Extramitochondrial cardiolipin suggests a novel function of mitochondria in spermatogenesis

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September 1, 2018

Re: JCB manuscript #201808131

Dr. Michael Schlame
NYU School of Medicine
550 First Avenue
New York, NY 10016

Dear Dr. Schlame,

Thank you for submitting your Report manuscript entitled "Extramitochondrial cardiolipin suggests a novel function of mitochondria in spermatogenesis" to the Journal of Cell Biology. As part of our normal reviewing procedure, your paper has been evaluated by at least two editors and an editorial statement is provided below. You will see that, in the consensus opinion of our editors, although we are interested in the concepts presented in this study, the manuscript is too preliminary for external review. We have thus decided not to subject your manuscript to a lengthy review process. We would be willing to consider a revised manuscript containing data addressing the detailed editorial comments below, assuming the novelty of the findings has not been compromised in the interim.

Because the Journal of Cell Biology addresses a wide and diverse audience of cell biologists, we must give priority to manuscripts that provide a substantial advance of broad appeal to the cell biology community, even though many others also present interesting and important advances for researchers in a particular field.

I am sorry that our answer on this occasion is not more positive, and I hope that this outcome will not dissuade you from submitting other manuscripts to us in the future.

Thank you for your interest in the Journal of Cell Biology.

With kind regards,

Jodi Nunnari, Ph.D.
Editor-in-Chief
Journal of Cell Biology

Editorial Statement:

This is an intriguing manuscript describing the presence of TPCL (harboring only saturated fatty acids) in acrosomes in spermatozoa. The authors show that this type of CL is also present in a specific subset of mitochondria to which they refer to as condensed. As CL synthase was only found in the mitochondrial fraction, the authors suggest that acrosomal TPCL is derived from condensed mitochondria. Further support for this hypothesis came from proteomic data that identified several mitochondrial proteins in the acrosomal fraction. The authors hypothesize based on these observations that mitochondria contribute to the biogenesis of acrosomes (besides the previously described role of the Golgi). These findings describe intriguing biology but the study

remains largely descriptive and correlative. To be competitive in peer review as a Report, we feel that additional evidence to more directly confirm the acrosomal mitochondrial components are truly derived from mitochondria, such as when and how the transfer happens, is required. Such support does not need to be mechanistic but could be for example EM data, or data indicating that the proteins are in mitochondria prior to transfer, to rule out the possibility of dual targeting.

January 7, 2019

Re: JCB manuscript #201808131R-A

Dr. Michael Schlame
NYU School of Medicine
550 First Avenue
New York, NY 10016

Dear Dr. Schlame,

Thank you for submitting your manuscript entitled "Extramitochondrial cardiolipin suggests a novel function of mitochondria in spermatogenesis". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

Your manuscript has now been seen by two experts who both agree on the novelty and potential interest of your study. However, they raise several issues that need to be addressed before we can make a final decision. Rev 1 raises questions concerning quantitative aspects (point 1) and the requirement of mitochondrial proteins for acrosome formation (point 3). Although a detailed mechanistic analysis is beyond the scope of a Report, we feel that you should discuss these points in your manuscript. This is deemed important to us as both ANT4 KO and TAZ-KO mice (largely) lack TPLC but apparently do not show male sterility. Moreover, it is key to address Rev 2´s concerns on the sample size and the statistical evaluation of your data (points 3, 4, 5 and 8). Please provide a point-by-point response to all comments of the reviewers and adjust your manuscript according to the guidelines for JCB Reports.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:
Text limits: Character count for a Report is < 20,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Reports may have up to 5 main text figures. To avoid delays in production, figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data.

Reports may have up to 3 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

Our typical timeframe for revisions is three months; if submitted within this timeframe, novelty will not be reassessed at the final decision. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Thomas Langer, Ph.D.
Monitoring Editor

Marie Anne O'Donnell, Ph.D.
Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

This is a fascinating study that provides strong evidence that a mitochondrial lipid and few mitochondrial proteins traffic to acrosomes and could be necessary for acrosome formation. This is an important and surprising finding and the study is well done and convincing. As the authors point out, there is no insight into the mechanism. Since this will require substantial additional work, the study could be more appropriate as a report instead of an article. There are a few, mostly minor issues to be addressed.

