EMBO Molecular Medicine

LAG3 is not expressed in human and murine neurons and does not modulate α-synucleinopathies

Marc Emmenegger, Elena De Cecco, Marian Hruska-Plochan, Timo Eninger, Matthias Schneider, Melanie Barth, Elena Tantardini, Pierre de Rossi, Mehtap Bacioglu, Rebekah Langston, Alice Kaganovich, Nora Bengoa-Vergniory, Andrés Gonzalez-Guerra, Merve Avar, Daniel Heinzer, Regina Reimann, Lisa Häsler, Therese Herling, Naunehal Matharu, Natalie Landeck, Kelvin Luk, Ronald Melki, Philipp Kahle, Simone Hornemann, Tuomas Knowles, Mark Cookson, Magdalini Polymenidou, Mathias Jucker, and Adriano Aguzzi
DOI: 10.15252/emmm.202114745

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(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.)

Review #1

1. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Less than 1 month

2. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

Very high evidence and clarity. Excellent scientific rigor.

The findings are important and reported clearly. The experiments are conducted in a rigorous way by numerous participating laboratories.

3. Significance:

Significance (Required)

Very high significance, both from a molecular biology and clinical standpoints.
This is an important manuscript that challenges the findings and conclusions of a prior high-profile paper in Science by Ma et al 2016, claiming that LAG3 is a receptor for aggregation-prone species of alpha-synuclein and that deletion of LAG3 results in reduced cell to cell propagation of alpha-synuclein aggregates.

The experiments in this paper are numerous and employ a variety of techniques. The overall conclusions are that LAG3 is not expressed by the relevant neurons and that LAG3 is not a receptor for alpha-synuclein fibrils (of different sizes). Therefore, the authors conclude that LAG3 is unlikely to play a role in the spread of alpha-synuclein pathology in Parkinson's disease and related disorders.

There are, however, some weaknesses. For example, the Introduction contains passages that are not written in a stringent way:

1. "Histologically, PD is characterized by α-synuclein aggregates known as Lewy Bodies in neurons of the substantia nigra," That is not a good description of PD neuropathology. Lewy pathology is present in numerous areas of the CNS and PNS, and is not restricted to the substantia nigra.
2. "Growing evidence suggests that α-synuclein fibrils spread from cell to cell". While alpha-synuclein pathology can spread from cell to cell, it is not known if the fibrils are

the species (alone or combined with other conformers) that cause the spreading of the pathology in a seeding fashion, or if smaller alpha-synuclein assemblies play that role.
3. "...by a "prionoid" process of templated conversion (Aguzzi, 2009; Aguzzi and Lakkaraju, 2016; Jucker and Walker, 2018; Kara, Marks and Aguzzi, 2018; Scheckel and Aguzzi, 2018; Uemura et al., 2020)." This sentence gives the impression that the corresponding author has led the field when it comes to alpha-synuclein's prionid properties. That is not really the case, and it would be appropriate to cite the literature in a more scholarly fashion that reflects how this part of the alpha-synuclein research field developed.
4. "Interrupting transmission of a-synuclein may slow down or abrogate the disease course." This is a bold statement and far from certain. While one might propose that this is the case, it is still just a hypothesis and the Introduction should reflect that.

Referee Cross-commenting

I concur with reviewers 2 and 3, and the new comment from reviewer 2. This paper should be published as soon as possible.

Review #2

1. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Less than 1 month

2. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

This study conclusively shows that LAG3 is not the receptor for a-synuclein that underlies the spread of synucleinopathic damage in various PD-related conditions. The paper is done extremely carefully and comprehensively. My only suggestion is to indicate the significance level in Figure 5a, as it may turn out that LAG3 is actually protective.

3. Significance:

Significance (Required)

This study is of extremely high significance - we need mechanisms to deal with spectacular results in the literature that should not have been published because they are were uncompelling to begin with, but were published for various sociological/political reasons. Science won't progress if we don't find correction mechanisms for wrong conclusions.

