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Cholesterol transfer via endoplasmic reticulum contacts mediates lysosome damage repair

Maja Radulovic, Eva Wenzel, Sania Gilani, Lya Katrine Holland, Alf Håkon Lystad, Santosh Phuyal, Vesa Olkkonen, Andreas Brech, Marja Jäättelä, Kenji Maeda, Camilla Raiborg, and Harald Stenmark

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Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

William Teale, PhD
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Referee #1:

Radulovic et al studied a new mechanism by which lysosomal membrane damage is repaired in ESCRT-independent manner by the formation of contacts between the ER and the lysosome that deliver cholesterol. According to the suggested model, the VAPA/B, an ER protein previously implicated in plasma membrane ER contacts, is needed for the formation of ER-lysosome contacts formed in response to lysosomal membrane damage. ORP1L, a cholesterol-binding protein is recruited to the membrane damage site by interacting with VAPA/B followed by accumulation of cholesterol in the lysosomal membrane. The authors also found that the PtdIns 4-kinase (PI4K2A) rapidly produce PtdIns4P on the damaged lysosomal membrane, serving to recruit ORP1L and cholesterol. The authors report that OSBP, a cholesterol-PtdIns4 transporter is also recruited to the damaged lysosomal membrane and in its absence the membrane repair is inhibited leading to cell death.

Overall, this is an interesting study that provide mechanistic details to the new lysosomal membrane repair process described last month by Tan and Finkel. The authors show a detailed lipid analysis of isolated control and damaged lysosomes. They show that ORP1L-mediated cholesterol transfer is essential to the repair process and showed that its recruitment to the damaged lysosomal membrane is regulated by PI4K2A and the accumulation of PtdIns4P on these membranes. In addition, the authors present data supporting the hypothesis that similar to its role in ER-Golgi contacts, OSBP may act to transfer cholesterol from the ER to the damaged lysosomal membrane in exchange to PtdIns4P.

There are few minor issues that require the authors' attention:
1. The data presented in Fig.2, showing the contacts between the ER and the damaged lysosomes is very exciting. The authors also tested for the effect of VAP knockout and claim that it prevents such contacts (Fig. EV4). It is important that this data should be quantified (similarly to that shown in Fig. 2B) and presented as part of Fig. 2. It would also be important to verify the presence of VAP proteins in the contacts shown in figure 2.
2. The authors should challenge their model by looking for VAP proteins in their purified lysosomes obtained from cells lacking (knockdown or knockout) either PI4K2A, ORP1L and or OSBP. Alternatively, biochemical approaches should be utilized to show interaction between VAPA/B and ORP1L or OSBP.
3. In the survival assay shown in Fig. 8C the authors describe LLOMe treatments of 0-180 min. It would be more suitable to test longer treatment periods.
4. Finally, and most importantly, the authors should better describe in the Discussion section the similarities and the differences between their and Tan and Finkel reports.

EMBOJ-2022-112677, response to reviewer's comments

Reviewer comments in black font, response in blue.

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We thank the reviewer for the excellent comments, and we have now quantified the EM data for the VAP double knockout cells as suggested. Indeed, the quantifications confirmed that there is no increase in ER-lysosome contact sites in VAP double knockout cells. Because of the importance of this result, we moved the complete VAP data set to a revised Figure 2C.

Regarding the presence of VAP proteins at the ER-Lysosome contacts, we performed immunofluorescence microscopy and detected co-occurrence between LAMP1 and VAPA positive lysosomes after 10 min of LLOMe treatment:

Ideally, we would have liked to confirm this result by immunoelectron microscopy, but the lack of antibodies suited for immunoelectron microscopy precluded this.

2. The authors should challenge their model by looking for VAP proteins in their purified lysosomes obtained from cells lacking (knockdown or knockout) either PI4K2A, ORP1L and or OSBP. Alternatively, biochemical approaches should be utilized to show interaction between VAPA/B and ORP1L or OSBP.