1. What percent of acrosomal protein and lipid is derived from the mitochondria?

2. It is premature to draw conclusions about how mitochondrial proteins reach the acrosome based on the static immunofluorescence images shown in Figure 5e. Also, what is meant by the Golgi phase of acrosome formation should be more fully explained.

3. Are the mitochondrial proteins in the acrosome intact and functional? Supplementary Fig. 6 suggests they may not be intact, but the image is confusing, and it is not clear why the authors believe the band indicated with the arrow is full-length protein or what the other bands are. If some of the mitochondrial proteins in acrosomes are degraded, then it seems possible that the mitochondrial proteins may not be required for acrosome formation.

4. The "atypical fragmentation of the matrix" shown in Fig. 5c is not obvious to me. The authors should use arrows to indicate the atypical structures and, if possible, quantify how frequently they are observed.

5. It is not clear what is being shown in Fig. 2c. It should be explained so that a reader not used to looking at spermatids can make sense of the images. Shouldn't all the structures stained with MitoTracker also be stained by Hoechst? Can the percent of NAO-positive, MitoTracker-negative structure be determined?

Reviewer #2 (Comments to the Authors (Required)):

This review constitutes critical analysis of an interesting and innovative study on cardiolipin (CL) and tetrapalmitoyl CL in human and murine sperm and spermatogenic cells. The former is highly unsaturated while the latter is a fully-saturated cardiolipin (CL) species that had previously been reported in rats to be found exclusively in the testes with implications to membrane fluidity, integrity, and function. The manuscript details a quite dense comprehensive and well-written study in which the authors conclude that mitochondria-derived membranes are assembled into the sperm's acrosome. The study uses several novel approaches and techniques that localize TPCL in sperm and spermatogenic cells. Very interestingly, the membranes with TPCL did not contain Cl synthase, indicating that the remodeling and unsaturation of palmitoyl chains apparently occurs prior to TPCL appearance in membranes. Further, TAZ-KO abolished formation of TPCL. While this manuscript addresses a current knowledge gap with a current burgeoning interest across many fields, there are major revisions that must be addressed before a more thorough critical review can be performed. The issues to be addressed are not a comprehensive list, but the above information is required for further critical review of this manuscript.

Major points to be addressed:
1. The introduction should be revised to clarify the functional implications TPCL versus CL.

2. The source listed for the exclusive synthesis and storage of CL in mitochondria is a review only focusing on mitochondrial CL, so I would recommend the authors include sources that also review the presence of CL species outside of the mitochondria, particularly in relative to post-synthesis modification.

3. The n values must be included for all experiments performed. Additionally, please specify whether n is referring to each testis, tissue from separate samples, etc. Any experimental replicates should be clearly noted.

4. Justification for only using a singular testicular tissue sample for the human tissue analysis. Were any replicates run? If lacking replicates and convincing justification of the results' validity, including mention of potential confounding effects of male-to-male differences, then the human tissue portion of this manuscript should be excluded.

5. All figure legends should include n, species, and mouse strain. Figure legends should all also include the methodologies used to generate the presented data.
6. Controls that were run should be clearly listed for all experiments. For example, what controls were included for fluorescence microscopy? For flow cytometry? What about controls for the KO models?

7. All probes and markers used for the various experimental techniques outlined in this manuscript must all be optimization and validated in the same species. At minimum, the approach used by this group for optimization and validation should be mentioned. If optimized and validated previously by another group, indicate such and include the appropriate citation.

8. A data and statistical analysis section is needed.

9. This manuscript lacks information regarding the statistical significance of results. When applicable, figures, such has bar charts, should include a symbol (*) to indicate significance. Statistical reporting should also be incorporated into the RESULTS section text. For example, when stating that there was a "correlation" between two parameters, the statistical method used for correlational analysis must be provided and appropriate statistics and significance values should be reported.