Referee Cross-commenting

I agree with reviewers 1 and 3, especially with the suggestions made by reviewer 1, which should be instituted. I think we all concur that the paper should be published without new experiments. I believe testing a-synuclein propagation in vivo in LAG3 KO mice would be useful, but given the complete lack of replication of LAG3 expression in brain and of a-synuclein binding to LAG3, this is not necessary.

Review #3

1. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Less than 1 month

2. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

It was proposed that LAG3 is important in the treatment of PD and related disorders, because it functions as a receptor of pathogenic α-synuclein and the treatment with anti-LAG3 antibodies attenuated the spread of pathological α-synuclein and drastically lowered the aggregation in vitro (Mao et al, Science 2016).

In this study, authors characterized 8 antibodies to LAG3 and investigated the presence of LAG3 in cultured cell lines, NSC-derived neural cultures, or organ homogenates for the presence of human or murine LAG3. But it was not detected in any of the neuronal samples tested. In addition, single cell (sc) RNAseq yielded only minimal counts for the LAG3 transcript in neurons, astrocytes, and mixed glial cells, and single-nucleus (sn) RNAseq human brain dataset for LAG3 expression across different cell types confirmed no LAG3 signals for any of 34 identified cell clusters, including 13 clusters of excitatory and 11 subtypes of inhibitory neurons, oligodendrocytes, oligodendrocyte precursor cells, microglia, astrocytes, and endothelial cells.

Authors also analyzed the binding of LAG3 with α-synuclein in mouse and human model

systems, and concluded that the affinity of LAG3 for α-synuclein fibrils, if any, is micromolar or less.

Furthermore, authors studied the propagation of pre-formed fibrils (PFFs) of α-synuclein in neural stem cell (NSC)-derived neural cultures in the presence or absence of LAG3, and the impact of LAG3 on survival in ASYNA53T transgenic mice expressing wild-type LAG3 as well as hemizygous or homozygous deletions thereof. However, they were unable to see any significant role for LAG3 in these in vitro and in vivo models of α-synucleinopathies.

In this connection, the reviewer would like to ask one question: Have you conducted any experiments of the propagation of PFFs of α-synuclein in LAG3-KO mice ? If they did, what were the results ?

Minor point

In Page 10, I think it's a typo: ASYYN mice must be ASYN mice.

3. Significance:

Significance (Required)

These negative findings about the LAG in α-synucleinopathies shown in this manuscript do not provide any new insight into the mechanisms of α-synuclein propagation. However, it is clear that LAG3 is not expressed in neuronal cells and the binding of LAG3 to α-synuclein fibrils appears limited. Overexpression of LAG3 in cultured human neural cells did not cause any worsening of α-synuclein pathology ex vivo. The overall survival of A53T α- synuclein transgenic mice was unaffected by LAG3 depletion and the seeded induction of α-synuclein lesions in hippocampal slice cultures was unaffected by LAG3 knockout. These data shown in this manuscript are convincing and the information is very important in terms of correcting the direction of disease treatment and research.

Referee Cross-commenting

I agree with reviewers 1 and 2. This paper should be published as soon as possible.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

1. "Histologically, PD is characterized by α-synuclein aggregates known as Lewy Bodies in neurons of the substantia nigra," That is not a good description of PD neuropathology. Lewy pathology is present in numerous areas of the CNS and PNS and is not restricted to the substantia nigra.

We have added a more detailed account:

"Histologically, PD is characterized by α-synuclein inclusions known as Lewy Bodies whose accumulation is associated with neurodegeneration (Dickson, 2012; Mullin and Schapira, 2015; Corbillé et al., 2016). These inclusions affect the Substantia nigra and other mesencephalic regions as well as, in some cases, the amygdala and neocortex (Dickson, 2018)."

2. "Growing evidence suggests that α-synuclein fibrils spread from cell to cell". While alpha-synuclein pathology can spread from cell to cell, it is not known if the fibrils are the species (alone or combined with other conformers) that cause the spreading of the pathology in a seeding fashion, or if smaller alpha-synuclein assemblies play that role.

