The work showed hyperactivity of Parp1 in XRCC1NES-CRE mice results in lethal seizures and shortened lifespan. And both defects are prevented by Parp1 inhibition. Overall, the work is of interest and well written, and highlights PARP inhibition as a possible therapeutic

approach in XRCC1-mutated neurological disease.
My comments are as follows:
1. The authors checked Parp1 protein level, as well as ADP-ribose in mice brains. It's better to show PARylation in neurons and tissues.
2. How Parp1 hyperactivity affects Ca2+ signaling, one possibility is that this is a result of NAD+ depletion. The authors should measure NAD+ levels in the cells and tissues.
3. The lifespan and seizure results are good. Did you perform other behavior assays on the mice? Such as cognition and motor function assays, which are closely related to neurological diseases.
4. Besides Parp1, it's better to measure other DNA repair related proteins in XRCC1NES-CRE and Parp1-/- XRCC1NES-CRE mice brains.
5. Parp1 inhibition seems to be a potential therapeutic target for XRCC1-mutated neurological disease. Then if treated XRCC1NES-CRE mice with Parp1 inhibitor, will the longevity and seizure be improved?
6. PARP1 and NAD+ axis in ageing-related disease and neurodegenerative diseases should be discussed, there are several relevant papers and reviews in the literature.

Dear Esther,

Please find below the point-by-point response to the referees of our manuscript. We would like to take this opportunity to thank the referees for their insightful comments, which I believe we have addressed in full and which have significantly improved the manuscript.

Referee #1:

The manuscript by Komulainen et al is an investigation of the role of Parp1 in promoting or alleviating the symptoms of conditional Xrcc1 deletion (Xrcc1Nes-Cre). The authors report that death is likely caused by lethal seizures leading to a dramatically shortened lifespan. They use electrophysiological and optical approaches to demonstrate that increased Parp1 activity (caused by Xrcc1 deficiency) triggers seizure-like activity in vivo, in slices and in isolated hippocampal neurons in vitro. Under these conditions, Parp1 inhibition and/or deletion in conditional Xrcc1 knockout mice suppresses seizures, restores electrophysiological activity and lengthens lifespan. The authors speculate as to how PARP inhibition might serve as a therapeutic approach to treatment of XRCC1-dependent neurological disease.

I found this to be an absolutely lovely paper. The model is well chosen, the problems addressed are significant and the experimental approaches are well thought out and well controlled. I was particularly impressed by the sophisticated activity measurements used - the MEAs and the modified GCaMP6 optical imaging. I literally have no changes to request (and I don't think I have said that in one my reviews in many years).

My only regret is that the authors focused all of their energies on the hippocampus and to a lesser extent on the cortex. This is logical and in the context of the large PARP1 increases in these areas, perfectly appropriate. But the human condition has a prominent cerebellar phenotype and it would have been of considerable interest to repeat some key experiments, particularly the MEA recordings, on slice preparations from this region to determine if there were lesser but still significant network changes there. Any answer would be interesting, even a negative one. For EMBO Reports, however, these additional observations are not required, and their absence does not diminish my strong enthusiasm for seeing this paper published in close to its current form.
We thank the referee for his/her support and enthusiasm. Indeed, we focused this work on the hippocampus, because of its link with seizures and our discovery that the latter are the cause of shortened lifespan. The cerebellum is of course of huge interest too, because as the referee correctly points out it is the source of the ataxia present in this mouse model (and in the associated and other related SSB repair-defective human diseases). We have presented data describing the elevated ADP-ribosylation in the cerebellum of this mouse model in our earlier paper (Hoch et al Nature, 2017), and also the presence of electrophysiological defects in cerebellar Purkinje cell (Supplementary Figure 9 in the Hoch paper). We plan to examine these in more detail using MEA in future work, when we will turn our focus once again to the ataxia phenotype.

Referee #2:

In this manuscript, Komulainen et al. show that lethal seizures and shortened lifespan due to loss of Xrcc1 in the nervous system can be rescued by deleting PARP1. The authors further show that deleting PARP1 can correct defects in presynaptic calcium signaling in Xrcc1-deficient neurons, and also decrease seizure like activity in Xrcc1-deficient hippocampal slices. The experiments are well designed and executed. Importantly, the reduction of seizure-like activity in Xrcc1Nes-Cre animals by PARP1 deletion is quite interesting and new. Addressing the comments below would further strengthen the points made in the manuscript:

1. Figure 1 shows that loss of PARP1 can cause a reduction in ADP-Ribose levels in Xrcc1 KO mice. Taken together with the reduced PARP1 staining, the authors conclude that PARP1 is hyperactive in Xrcc1 KO brains. However, the figure does not directly show that the remaining PARP1 protein in these brains is actually hyperactive. The authors say throughout the text and figures that PARP1 is aberrant/hyperactive in Xrcc1 KO brains, but this is never directly demonstrated biochemically.
We have now addressed this question biochemically, as suggested by the referee, by incubating hippocampal and cerebellar tissue extracts with NAD+. We show that PARP1-mediated ribosylation is

elevated in Xrcc1-defective hippocampal and cerebellar tissue extracts, when compared to that present in the corresponding wild type extracts. We have added the biochemical experiments to Fig.1b,c (replacing the anti-PARP1 IF, which has been reported previously by us in Lee et al 2009) In addition, we note that the elevated ADP-ribose that is uncovered in cultured *Xrcc1*$^{Nes\text{-}Cre}$ neurons by incubation for 1-hr with PARG inhibitor (Fig.5a) is the result of hyperactive PARP1, because this elevated ADP-ribose is prevented by PARP inhibitor. This is discussed in the text (bottom of Page 6).

2. Related to the point above, the quantification in Figure 1 shows that the loss of PARP1 causes a general reduction in ADP-Ribose levels unrelated to the loss of Xrcc1 (blue vs gray bars). This could account for the lack of detectable ADP-ribose staining instead of a specific PARP1 hyperactivation in Xrcc1 KO brains. Both points could be addressed by assessing the biochemical activity of PARP1 from these tissues. I

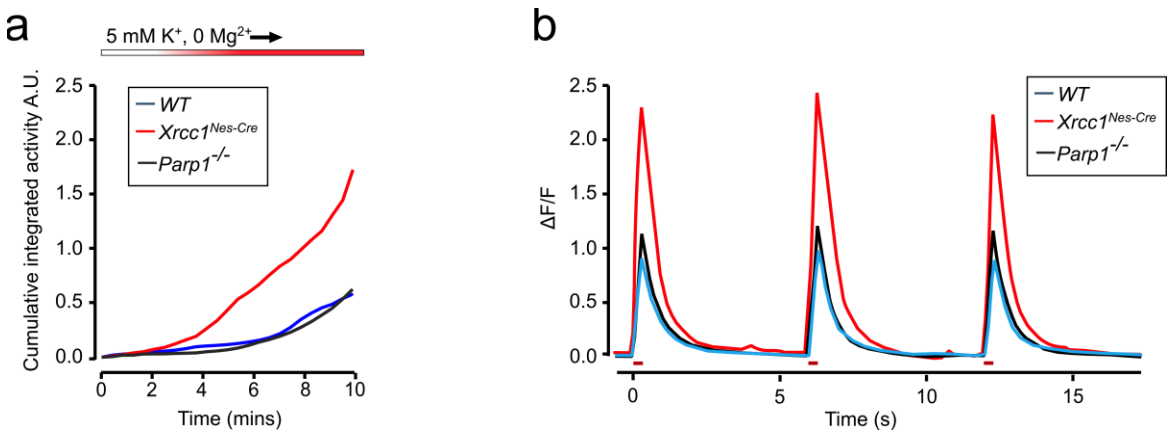
I apologise, I'm not sure if I understand this point. ADP-ribose staining is elevated in the *Xrcc1*$^{Nes\text{-}Cre}$ brain, and is suppressed as expected by additional deletion of one or both alleles of Parp1 (Fig.1a). However, we have as requested added the suggested biochemical experiments (Fig.1b,c, and see above).

3. Several correlations described by the authors need further clarification. For instance, in Figure 2, the observation is made that the loss of a single PARP1 copy is protective. However, in Figure 1, poly-ADP ribose levels are significantly elevated under these conditions. On the other hand, whereas the loss of PARP1 alone does not seem to affect mortality, its loss in the context of Xrcc1 seems toxic. Based on these results, the correlation between PARP1 levels, PARP1 activity, and mortality seem unclear. Finally, the loss of a single copy of PARP1 is sufficient to reduce seizures even though this PARP1 would seem to be hyperactive based on the authors interpretations of Figure 1.

