nature research

Peer Review Information

Journal: NSMB
Manuscript Title: A helical assembly of human ESCRT-I scaffolds reverse-topology membrane scission
Corresponding author name(s): James H. Hurley

Reviewer Comments & Decisions:

Decision Letter, initial version:

Dear Jim,

Thank you again for submitting your manuscript "A helical assembly of human ESCRT-I scaffolds reverse-topology membrane scission". We now have comments (below) from 2 reviewers who evaluated your paper; both are experts in ESCRT systems. In light of those reports, we remain interested in your study and would like to see your response to the comments of the referees, in the form of a revised manuscript.

You will see that reviewer 1 is quite positive about the interest of the work, and has requests for clarifications on reproducibility of some of the data presented. Reviewer 2 is concerned about the physiological relevance of the helical assembly, and that the mutations that disrupt such assembly could affect interaction of ESCRT-I with other factors. We would like to know the availability (or feasibility) of cellular data that could address such concerns. We'd be happy to discuss a revision, in which case a point-by-point response (for editorial assessment) would be very useful.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We expect to see your response within 1 week; you may send that to me directly by e-mail.

As you already know, we put great emphasis on ensuring that the methods and statistics reported in our papers are correct and accurate. As such, if there are any changes that should be reported, please

submit an updated version of the Reporting Summary along with your revision.

Please follow the links below to download these files:

Reporting Summary:
https://www.nature.com/documents/nr-reporting-summary.pdf

Please note that the form is a dynamic 'smart pdf' and must therefore be downloaded and completed in Adobe Reader.

UNCROPPED IMAGES: Please note that all key data shown in the main figures as cropped gels or blots should be presented in uncropped form, with molecular weight markers. These data can be aggregated into a single supplementary figure item. While these data can be displayed in a relatively informal style, they must refer back to the relevant figures. These data should be submitted with the final revision, as source data, prior to acceptance, but you may want to start putting it together at this point.

SOURCE DATA: we urge authors to provide, in tabular form, the data underlying the graphical representations used in figures. This is to further increase transparency in data reporting, as detailed in this editorial (http://www.nature.com/nsmb/journal/v22/n10/full/nsmb.3110.html). Spreadsheets can be submitted in excel format. Only one (1) file per figure is permitted; thus, for multi-paneled figures, the source data for each panel should be clearly labeled in the Excel file; alternately the data can be provided as multiple, clearly labeled sheets in an Excel file. When submitting files, the title field should indicate which figure the source data pertains to. We encourage our authors to provide source data at the revision stage, so that they are part of the peer-review process.

DATA AVAILABILITY: this journal strongly supports public availability of data. All data used in accepted papers should be available via a public data repository, or alternatively, 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:
https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data

We require deposition of coordinates (and, in the case of crystal structures, structure factors) into the Protein Data Bank with the designation of immediate release upon publication (HPUB). Electron microscopy-derived density maps and coordinate data must be deposited in EMDB and released upon publication. 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. To avoid delays in publication, dataset accession numbers must be supplied with the final accepted manuscript and appropriate release dates must be indicated at the galley proof stage.

While we encourage the use of color in preparing figures, please note that this will incur a charge to partially defray the cost of printing. Information about color charges can be found at http://www.nature.com/nsmb/authors/submit/index.html#costs

Nature Structural & Molecular Biology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

Please use the link below to submit your revised manuscript and related files:

[REDACTED]