We have reformulated the sentence to credit the fact that we do not know which synuclein species is the one that is transmitted:

"Growing evidence suggests that α-synuclein aggregates spread from cell to cell (Volpicelli-Daley et al., 2011; Volpicelli-Daley, Luk and Lee, 2014)… "

3. "...by a "prionoid" process of templated conversion (Aguzzi, 2009; Aguzzi and Lakkaraju, 2016; Jucker and Walker, 2018; Kara, Marks and Aguzzi, 2018; Scheckel and Aguzzi, 2018; Uemura et al., 2020)." This sentence gives the impression that the corresponding author has led the field when it comes to alpha-synuclein's prionid properties. That is not really the case, and it would be appropriate to cite the literature in a more scholarly fashion that reflects how this part of the alpha-synuclein research field developed.

I cannot disagree, and in fact I suspect that the present paper may be my second and possibly last experimental contribution to the synuclein field! However, I do claim intellectual parenthood of the prionoid (not "prionid") concept, which I first expounded in a 2009 Nature paper. Anyway, we now provide a more balanced citation:

"…by a "prionoid" process of templated conversion (Aguzzi, 2009; Jucker and Walker, 2018; Kara, Marks and Aguzzi, 2018; Henderson, Trojanowski and Lee, 2019; Karpowicz, Trojanowski and Lee, 2019; Uemura et al., 2020; Kara et al., 2021)."

4. "Interrupting transmission of a-synuclein may slow down or abrogate the disease course." This is a bold statement and far from certain. While one might propose that this is the case, it is still just a hypothesis and the Introduction should reflect that.

We have rewritten the sentence in a more subdued manner:

"It is thought that interrupting transmission of a-synuclein may slow down or abrogate the disease course."

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

This study conclusively shows that LAG3 is not the receptor for a-synuclein that underlies the spread of synucleinopathic damage in various PD-related conditions. The paper is done extremely carefully and comprehensively. My only suggestion is to indicate the significance level in Figure 5a, as it may turn out that LAG3 is actually protective.

We have added the significance level in Fig. 5A, in the legend: "The survivals of $ASYN^{A53T}$ $LAG3^{-/-}$, $LAG3^{+/-}$ and $LAG3^{+/+}$ mice were similar (Mantel-Cox log-rank test, p-value = 0.165)."

Referee Cross-commenting

I agree with reviewers 1 and 3, especially with the suggestions made by reviewer 1, which should be instituted. I think we all concur that the paper should be published without new experiments. I believe testing a-synuclein propagation in vivo in LAG3 KO mice would be useful, but given the complete lack of replication of LAG3 expression in brain and of a-synuclein binding to LAG3, this is not necessary.

We considered running experiments in addition to those performed in vivo in $ASYN^{A53T}$ transgenic mice (including LAG3 KO) and ex vivo in organotypic slices, the latter using pre-formed fibrils. However, the outcome of these experiments, along with the absence of LAG3 expression in neurons and its unclear binding, convinced us that the usage of further animals and reagents would be unwarranted.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

Have you conducted any experiments of the propagation of PFFs of α-synuclein in LAG3-KO mice ? If they did, what were the results ?

We did consider the possibility of replicating the experiments using PFFs in LAG3 KO mice. However, as stated above, we felt that our experiments – including the survival study in vivo in $ASYN^{A53T}$ transgenic mice – were unambiguous. After critical consideration, we remained unconvinced that this additional experiment would change the weight of our evidence in a substantial manner that would justify the inoculation of other animals and the utilisation of more resources.

Minor point

In Page 10, I think it's a typo: ASYYN mice must be ASYN mice.

Thank you for pointing this out. We corrected it.

