

Peer Review Information

Journal: Nature Structural and Molecular Biology

Manuscript Title: Atg9 is a lipid scramblase that mediates autophagosomal membrane expansion

Corresponding author name(s): Dr. Nobuo Noda

Editorial Notes:

Transferred manuscripts This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments, rebuttal and decision letters for versions considered at Nature Structural and Molecular Biology.

Reviewer Comments & Decisions:

Decision Letter, initial version:
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4th Sep 2020

Dear Dr. Noda,

Thank you again for submitting your manuscript "A lipid scramblase mediates autophagosomal membrane expansion". I am very sorry for the delay in reaching this decision. We have now the reports from 2 of the original reviewers at Nature (below), and based on these comments, we are happy to accept your paper, in principle, for publication as an Article in Nature Structural & Molecular Biology, on the condition that you revise your manuscript in response to the comments of the referees and our editorial requirements.

You will see that the reviewers are fully satisfied with the revisions and response; in comments to editors, one reviewer recommends avoiding overstatements on the *in vivo* scramblase activity of Atg9, since that was not directly assessed.

In addition, the text and figures require revisions. We are closed on Monday but you should receive detailed instructions for the final revision, along with information on editorial and formatting requirements by Tuesday. We recommend that you do not start revising the manuscript until you receive this additional information.

Data availability: this journal strongly supports public availability of data. Please place the data used in your paper into a public data repository, or alternatively, present the data as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found below:
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<http://platformsupport.nature.com/>>Platform Support Helpdesk.

We hope that you will support this initiative and supply the required information. Should you have any query or comments, please do not hesitate to contact me.

In recognition of the time and expertise our reviewers provide to Nature Structural & Molecular Biology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "A lipid scramblase mediates autophagosomal membrane expansion". For those reviewers who give their assent, we will be publishing their names alongside the published article.

If you have any questions, please do not hesitate to contact me directly.

Sincerely,
Ines

Ines Chen, Ph.D.
Chief Editor
Nature Structural & Molecular Biology

ORCID 0000-0002-1405-9703

Reviewer #1 (Remarks to the Author):

The manuscript by Noda and colleagues have addressed all my concerns in the rebuttal and the revised version of the manuscript.

Reviewer #2 (Remarks to the Author):

NONE

8th Sep 2020

Dear Dr. Noda,

I am writing as the secondary editor of this manuscript, as Anke Sparmann is currently out of the office.

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Structural & Molecular Biology manuscript, "A lipid scramblase mediates autophagosomal membrane expansion" (NSMB-A43813-T). Please follow the instructions provided here and in the attached files (sent separately), as the formal acceptance of your manuscript will be delayed if these issues are not addressed.

When you upload your final materials, please include a concise point-by-point response to the points below.

SCIENTIFIC ISSUES:

1. The remaining concerns of referee should be addressed, by avoiding overstatements about the scramblase activity in vivo.

POLICY ISSUES:

1. Data availability: this journal strongly supports public availability of data. Please place the data used in your paper into a public data repository, or alternatively, present the data as supplementary information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory.

2. DATA DEPOSITION: We require deposition of coordinates (and structure factors) into the Protein Data Bank with the designation of immediate release upon publication (HPUB). EM maps must be deposited in the EMDB. Deposition and immediate release of NMR chemical shift assignments are highly encouraged. Deposition of deep sequencing and microarray data is mandatory, and the datasets must be released prior to or upon publication. Proteomic datasets should be deposited in PRIDE. Accession codes must be provided in your final submission for acceptance, and entries must be accessible or HPUB at the galley proof stage.

3. Nature Research is taking an active approach to improving our transparency standards and increasing the reproducibility of all of our published results. Detailed information on experimental design and reagents is now collected on our Life Sciences Reporting Summary, which will be published alongside your paper.

Please provide an updated version of the Reporting Summary (which will be published with the paper) with your final files.