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,
Ines

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

ORCID 0000-0002-1405-9703

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
Flower et al reports the structure of the headpiece of human ESCRT-I. The subcomplex serendipitously crystallized as a helical assembly. The authors propose that this assembly recapitulates a key functional feature of ESCRT-I during its role in vesicle budding. Evidence that the helical arrangement observed in the crystals include showing that ESCRT-I subcomplexes form helical assemblies in solution and that mutagenesis of helical interface residues affect assembly in vitro and abrogate function during membrane fission in cells. The authors then used molecular simulations of modeled full-length ESCRT-I helices in context of HIV-1 budding. They conclude that ESCRT-I may have a scaffolding and mechanical role during vesicle formation. Whereas the structure of the equivalent complex from yeast has been previously reported by the Hurley group, this paper is still quite novel and substantially extends those studies. The finding that ESCRT-I forms helical assemblies is the most impactful aspect of the paper as it significantly changes understanding of its role in membrane fission and will inform future studies in this area. The paper also clarifies the relationship between yeast Mvb12 and human MVB12A which is not apparent from sequence, the role of MVB12A (VPF motif) in assembly of the headpiece, and the relationship between the UEV domain and PTAP motifs of TSG101 – while these aspects are of more specialized interest, they remain an important contribution and fill in gaps in understanding of ESCRT-I function.

The paper is very well written throughout; concepts are presented clearly and logically. The biochemical and structural data are all of high quality. I have no changes to suggest, except that for the biochemical and functional experiments the figure legends 2d, 2f, 3e, 3f, 5 and S1 should indicate the number of biological/technical repeats if any.

Reviewer #2:
Remarks to the Author:
Flower et al define the structure of the human ESCRT-I headpiece, a fragment of the full ESCRT-I complex that is composed of ~80 amino acids of Tsg101, ~70 amino acids of Vps37B, ~120 amino acids of Vps28, and ~60 amino acids of Mvb12A (small domains of each subunit). Despite lacking sequence identity with yeast Mvb12, human Mvb12A occupies a similar location on the ESCRT-I headpiece. Overall, the complex solved exhibits a highly similar conformation as compared to the previously published yeast ESCRT-I headpiece. The structure shown is well characterized, and on its own, is publishable at another more appropriate venue, given its relatively modest novelty. During analysis, the authors find that the headpiece is capable of oligomerization and filament formation in vitro, mediated largely by electrostatic interactions between Vps28 subunits within each headpiece. Although they are able to disrupt this interface with a series of significant charge reversals (K54D and K58D) and charge neutralization (D59A) mutations, there is no evidence that these mutations do not also impact other

contacts needed for Vps28 to function in cells. Unfortunately, all assays performed in cells leverage these mutations, making them inconclusive. Ultimately, the data do not directly support a putative role for ESCRT-I oligomerization in autophagy or HIV-1 budding. In its present form, the work is not suitable for publication in NSMB, as the findings seem overinterpreted and remain ambiguous.

Major comments:

1. Currently there is no evidence that ESCRT-I forms anything larger than a heterotetramer in cells. Specifically, the authors fail to provide data using biochemical methods or imaging that directly demonstrate ESCRT-I oligomerizes during autophagosome closure or HIV-1 budding, which is absolutely necessary to make their model plausible.
2. Could the K54D, K58D, D59A mutations in Vps28 not impair its other associations in cells? The authors seem to overinterpret the impact of these mutations, as they may act in other ways beyond specifically interfering with Vps28-Vps28 interactions, which may only be an artifact of crystallization.
3. The indirect assay examining Vps37 accumulation following CHMP2A depletion is insufficient evidence that "…the mutations within the helical interface of VPS28 disrupt the higher order oligomerization of ESCRT-I." To make any strong conclusions regarding the ability of ESCRT-I to oligomerize or not, the authors need to examine wild-type ESCRT-I assembly in a manner similar to that of Teis and Kirchhausen (at the single molecule level; Adell et al., 2017).

Author Rebuttal to Initial comments

Response to Reviewers' Comments:

Reviewer #1:
Remarks to the Author:

We greatly appreciate Reviewer 1's thoughtful comments and enthusiasm for the work.

The paper is very well written throughout; concepts are presented clearly and logically. The biochemical and structural data are all of high quality. I have no changes to suggest, except that for the biochemical and functional experiments the figure legends 2d, 2f, 3e, 3f, 5 and S1 should indicate the number of biological/technical repeats if any.