For the phenotypes of elevated ADP-ribose levels, dysfunctional calcium signalling, and elevated seizures (whether measured by electrophysiology or by video imaging) the relationship with *Parp1* is straightforward. In *Xrcc1*$^{Nes\text{-}Cre}$ brain, the deletion of one *Parp1* allele partially rescues these phenotypes and the deletion of both *Parp1* alleles fully rescues them. However, we agree that the relationship between *Parp1* genotype and lifespan is complex. The confusing result is that deletion of one *Parp1* allele rescues lifespan in *Xrcc1*$^{Nes\text{-}Cre}$ mice more than does deletion of both *Parp1* alleles. Our interpretation of this is that whilst deletion of both *Parp1* alleles ablates the elevated ADP-ribose levels, calcium dysfunction, and seizure-induced death in *Xrcc1*$^{Nes\text{-}Cre}$ mice, the complete absence of Parp1 imposes a new (as yet undefined) defect; resulting in mice that live longer than *Xrcc1*$^{Nes\text{-}Cre}$ mice (because the seizure-dependent death is prevented) but younger than *Xrcc1*$^{Nes\text{-}Cre}$ mice that retain one Parp1 allele. We do not yet now why it is important to retain one Parp allele in *Xrcc1*$^{Nes\text{-}Cre}$ mice, but it is clearly related to the absence of Xrcc1 because as the referee noted single Parp1 KO mice have a normal lifespan. It will now be of great interest to identify the mechanism of this (seizure-independent) death in the double KO mice. This is now discussed on Page 5 & 9 of the revised manuscript.

4. The results in Figure 4 showing the reduction of seizures following the loss of PARP1 in Xrcc1 KO mice is indeed interesting. Does the loss of PARP1 also affect the ability to induce seizures in a WT background? The same comment extends to the results shown in Figure 6. Does the loss of PARP1 affect calcium signaling in a WT background?

This is a great question. We have now conducted some initial analysis of our current *Parp1*$^{-/-}$ animals, but cannot see a difference in either seizure frequency by MEA or in calcium signalling (see the Figure below). This suggests that Parp1 activity does not contribute to these phenotypes in wild type mice, at least at the juvenile mouse age we have examined. If the Referee agrees, we would rather not include this negative data in the manuscript because it might be that the situation is different if we examine aged *Parp1*$^{-/-}$ mice (e.g. 1-2 yr old aged mice), in which endogenous levels of DNA damage have had a chance to accumulate. That would be really exciting but is beyond the time-frame of this manuscript.



[a] Acute brain slices of the indicated genotypes were recorded on MEA. Mean cumulative activity plots in the CA3 region of hippocampus over 10 min of recording in epileptogenic buffer. *WT* (n = 13 slices from 3 mice), *Xrcc1*$^{Nes\text{-}Cre}$ (n = 9, 3), Parp1$^{-/-}$ (n=3, 1). **[b]** Mean SyGCaMP6f calcium responses of cultured hippocampal primary neurons to three rounds of 10 APs stimulation from mice of the following genotypes; *WT* (n = 1257 synapses, 5 coverslips, 2 animals), *Xrcc1*$^{Nes\text{-}Cre}$ (n =3313,12, 4)* Parp1$^{-/-}$ (n = 1251, 5, 2). *Data transposed from Fig.6 of the manuscript for comparison.

5. The results in Figure 5 further suggest the importance of directly assessing PARP1 activity in WT and Xrcc1 KO neurons and tissues.
This we have now done, as suggested above.

Referee #3:

Komulainen et al. investigated the mechanism linking defects in DNA SSBR with neurological dysfunction. The work showed hyperactivity of Parp1 in XRCC1NES-CRE mice results in lethal seizures and shortened lifespan. And both defects are prevented by Parp1 inhibition. Overall, the work is of interest and well written, and highlights PARP inhibition as a possible therapeutic approach in XRCC1-mutated neurological disease.
My comments are as follows:
1. The authors checked Parp1 protein level, as well as ADP-ribose in mice brains. It's better to show PARylation in neurons and tissues.
We employed a pan-ADP-ribose detection reagent for most of this work because of the sensitivity and reliability of the regent. However, we have also detected the elevated ADP-ribosylation in *Xrcc1*$^{Nes\text{-}Cre}$ mouse brain sections by IHC using anti-poly(ADP-ribose) antibodies (Figure EV1) and in *Xrcc1*$^{Nes\text{-}Cre}$ tissue extracts by WB using poly(ADP-ribose)-specific detection reagent (Fig.1b,c). We also note that, in cultured neurons at least, we require a short incubation with PARG inhibitor to detect the elevated ADP-ribose, confirming that this signal is also poly(ADP-ribose) (Fig.5a).

2. How Parp1 hyperactivity affects Ca2+ signaling, one possibility is that this is a result of NAD+ depletion. The authors should measure NAD+ levels in the cells and tissues.
Indeed, we have now measured NAD^{+} levels and find that these are reduced by ~50% in *Xrcc1*$^{Nes\text{-}Cre}$ brain. We have added these data to Fig.1d and discussed this finding as a possible explanation for the calcium signaling defect on Page 10.