<https://www.nature.com/authors/policies/ReportingSummary.pdf>

Please also upload a revised Editorial policy checklist. Unless there were changes from the previous version, it is not necessary to upload the PDB validation reports again.

<https://www.nature.com/authors/policies/Policy.pdf>

GENERAL FORMATTING:

4. The manuscript is currently 4064 words (main text; Introduction and Results: 3595 words; Discussion: 469 words). That's fine, though some reorganization is required and might increase the word count a bit, which is also fine, keep it under 4,500. See attached file for specific editing

suggestions.

5. Current title: A lipid scramblase mediates autophagosomal membrane expansion (7 words, 62 characters, spaces included). I suggest modifying it slightly, to say "Atg9 is a lipid scramblase that mediates autophagosomal membrane expansion" (our guidelines for title are fewer than 100 characters including spaces and no punctuation).

6. Your abstract is currently 212 words. It must be under 150 words and should not include citations. Move most of the background (and references) to introduction, and make sure to keep the species of origin of the system being studied.

7. The Online methods section is currently 4194 words. We do not have a strict limit for this section but we suggest 3000 words as a target. I suggest moving some methods to Supplementary Note 1 (e.g., strains and plasmids, protein expression and purification, preparation of antibody fragments), and just having a short section pointing to the SUPpl Note, see attached.

8. References: the current manuscript has 25 references in main text and 27 in methods. This is a bit outside of our usual limits (up to 60 references in main text, and additional 20 references in online Methods), but overall it should be OK. Please make sure all references are cited in numerical order and place Methods-only references after the Methods section, following the numbering of the main reference list (i.e. do not start at 1). Again, please ensure that there are no citations in the abstract.

9. References: the reference list should contain papers that have been published or accepted by a named publication or recognized preprint server. Published conference abstracts, numbered patents and research datasets that have been assigned a digital object identifier may also be included in the reference list.

FIGURES AND TABLES:

10. There are currently 4 Figures and 0 Tables in main article. The table with cryo-EM data and statistics should be presented in main article, using the provided template. Figure 3 is quite large and complex, and it should be organized for optimal layout: the embedded table in 3a should be presented as a Supplementary Table or a stand-alone Table 2 (presented in main article word document, as editable table, not image). The remainder of the figure should be divided in 2 (e.g., panels k–m could be a new figure, that would allow panel k to be larger). These changes would result in 5 Figures and 2 Tables in main (and no additional color charges).

11. Please make sure all figures and tables, including Extended Data Figures, are cited in the text in numerical order.

12. Figure legends should be concise (i.e., under 300 words). Begin with a brief title and then describe what is presented in the figure and detail all relevant statistical information, avoiding inappropriate methodological detail or interpreting the data. See editing suggestions.

13. Red/green color contrasts can confuse our colorblind readers; please consider recoloring figures, if possible.

14. Cropping of gel and/or blot images: gel pieces should be separated with white space (do not add borders). When cropped gels or blots are shown in the main figures, all key data should be presented in uncropped form with molecular weight markers, as Source Data, as instructed below. These data can be displayed in a relatively informal style, but must refer back to the relevant figures; figure legend text should refer to the uncropped image and cite the Source Data (e.g., Uncropped blot/gel images are shown in the Source Data”.

15. When submitting the revised version of your manuscript, please pay close attention to our [Digital Image Integrity Guidelines](https://www.nature.com/nature-research/editorial-policies/image-integrity) and to the following points below:

- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
 - that control panels for gels and western blots are appropriately described as loading or sample processing controls
 - that all images in the paper are checked for duplication of panels and for splicing of gel lanes.
- Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the production process or after publication if any issues arise.

SUPPLEMENTARY INFORMATION

All Supplementary Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of three categories:

1. EXTENDED DATA FIGURES: you can have up to 10 such items, and the current Extended Data Fig 2 is very large, I suggest breaking it up (i.e. make panels g–o and p–u could be individual Extended Data Figures). On the other hand, the alignment in Extended Data Fig. 3 is not going to display properly, and should thus be moved to a Supplementary Figure (PDF format). Current Extended Data Fig 4 might not fit into one page (with legend), so you could consider breaking it up into 2 figures or resizing panels appropriately.