10. It would be helpful if data and distribution statistics for purity of testicular cell populations were included in the supplemental materials. Does purity impact results interpretation?

11. It needs to be clarified whether epididymal sperm evaluated were exclusively from the cauda or the entirety of the epididymal occupying sperm. Significant membrane remodeling, motility acquisition, and many other changes are well-documented through epididymal transit, which could result in high cell-to-cell variation and complicate results interpretation. Was this addressed at all?

12. Methods:
Specimens: more information needed to define epididymal isolations: How many mice (sample numbers) were used? Were samples from whole epididymis or just tails? This is critical to understanding due to known lipid redistribution associated with plasma membrane maturation during epididymal maturation and transit. Authors must address adequately as this is critical to making conclusions about lipid content and distribution during and following spermiogenesis.

13. Not mentioned in methods are age groups of mice although data is shown and described in Fig legend for Fig1. Need more description of mouse testicular ages and why selected in Methods

14. Was the CL synthase assay validated for mouse, or referenced to mouse testis? What was the sensitivity and specificity of the assay used?

Re: JCB manuscript #201808131R-A

January 28, 2019

Dear Dr. Langer and Dr. O'Donnell:
Thank you for reviewing our manuscript entitled "Extramitochondrial cardiolipin suggests a novel function of mitochondria in spermatogenesis". We are excited that the reviewers found our work interesting and we appreciate their comments and criticism.

In response to the critique, we made revisions in the manuscript, which are highlighted in yellow. We also prepared a point-by-point reply addressing each of the issues raised by the two reviewers.

We would like to express our gratitude to the reviewers for their insightful comments and we would like to thank you for your work on this submission.

With kind regards,

Michael

Editorial comments
1. Rev 1 raises questions concerning quantitative aspects (point 1) and the requirement of mitochondrial proteins for acrosome formation (point 3). Although a detailed mechanistic analysis is beyond the scope of a Report, we feel that you should discuss these points in your manuscript. This is deemed important to us as both ANT4 KO and TAZ-KO mice (largely) lack TPLC but apparently do not show male sterility.
The questions by reviewer 1 are addressed below. We would like to point out though that both ANT4-KO and TAZ-KO are male sterile. This is now clearly spelled out in the Discussion of the revised manuscript.

2. Moreover, it is key to address Rev 2´s concerns on the sample size and the statistical evaluation of your data (points 3, 4, 5 and 8). Please provide a point-by-point response to all comments of the reviewers and adjust your manuscript according to the guidelines for JCB Reports.
All concerns of reviewer 2 are addressed below.

Reviewer #1
This is a fascinating study that provides strong evidence that a mitochondrial lipid and few mitochondrial proteins traffic to acrosomes and could be necessary for acrosome formation. This is an important and surprising finding and the study is well done and convincing. As the authors point out, there is no insight into the mechanism. Since this will require substantial additional work, the study could be more appropriate as a report instead of an article. There are a few, mostly minor issues to be addressed.

1. What percent of acrosomal protein and lipid is derived from the mitochondria?
TPCL comprised about 6% of lipids in acrosomal rafts and the combined intensities of proteins with mitochondrial annotation in the acrosome was 7% of the proteins with acrosomal annotation. These figures are the best estimate we can give at this point. They are included in the Discussion of the revised manuscript.

2. It is premature to draw conclusions about how mitochondrial proteins reach the acrosome based on the static immunofluorescence images shown in Figure 5e. Also, what is meant by the Golgi phase of acrosome formation should be more fully explained.
We agree. The phases of acrosome formation are explained in the revised manuscript and the images in Fig. 5e are interpreted more carefully, essentially saying that our data tentatively indicate that Ant4 enters the acrosomal compartment during the Golgi phase, the first phase of acrosome biogenesis (last paragraph of Results).