We tested the purity of immunoprecipitated lysosomes by excluding contamination from the ER membranes (as probed by Calnexin shown in Fig EV1). Since VAP proteins are ER-resident, we did not look for them in the purified lysosomal fractions. In our model, we assume that lysosomes are in contact with the ER membrane and that this contact is mediated by VAP proteins among others. However, we have performed the suggested co-IP experiment with GFP-ORP1L and GFP-OSBP (see figure for reviewer, below).. In agreement with previous publications, we detected interactions of VAPB with both ORP1L and OSBP. Because interactions between VAP proteins and ORP1L and OSBP have been demonstrated in multiple papers previously, we suggest not to include this figure in our manuscript.

3. In the survival assay shown in Fig. 8C the authors describe LLOMe treatments of 0-180 min. It would be more suitable to test longer treatment periods.

We thank the reviewer for this suggestion, but we refrained from performing longer time points since cell viability was already quite impaired in OSBP depleted cells after 3 h of LLOMe treatment. However, we did strengthen our current 3h dataset by performing more experiments to reach higher cell numbers for the quantification.

4. Finally, and most importantly, the authors should better describe in the Discussion section the similarities and the differences between their and Tan and Finkel reports.

As suggested, in the revised manuscript we have better described similarities and differences between our and Tan & Finkel´s studies (second paragraph of the Discussion). Overall, the two papers complement each other well and jointly show the importance of PI4K2A and ER-lysosome contacts in lysosome repair. Differences partially exist in use of methodology (proximity biotinylation and in vitro assays in the Finkel paper, vs lipidomics and electron microscopy in our manuscript), and also in the fact that the Finkel paper focuses on phospholipid transfer whereas our paper focuses on cholesterol transfer. Finally, the Finkel paper identified a potential role for Ca^{2+} in PI4K2A recruitment whereas our manuscript shows a role for OSBP in removal of PtdIns4P on lysosomes and demonstrates its importance for viability.

1st Revision - Editorial Decision **4th Nov 2022**

Dear Harald,

Thank you for submitting a revised version of your manuscript. There are now only a couple of minor revisions to be made. Would you please:

- Format the manuscript as a .doc file with no figures and no track changes
- Change the Conflict of interest section title to "DISCLOSURE AND COMPETING INTERESTS STATEMENT"
- Remove the author contributions section from the manuscript and add names on the AC/CRediT section of our submission website.
- Consider adding Source Data files containing the original photographs of the few western blots shown in the manuscript, and
- Add a synopsis image and text. The image could be take from the manuscript, and the text need only be two sentences and three or four bullet points.

Best wishes,

William

William Teale, PhD
Editor
The EMBO Journal
w.teale@embojournal.org

Use the link below to submit your revision:

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All editorial and formatting issues were resolved by the authors.

2nd Revision - Editorial Decision **7th Nov 2022**

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Corresponding Author Name: Harald Stenmark
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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.
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- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
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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.
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- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
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- 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 χ^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?
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Select "Not Applicable" only when the requested information is not relevant for your study.

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

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

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	HeLa (Kyoto) cells were obtained from D. Gerlich, Institute of Molecular Biotechnology, Wien, Austria. A stable HeLa cell line expressing CHMP4B-eGFP was obtained from Anthony A. Hyman (Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany). The HeLa-VAP-
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	NA
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	The cell lines are routinely tested for mycoplasma infections every sixth week by the cell lab-manager.

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If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgements setion

Design

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Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	NA

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

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Include a statement about **sample size** estimate even if no statistical methods were used.	Not Applicable	NA
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Not Applicable	NA
Include a statement about **blinding** even if no blinding was done.	Not Applicable	NA
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Not Applicable	NA
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	All statistical test are described in figure legends

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If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	Most regents used in this manuscript are security level1, except where we used lentiviruses which are biosecurity level 2.
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	NA

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If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	NA