Extended Data Figures are an integral part of the paper and only data that directly contribute to the main message should be presented. These figures will be integrated into the full-text HTML version of your paper and will be appended to the online PDF. There is a limit of 10 Extended Data Figures, and each must be referred to in the main text. Each Extended Data Figure should be of the same quality as the main figures, and should be supplied at a size that will allow both the figure and legend to be presented on a single legal-sized page. Each figure should be submitted as an individual .jpg, .tif or .eps file with a maximum size of 10 MB each. All Extended Data figure legends must be provided in the attached Inventory of Accessory Information, not in the figure files themselves.

All Extended Data Figure must be called out in order as Extended Data Fig. 1, Extended Data Fig 2, etc.

2. SUPPLEMENTARY INFORMATION: this includes

- flat items: Supplementary Note 1 (with part of Methods for example; Supplementary Tables 1-2); Supplementary Figure 1 (with current Extended Data Fig 3); code script now in Supplementary Information could be another Supplementary Note (PDF format). All these items should be aggregated

into a single PDF file and listed in the inventory section 2A. Avoid having another numbered list of references; if those references are the same as in main, just use that number; if they are new ones, you could continue from the last number of Methods references.

-non-flat items: these would be the current Supplementary Videos 1–7, listed in section 2B of the inventory.

Supplementary Information is material that is essential background to the study but which is not practical to include in the printed version of the paper (for example, large figures, video files, large data sets and calculations). Each item must be referred to in the main manuscript and detailed in the attached Inventory of Accessory Information. Supplementary Tables containing large data sets should be in Excel format, with the table number and title included within the body of the table. All textual information and any additional Supplementary Figures (which should be presented with the legends directly below each figure) should be provided as a single, combined PDF. Please note that we cannot accept resupplies of Supplementary Information after the paper has been formally accepted unless there has been a critical scientific error.

Supplementary items (such as Supplementary Tables, Videos, Notes, and additional Supplementary Figures if permitted), should be numbered and called out in main article, as Supplementary Figure 1 (not SI1) and so on.

3. SOURCE DATA: I did not see source data files in the system. For uncropped images, you are welcome to aggregate all images into one single Supplementary Figure.

We encourage you to provide source data for your figures. Full-length, unprocessed gels and blots must be provided as source data for any relevant figures, and should be provided as individual PDF files for each figure containing all supporting blots and/or gels with the linked figure noted directly in the file. Statistical source data (i.e., data behind graphs) should be provided in Excel format, one file for each relevant figure, with the linked figure noted directly in the file. For imaging source data, we encourage deposition to a relevant repository, such as figshare (<https://figshare.com/>) or the Image Data Resource (<https://idr.openmicroscopy.org>).

Source data should be cited in the legend text (e.g., “Uncropped images for panels a-c are available as source data” or “Data for graphs in d-f are available as source data”).

STATISTICS and REPRODUCIBILITY

1. Bar graphs: we encourage replacing bar graphs with a dot-plot format (showing individual data points), particularly for sample sizes of $n < 10$, but it's fine to keep the current ones. For all error bars, make sure they are properly defined (e.g., s.d. or s.e.m.) together with a measure of center (e.g., mean or median) and with precise n number, using the wording “n=X cell cultures/animals/independent experiments” etc. as applicable; for biological replicates, please be as specific as possible.

2. For box plots, make sure they are properly defined in terms of minima, maxima, center, and percentiles, and should be accompanied by their precise n number defined as noted above.

3. Wherever statistical significance has been derived, precise P values should be provided if possible and appropriate. The type of statistical test used needs to be defined in the legend, whether they were one-sided or two-sided or whether adjustments were made for multiple comparisons.