1st Editorial Decision 22nd Jun 2021

22nd Jun 2021

Thank you for the submission of your manuscript to EMBO Molecular Medicine. I have now had a chance to carefully read your manuscript and the point-by-point response to the concerns raised by Review Commons referees. I also discussed your work and your response to the referees' comments with the other members of our editorial team. We are satisfied with the modifications made and would like to invite a minor revision of your manuscript.

We noticed that in your point-by-point response, some of the referees' comments are missing. Please make sure that you include all the comments from referees, including the *Referee Cross-commenting*.

Please read below for important editorial formatting and consult our author's guidelines for proper formatting of your revised article for EMBO Molecular Medicine.

I look forward to receiving your revised manuscript soon.

Link Not Available

Please do not share this URL as it will give anyone who clicks it access to your account.

Kind regards,
Jingyi

Jingyi Hou
Editor
EMBO Molecular Medicine

*** IMPORTANT INFORMATION ***

When submitting your revised manuscript, please carefully review the instructions that follow below. We perform an initial quality control of all revised manuscripts before re-review; failure to include requested items will delay the evaluation of your revision.

We 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). For guidance, download the 'Figure Guide PDF' (https://www.embopress.org/page/journal/17574684/authorguide#figureformat).

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 (https://www.embopress.org/page/journal/17574684/authorguide#submissionofrevisions). 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.

6) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see https://www.embopress.org/page/journal/17574684/authorguide#dataavailability).

In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.

7) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

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

.

9) 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 .

10) 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.

- 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.

See detailed instructions here:

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11) The paper explained: EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting

- the medical issue you are addressing,

- the results obtained and

- their clinical impact.

This may be edited to ensure that readers understand the significance and context of the research. Please refer to any of our published articles for an example.

12) For more information: There is space at the end of each article to list relevant web links for further consultation by our readers. Could you identify some relevant ones and provide such information as well? Some examples are patient associations, relevant databases, OMIM/proteins/genes links, author's websites, etc...

13) Author contributions: the contribution of every author must be detailed in a separate section (before the acknowledgments).

14) A Conflict of Interest statement should be provided in the main text.

15) Every published paper now includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short stand first (maximum of 300 characters, including space) as well as 2-5 one-sentences bullet points that summarizes the paper. Please write the bullet points to summarize the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice. Please attach these in a separate file or send them by email, we will incorporate them accordingly.

Please also suggest a striking image or visual abstract to illustrate your article as a PNG file 550 px wide x 300-600 px high.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

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Rev_Com_number: RC-2021-00804
New_manu_number: EMM-2021-14745
Corr_author: Aguzzi
Title: LAG3 is not expressed in human and murine neurons and does not modulate α-synucleinopathies

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Reviewer #1 (Evidence, reproducibility and clarity (Required)):

Very high evidence and clarity. Excellent scientific rigor.

The findings are important and reported clearly. The experiments are conducted in a rigorous way by numerous participating laboratories.

Reviewer #1 (Significance (Required)):

Very high significance, both from a molecular biology and clinical standpoints.
This is an important manuscript that challenges the findings and conclusions of a prior high-profile paper in Science by Ma et al 2016, claiming that LAG3 is a receptor for aggregation-prone species of alpha-synuclein and that deletion of LAG3 results in reduced cell to cell propagation of alpha-synuclein aggregates.

The experiments in this paper are numerous and employ a variety of techniques. The overall conclusions are that LAG3 is not expressed by the relevant neurons and that LAG3 is not a receptor for alpha-synuclein fibrils (of different sizes). Therefore, the authors conclude that LAG3 is unlikely to play a role in the spread of alpha-synuclein pathology in Parkinson's disease and related disorders.

There are, however, some weaknesses. For example, the Introduction contains passages that are not written in a stringent way:

1. "Histologically, PD is characterized by α-synuclein aggregates known as Lewy Bodies in neurons of the substantia nigra," That is not a good description of PD neuropathology. Lewy pathology is present in numerous areas of the CNS and PNS, and is not restricted to the substantia nigra.