The pull down experiments shown in figure 2d and f were repeated twice with the same results. Gel filtration experiments shown in figure 3 were repeated three times. The experiment in figure 5 was repeated three times and the Western blot is representative of the outcome on all occasions. The gel in figure S1 represents two biological replicates. All of this information is now noted in the respective figure legends.

Reviewer #2:
Remarks to the Author:

We appreciate Reviewer 2's comments on the high quality of the structural work. However, the reviewer's concerns about the cellular function are overstated, as detailed below.

Major comments:

1. Currently there is no evidence that ESCRT-I forms anything larger than a heterotetramer in cells. Specifically, the authors fail to provide data using biochemical methods or imaging that directly demonstrate ESCRT-I oligomerizes during autophagosome closure or HIV-1 budding, which is absolutely necessary to make their model plausible.

We would like to call the referee's attention to a recent publication demonstrating that 3-11 copies of ESCRT-I mediate HIV-1 release, consistent with our findings. Please see Hoffman et al. https://www.jbc.org/content/294/44/16266.long. This publication is cited on pg. 17 as ref. 56. We have expanded the discussion of the Hoffman et al. findings.

2. Could the K54D, K58D, D59A mutations in Vps28 not impair its other associations in cells? The authors seem to overinterpret the impact of these mutations, as they may act in other ways beyond specifically interfering with Vps28-Vps28 interactions, which may only be an artifact of crystallization.

We are not aware of any other interactors reported with the N-terminal domain of VPS28. With respect to the possibility of a crystallization artifact, this is ruled out by the EM of filaments assembled in solution, which we provide in Fig. 3.

3. The indirect assay examining Vps37 accumulation following CHMP2A depletion is insufficient evidence that "…the mutations within the helical interface of VPS28 disrupt the higher order oligomerization of ESCRT-I." To make any strong conclusions regarding the ability of ESCRT-I to oligomerize or not, the authors need to examine wild-type ESCRT-I assembly in a manner similar to that of Teis and Kirchhausen (at the single molecule level; Adell et al., 2017).

The suggested wild-type ESCRT assembly imaging was already published by Hoffman et al. https://www.jbc.org/content/294/44/16266.long and the results are consistent with our

conclusions. Out of respect to the reviewer's concern we have however rephrased the quoted sentence and now state that the mutations "inhibit the accumulation" of ESCRT-I instead of "disrupt the higher order oligomerization of ESCRT-I."

Decision Letter, first revision:

Dear Jim,

Thank you again for submitting your manuscript "A helical assembly of human ESCRT-I scaffolds reverse-topology membrane scission". As previously communicated, after editorial assessment of the revision and response, 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 our editorial requirements.

The text and figures require revisions. Note that, within a few days, we will send you detailed instructions for the final revision, along with information on editorial and formatting requirements. 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:
https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data

TRANSPARENT PEER REVIEW
Nature Structural & Molecular Biology offers a transparent peer review option for new original research manuscripts submitted from 1st December 2019. We encourage increased transparency in peer review by publishing the reviewer comments, author rebuttal letters and editorial decision letters if the authors agree. Such peer review material is made available as a supplementary peer review file. Please state in the cover letter ‘I wish to participate in transparent peer review’ if you want to opt in, or ‘I do not wish to participate in transparent peer review’ if you don’t. Failure to state your preference will result in delays in accepting your manuscript for publication.
Please note: we allow redactions to authors’ rebuttal and reviewer comments in the interest of confidentiality. If you are concerned about the release of confidential data, please let us know specifically what information you would like to have removed. Please note that we cannot incorporate

redactions for any other reasons. Reviewer names will be published in the peer review files if the reviewer signed the comments to authors, or if reviewers explicitly agree to release their name. For more information, please refer to our FAQ page.

ORCID

Nature Structural & Molecular Biology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS) prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. For more information please visit http://www.springernature.com/orcid

For all corresponding authors listed on the manuscript, please follow the instructions in the link below to link your ORCID to your account on our MTS before submitting the final version of the manuscript. If you do not yet have an ORCID you will be able to create one in minutes.
https://www.springernature.com/gp/researchers/orcid/orcid-for-nature-research

IMPORTANT: All authors identified as 'corresponding author' on the manuscript must follow these instructions. Non-corresponding authors do not have to link their ORCIDs but are encouraged to do so. Please note that it will not be possible to add/modify ORCIDs at proof. Thus, if they wish to have their ORCID added to the paper they must also follow the above procedure prior to acceptance.