4. When representative experiments are shown, you should state in the legends how many times each experiment was repeated independently with similar results. Please indicate number of times experiments were repeated, number of images collected, etc. If space in the legends is limiting, this information can be included in the "Statistics and Reproducibility" subsection in Methods.

5. Competing interests statement: Please include a competing interests statement as a separate section after the Author Contributions, under the heading "Competing interests", and enumerate any such circumstances there, or read: The authors declare no competing interests.

6. Reporting Summary statement: This should be placed after Online Methods section and read: Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

7. Code Availability Statement: This should be placed after Reporting Summary statement; you can include the statement about the script currently in "Supplementary Information" (but it should be changed to a SUPplementary Note, as noted above) and information on any other custom code used.

8. Data Availability statement: This should be placed after Code Availability / Reporting Summary statement (before Methods-only references). We suggest that you list in this order:

- data deposited in public repositories, with accession codes or DOIs.
- data available as Source Data (e.g. "Source data are available with the paper online.")
- if any data can only be shared upon request, please specify what those data are and explain why.

More information and examples can be found at

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TRANSPARENT PEER REVIEW

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AUTHORSHIP AND OTHER REQUIREMENTS

10. Ensure that all required forms found in the Policy Worksheet are uploaded to our Journal Processing system as "Supplementary Materials".

ORCID: Dr. Noda's account is already linked to the ORCID, as per our requirement; we do encourage all coauthors to do the same, using the instructions here:

<https://www.springernature.com/gp/researchers/orcid/orcid-for-nature-research>

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Please use the following link for uploading these materials:

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We ask that you aim to return your revised paper within 7 days. If you have any further questions, please feel free to contact me.

Best regards,

Ines Chen, Ph.D.
Chief Editor
Nature Structural & Molecular Biology

ORCID 0000-0002-1405-9703

Reviewer #1:

Remarks to the Author:

The manuscript by Noda and colleagues have addressed all my concerns in the rebuttal and the revised version of the manuscript.

Reviewer #2:

Remarks to the Author:

NONE

Author Rebuttal to Initial comments

1. The remaining concerns of referee should be addressed, by avoiding overstatements about the scramblase activity in vivo.

Response: We rewrote the first paragraph of Discussion in order to avoid overstatements about the scramblase activity in vivo as follows:

“Here, we describe the lipid scramblase activity of Atg9 in vitro, a critical finding that has important implications for this model. Following transfer of phospholipids from the ER to the cytoplasmic leaflet of the IM by Atg2, Atg9 would translocate superfluous phospholipids from the cytoplasmic leaflet to the luminal leaflet using its lipid scramblase activity. Atg2 and Atg9 therefore work cooperatively to enable lipid supply to IMs and, ultimately, IM expansion. For further confirming this model, measurement of lipid transfer and scrambling activities of Atg2 and Atg9, respectively, in vivo is the challenge we must work on in the future.”

Final Decision Letter:

15th Sep 2020

Dear Dr. Noda,

We are now happy to accept your revised paper "Atg9 is a lipid scramblase that mediates autophagosomal membrane expansion" for publication as a Article in Nature Structural & Molecular Biology.

Acceptance is conditional on the manuscript's not being published elsewhere and on there being no announcement of this work to the newspapers, magazines, radio or television until the publication date in Nature Structural & Molecular Biology.

Before the manuscript is sent to the printers, we shall make any detailed changes in the text that may be necessary either to make it conform with house style or to make it intelligible to a wider readership. If the changes are extensive, we will ask for your approval before the manuscript is laid out for production. Once your manuscript is typeset you will receive a link to your electronic proof via email within 20 working days, with a request to make any corrections within 48 hours. Please read proofs with great care to make sure that the sense has not been altered. If you have queries at any point during the production process then please contact the production team at rjsproduction@springernature.com. Once your paper has been scheduled for online publication, the Nature press office will be in touch to confirm the details.

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Note the policy of the journal on data deposition:
<http://www.nature.com/authors/policies/availability.html>.

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Ines Chen, Ph.D.
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