We have added a more detailed account:

“Histologically, PD is characterized by α-synuclein inclusions known as Lewy Bodies whose accumulation is associated with neurodegeneration (Dickson, 2012; Mullin and Schapira, 2015; Corbillé et al., 2016). These inclusions affect the Substantia nigra and other mesencephalic regions as well as, in some cases, the amygdala and neocortex (Dickson, 2018).”

2. "Growing evidence suggests that α-synuclein fibrils spread from cell to cell". While alpha-synuclein pathology can spread from cell to cell, it is not known if the fibrils are the species (alone or combined with other conformers) that cause the spreading of the pathology in a seeding fashion, or if smaller alpha-synuclein assemblies play that role.

We have reformulated the sentence to credit the fact that we do not know which synuclein species is the one that is transmitted:

“Growing evidence suggests that α-synuclein aggregates spread from cell to cell (Volpicelli-Daley et al., 2011; Volpicelli-Daley, Luk and Lee, 2014)… “

3. "...by a "prionoid" process of templated conversion (Aguzzi, 2009; Aguzzi and Lakkaraju, 2016; Jucker and Walker, 2018; Kara, Marks and Aguzzi, 2018; Scheckel and Aguzzi, 2018; Uemura et al., 2020)." This sentence gives the impression that the corresponding author has led the field when it comes to alpha-synuclein's prionid properties. That is not really the case, and it would be

appropriate to cite the literature in a more scholarly fashion that reflects how this part of the alpha-synuclein research field developed.

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"…by a "prionoid" process of templated conversion (Aguzzi, 2009; Jucker and Walker, 2018; Kara, Marks and Aguzzi, 2018; Henderson, Trojanowski and Lee, 2019; Karpowicz, Trojanowski and Lee, 2019; Uemura et al., 2020; Kara et al., 2021)."

4. "Interrupting transmission of a-synuclein may slow down or abrogate the disease course." This is a bold statement and far from certain. While one might propose that this is the case, it is still just a hypothesis and the Introduction should reflect that.

We have rewritten the sentence in a more subdued manner:

"It is thought that interrupting transmission of a-synuclein may slow down or abrogate the disease course."

Referee Cross-commenting

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Reviewer #2 (Evidence, reproducibility and clarity (Required)):

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complete lack of replication of LAG3 expression in brain and of a-synuclein binding to LAG3, this is not necessary.

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In this connection, the reviewer would like to ask one question: Have you conducted any experiments of the propagation of PFFs of α-synuclein in LAG3-KO mice ? If they did, what were the results ?

We did consider the possibility of replicating the experiments using PFFs in LAG3 KO mice. However, as stated above, we felt that our experiments – including the survival study in vivo in ASYN^{A53T} transgenic mice – were unambiguous. After critical consideration, we remained unconvinced that this additional experiment would change the weight of our evidence in a substantial manner that would justify the inoculation of other animals and the utilisation of more resources.

Minor point

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Thank you for pointing this out. We corrected it.

Reviewer #3 (Significance (Required)):

These negative findings about the LAG in α-synucleinopathies shown in this manuscript do not provide any new insight into the mechanisms of α-synuclein propagation. However, it is clear that LAG3 is not expressed in neuronal cells and the binding of LAG3 to α-synuclein fibrils appears limited. Overexpression of LAG3 in cultured human neural cells did not cause any worsening of α-synuclein pathology ex vivo. The overall survival of A53T α- synuclein transgenic mice was unaffected by LAG3 depletion and the seeded induction of α-synuclein lesions in hippocampal slice cultures was unaffected by LAG3 knockout. These data shown in this manuscript are convincing and the information is very important in terms of correcting the direction of disease treatment and research.

Referee Cross-commenting

I agree with reviewers 1 and 2. This paper should be published as soon as possible.