3. Are the mitochondrial proteins in the acrosome intact and functional? Supplementary Fig. 6 suggests they may not be intact, but the image is confusing, and it is not clear why the authors believe the band indicated with the arrow is full-length protein or what the other bands are. If some of the mitochondrial proteins in acrosomes are degraded, then it seems possible that the mitochondrial proteins may not be required for acrosome formation.
The Suox band in Fig. S6 was assigned based on the size of the full-length protein (61 kDa) and the enrichment of this band in mitochondria, which is the established intracellular compartment of Suox. The 75 kDa band present in the blot seems to represent a cross-reacting protein, which is about equally abundant in all fractions. We agree that Suox degradation products may be present in the acrosomes but we feel that it is premature to speculate about any functional implication because (i) Suox may have a different function in the acrosome than in mitochondria and (ii) despite the degradation products, full length Suox is still the dominant band in the acrosome. Revisions were made in the legend of Figure S6 and in the Discussion to address these issues.

4. The "atypical fragmentation of the matrix" shown in Fig. 5c is not obvious to me. The authors should use arrows to indicate the atypical structures and, if possible, quantify how frequently they are observed.
The arrow was added to Fig. 5c. The “atypical fragmentation” is perhaps better described as “incomplete matrix contraction”, the expression we use now in the revised manuscript. In order to quantify this phenomenon, we analyzed 11 electron micrographs, in which we found that 198 out of 217 intermediate mitochondria (91.2%) showed incomplete matrix contraction. This is stated in the Results section of the revised manuscript (p. 6).

5. It is not clear what is being shown in Fig. 2c. It should be explained so that a reader not used to looking at spermatids can make sense of the images. Shouldn't all the structures stained with MitoTracker also be stained by Hoechst? Can the percent of NAO-positive, MitoTracker-negative structure be determined?
Fig. 2c shows two round spermatids. They were stained with Hoechst (specific for nuclei), MitoTracker (specific for mitochondria), and NAO (specific for CL). This is now better explained in the legend. In 19 cells, we observed 638 NAO-positive organelles, of which 38

(6.0%) did not stain with MitoTracker (stated in the Results section, p. 4).

Reviewer #2

This review constitutes critical analysis of an interesting and innovative study on cardiolipin (CL) and tetrapalmitoyl CL in human and murine sperm and spermatogenic cells. The former is highly unsaturated while the latter is a fully-saturated cardiolipin (CL) species that had previously been reported in rats to be found exclusively in the testes with implications to membrane fluidity, integrity, and function. The manuscript details a quite dense comprehensive and well-written study in which the authors conclude that mitochondria-derived membranes are assembled into the sperm's acrosome. The study uses several novel approaches and techniques that localize TPCL in sperm and spermatogenic cells. Very interestingly, the membranes with TPCL did not contain Cl synthase, indicating that the remodeling and unsaturation of palmitoyl chains apparently occurs prior to TPCL appearance in membranes. Further, TAZ-KO abolished formation of TPCL. While this manuscript addresses a current knowledge gap with a current burgeoning interest across many fields, there are major revisions that must be addressed before a more thorough critical review can be performed. The issues to be addressed are not a comprehensive list, but the above information is required for further critical review of this manuscript.

Major points to be addressed:

1. The introduction should be revised to clarify the functional implications TPCL versus CL.

The Introduction was revised: “Mitochondrial CL is known to be one of the most fluid phospholipids in contrast to TPCL that remains in the gel phase at body temperature (Lewis & McElhaney, 2009) and therefore is expected to segregate from mitochondrial CL.”

2. The source listed for the exclusive synthesis and storage of CL in mitochondria is a review only focusing on mitochondrial CL, so I would recommend the authors include sources that also review the presence of CL species outside of the mitochondria, particularly in relative to post-synthesis modification.

We added 4 citations, in which extramitochondrial CL is mentioned on p. 4: “Although there have been rare reports of extramitochondrial CL in the peroxisomes of yeast (Zinser et al, 1991; Wriessnegger et al, 2007) and in the plasma membrane of apoptotic cells (Sorice et al, 2000; 2004), their significance has remained unclear.” None of these papers addresses issues related to post-synthetic modifications. We are not aware of any additional papers on extramitochondrial CL.