To support ORCID's aims, we only allow a single ORCID identifier to be attached to one account. If you have any issues attaching an ORCID identifier to your MTS account, please contact the 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.

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

Final Decision Letter:

Dear Jim,

Hope all is well with you and your team.

We are now happy to accept your revised paper "A helical assembly of human ESCRT-I scaffolds reverse-topology membrane scission" 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.

Please note that due to tight production schedules, proofs should be returned as quickly as possible to avoid delaying publication. If you anticipate any limitations to your availability over the next 2-4 weeks, please e-mail rjsproduction@springernature.com as soon as possible. Please provide specific dates that you will be unavailable and provide detailed contact information for an alternate corresponding author if necessary.

To assist our authors in disseminating their research to the broader community, our SharedIt initiative provides all co-authors with the ability to generate a unique shareable link that will allow anyone (with or without a subscription) to read the published article. Recipients of the link with a subscription will also be able to download and print the PDF.

As soon as your article is published, you can generate your shareable link by entering the DOI of your article here: <a href="http://authors.springernature.com/share">http://authors.springernature.com/share<a>. Corresponding authors will also receive an automated email with the shareable link

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

Your paper will be published online soon after we receive proof corrections and will appear in print in the next available issue. You can find out your date of online publication by contacting the production team shortly after sending your proof corrections. The embargo is set at 16:00 London time (GMT) / 11:00 am US Eastern time (EST), on the Monday of publication. Now is the time to inform your Public Relations or Press Office about your paper, as they might be interested in promoting its publication. This will allow them time to prepare an accurate and satisfactory press release. Include your manuscript tracking number (NSMB-A42885B) and our journal name, which they will need when they contact our press office.

About one week before your paper is published online, we shall be distributing a press release to news organizations worldwide, which may very well include details of your work. We are happy for your institution or funding agency to prepare its own press release, but it must mention the embargo date and Nature Structural & Molecular Biology. If you or your Press Office have any enquiries in the meantime, please contact press@nature.com.

You can now use a single sign-on for all your accounts, view the status of all your manuscript submissions and reviews, access usage statistics for your published articles and download a record of your refereeing activity for the Nature journals.

If you have not already done so, we strongly recommend that you upload the step-by-step protocols used in this manuscript to the Protocol Exchange. Protocol Exchange is an open online resource that allows researchers to share their detailed experimental know-how. All uploaded protocols are made freely available, assigned DOIs for ease of citation and fully searchable through nature.com. Protocols can be linked to any publications in which they are used and will be linked to from your article. You can also establish a dedicated page to collect all your lab Protocols. By uploading your Protocols to Protocol Exchange, you are enabling researchers to more readily reproduce or adapt the methodology you use, as well as increasing the visibility of your protocols and papers. Upload your Protocols at www.nature.com/protocolexchange/. Further information can be found at www.nature.com/protocolexchange/about.

An online order form for reprints of your paper is available at https://www.nature.com/reprints/author-reprints.html. Please let your coauthors and your institutions' public affairs office know that they are also welcome to order reprints by this method.

Please note that we encourage the authors to self-archive their manuscript (the accepted version before copy editing) in their institutional repository, and in their funders' archives, six months after publication. Nature Research Group recognizes the efforts of funding bodies to increase access of the research they fund, and strongly encourages authors to participate in such efforts. For information about our editorial policy, including license agreement and author copyright, please visit www.nature.com/nsmb/ about/ed_policies/index.html

The Author's Accepted Manuscript (the accepted version of the manuscript as submitted by the author) may only be posted 6 months after the paper is published, consistent with our <a

Sincerely,
Ines

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

ORCID 0000-0002-1405-9703