30th Jun 2021 ,

Thank you for submitting your revised manuscript to EMBO Molecular Medicine, and thank you for addressing the editorial issues. I am pleased to inform you that we will be able to accept your manuscript pending the following amendments:

1. in the main manuscript file:
- please provide up to 5 keywords and incorporate them in the main text.
- please rename "Competing Interests" to "Conflicts of interest".
- Reference format: list all 10 co-authors of a paper before to add et al. in the reference list.
- 'FUNDING' needs to be merged with 'Acknowledgements'.
- In the Materials and Methods, include a statement that informed consent was obtained from all subjects.
- For animal work, the manuscript must include a statement in the Materials and Methods identifying the institutional and/or licensing committee approving the experiments. Housing conditions should be indicated.

2. Figures: Panel A can be removed for Figs EV3 and EV5 since there are no other panels. Please update the callouts accordingly.

3. Tables: The antibodies tables at the beginning of Materials and Methods need a title and callouts, and should be moved to the end of the manuscript.

4. Data Availability:

- Please rename "Data and material availability" into "Data availability", and place this section after the Materials & Methods section.
- Since this study does not generate large-scale datasets, please only include the following sentence in this section- "This study includes no data deposited in external repositories".

5. Please move "The paper explained" to the main manuscript file.

6. 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/17574684/authorguide#sourcedata

7. I only made a minor change in the synopsis text (see attached). I have also adjusted your synopsis image to the requested resolution (see attached).

Can you please let us know whether you are okay with the modified text and resized image, or if you would like to introduce future modifications?

Please note that this would be the final version, and synopsis text and image changes during

proofing are usually not allowed.

8. As part of the EMBO Publications transparent editorial process initiative (see our Editorial at http://embomolmed.embopress.org/content/2/9/329), EMBO Molecular Medicine will publish online a Review Process File (RPF) to accompany accepted manuscripts.

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9. Our data editors have seen the manuscript, and they have made some comments and suggestions that need to be addressed (see attached). Please send back a revised version (in track change mode), as we will need to go through the changes.

I look forward to receiving your revised manuscript soon.

Kind regards,
Jingyi

Jingyi Hou
Editor
EMBO Molecular Medicine

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When submitting your revised manuscript, please include:

1) a .docx formatted version of the manuscript text (including Figure legends and tables)

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4) a letter INCLUDING the reviewer's reports and your detailed responses to their comments (as Word
file).

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- please provide up to 5 keywords and incorporate them in the main text.

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Done.

- For animal work, the manuscript must include a statement in the Materials and Methods identifying the institutional and/or licensing committee approving the experiments. Housing conditions should be indicated.

Done.

2. Figures: Panel A can be removed for Figs EV3 and EV5 since there are no other panels. Please update the callouts accordingly.

Done.

3. Tables: The antibodies tables at the beginning of Materials and Methods need a title and callouts, and should be moved to the end of the manuscript.

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4. Data Availability:

- Please rename "Data and material availability" into "Data availability", and place this section after the Materials & Methods section.

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Jingyi

Jingyi Hou
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Corresponding Author Name: Adriano Aguzzi
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2021-14745

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 justified
- 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?	We adhered to best practice guidelines established in our field of science and chose sample sizes accordingly.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	We used standard statistical methods for analyses in all animal experiments. N were estimaed based on our previous work
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	We have not excluded samples or animals from our analyses. The criteria would have been pre-established as is standard in the field.
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.	In all analysis includng animal experimentation, the experimenter was blinded.
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4.b. For animal studies, include a statement about blinding even if no blinding was done	In all analysis includng animal experimentation, the experimenter was blinded.
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.
Is there an estimate of variation within each group of data?	Yes.

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

http://datadryad.org

http://figshare.com

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

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
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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).	Details are provided in materials and methods section as table.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Details are provided in materials and methods section as table.

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

D- Animal Models

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9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	This is included in the methods section.
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.	Our study complies with ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	This is included in the methods section.
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.	This is included in the methods section.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	Not applicable.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	We state this in the manuscript.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not applicable.
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.	Not applicable.
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.	Not applicable.

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	Not applicable.
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).	For Western Blot as well as for microscopy images, images are uncropped and directly inserted in the figures. All processing steps are described in the methods.
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