3. The lifespan and seizure results are good. Did you perform other behaviour assays on the mice? Such as cognition and motor function assays, which are closely related to neurological diseases.
Since this work is focused on the hippocampal/seizure phenotype and its impact on mortality, we have not conducted other behavioural tests. However, we have tested motor function and described the ataxia in this mouse model in our previous work (Lee et al Nat. Neuroscience 2009; Hoch et al, Nature 2017).

4. Besides Parp1, it's better to measure other DNA repair related proteins in XRCC1NES-CRE and Parp1-/- XRCC1NES-CRE mice brains.
As requested, we have now added IHC of another DNA repair protein (Atm), in Figure EV1

5. Parp1 inhibition seems to be a potential therapeutic target for XRCC1-mutated neurological disease. Then if treated XRCC1NES-CRE mice with Parp1 inhibitor, will the longevity and seizure be improved?

We have now conducted these experiments as suggested. We now show that, in addition to correcting the defect in calcium signaling in cultured neurons, Parp1 inhibitor also prevents the elevated seizure-like activity in $Xrcc1^{Nes\text{-}cre}$ brain slices, as measured by MEA (Fig.4e). This is an exciting result. This was achieved by inclusion of the inhibitor in the drinking water of the mother, since the tissues were extracted an analysed prior to weaning. This is an exciting finding, because it supports the possibility that PARP inhibition might provide a therapeutic approach for the treatment of XRCC1-defective, and possibly other, neurological diseases. However, we have not yet observed lifespan rescue. Currently available inhibitors may not be suitable for this purpose, because they 'trap' PARP enzymes on unrepaired SSBs and thereby exacerbate the DNA repair defect in SSB repair-defective cells, causing increased DNA replication fork stalling and/or collapse during S phase. Whilst this is not a problem for post-mitotic neurons, it is likely to be cytotoxic in proliferating neural and other cell types. Indeed, proliferating XRCC1-defective cells are hypersensitive to current PARP inhibitors, perhaps explaining we have so far been unable to extend significantly the lifespan in $\textit{XRCC1}^{Nes\text{-}Cre}$ mice. We have discussed this in the text (Page 10/11).

6. PARP1 and NAD+ axis in ageing-related disease and neurodegenerative diseases should be discussed, there are several relevant papers and reviews in the literature.

Thanks you, we have now discussed this in the text (Page 10).

1st Revision - Editorial Decision 1st Mar 2021

Dear Keith,

Thank you for the submission of your revised manuscript. We have now received the enclosed reports from the referees that were asked to assess it, and I am happy to say that both support its publication now. Only a few more minor editorial changes will be required before we can proceed with the official acceptance of your manuscript.

- Please add up to 5 keywords to the manuscript.

- Please correct the subheading to "Conflict of interest".

- Please add all author contributions.

- I attach to this email a related manuscript file with comments by our data editors. Please address all comments in the final manuscript (e.g. the number of replicates is not clearly defined in Fig 3C & Fig 6G).

- The manuscript sections need re-ordering, please move the figure legends to after the references.

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

Best wishes,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #2:

The authors have addressed all my concerns satisfactorily. I think the manuscript is suitable for publication in EMBO reports without additional revision.

Referee #3:

The authors have now adequately revised and the paper is suitable for publication

The authors have addressed all minor editorial requests.

2nd Revision - Editorial Decision 5th Mar 2021

Prof. Keith Caldecott
University of Sussex
Genome Damage and Stability Centre
Science Park Road
Falmer
Brighton, Sussex BN1 9RQ
United Kingdom

Dear Prof. Caldecott,

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.

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Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

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Corresponding Author Name: Keith Caldecott
Journal Submitted to: EMBO Reports
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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 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.

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?	sample sizes were as large a feasibly possible, given limitations in generating the genotypes of interest. All sample sizes are indicated in the text.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	See above
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Samples/animals were not excluded
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.	where possible samples were scored blind.
For animal studies, include a statement about randomization even if no randomization was used.	randomisation was not necessary for this study
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.	group allocation was based strictly on genotype, so there was no opportunity for user bias.
4.b. For animal studies, include a statement about blinding even if no blinding was done	where possible experimental samples were scored blind.
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, as far as we are aware. Appropriate statistical software (e.g. Prism) was used thoughout.
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, as far as we are aware.

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).	all reagent/antibody sources are indicated in the text
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	all cutured neurons employed here were derived ex vivo from the corresponding mice.

* 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.	mouse details are indicated in the text
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	all animal experments were carried out in accordance with the UK-Animal (Scientific Procedures) Act 1986 and satisfied local institutional ethical regulations/governance at the University of Sussex.
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.	Compliance confrimed

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).	We confirm in our data statement that all data and materials associated with the manuscript are available upon request
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