3. The n values must be included for all experiments performed. Additionally, please specify whether n is referring to each testis, tissue from separate samples, etc. Any experimental replicates should be clearly noted.

The N values are now listed in the legends of the revised manuscript. In all cases, replicas represent measurements in independent animals. This is stated in the new Data and Statistical Analysis section of the revised manuscript.

4. Justification for only using a singular testicular tissue sample for the human tissue analysis. Were any replicates run? If lacking replicates and convincing justification of the results' validity,

including mention of potential confounding effects of male-to-male differences, then the human tissue portion of this manuscript should be excluded.
The human testis biopsy was purchased from Proteogenex. Only one specimen was obtained. We verified the presence of TPCL in this sample by two different mass spectrometry methods (MALDI-TOF and LC-MS/MS). We agree that variations between individuals are likely to be present. However, we did not make any statements about the quantity of TPCL in human testis, we only pointed out that it exists because it makes our work more relevant. In light of that, we prefer to keep the human data in Figure S1.

5. All figure legends should include n, species, and mouse strain. Figure legends should all also include the methodologies used to generate the presented data.
The N values are now listed in the legends of the revised manuscript and the methods are better explained (see highlighted parts). The mouse strains are specified in the first two paragraphs of the Materials and Methods section.

6. Controls that were run should be clearly listed for all experiments. For example, what controls were included for fluorescence microscopy? For flow cytometry? What about controls for the KO models?
Immunofluorescence images obtained without primary antibodies (secondary antibodies only) served as negative control. Wild-type mice of the same genetic background (C57BL/6) were the controls for the two KO mouse models. Flow cytometry was performed by an established Hoechst dye-based method that separates germ cells by size and DNA content (Bastos et al, 2005; Getun et al, 2011; Gaysinskaya et al, 2014). The cell populations are different from each other because they have different lipid profiles (Fig. 1f). For further comments on flow cytometry, please refer to the answer to point 10. All this information is specified in the Methods section of the revised manuscript.

7. All probes and markers used for the various experimental techniques outlined in this manuscript must all be optimization and validated in the same species. At minimum, the approach used by this group for optimization and validation should be mentioned. If optimized and validated previously by another group, indicate such and include the appropriate citation.
All fluorescence dyes were applied according to published protocols. A new reference is included in the Methods section of the revised paper (Keij et al, 2000). Primary antibodies were applied according to the manufacturer's instructions, typically at a dilution of 1:200, which is now stated in the revised manuscript.

8. A data and statistical analysis section is needed.
A Data and Statistical Analysis paragraph has been included at the end of the Methods section. It reads: "Continuous variables are presented as mean values with the standard error of the mean (SEM). The number of replicas is given in the legends of the figures. Replicas refer to measurements made in separate animals. Mean values were compared by Student's t-test or by analysis of variance (ANOVA). Distributions between different types of mitochondria or cells were performed by the chi-squared test. For each genotype, the distribution data were collected from >30 electron micrographs prepared from 4 testes (2 separate animals)."

9. This manuscript lacks information regarding the statistical significance of results. When

applicable, figures, such has bar charts, should include a symbol (*) to indicate significance. Statistical reporting should also be incorporated into the RESULTS section text. For example, when stating that there was a "correlation" between two parameters, the statistical method used for correlational analysis must be provided and appropriate statistics and significance values should be reported.
In the revised manuscript, we included rigorous statistical testing. The statistical significances (*P* values) are presented in the revised figures or in their legends rather than in the text to keep the manuscript succinct. We used Student's t-test, the chi-squared test, and ANOVA as warranted by the type of data. Correlation analyses were not performed.

10. It would be helpful if data and distribution statistics for purity of testicular cell populations were included in the supplemental materials. Does purity impact results interpretation?
We used flow cytometry according to Bastos et al (Cytometry 65A:40-49, 2005) to separate germ cells but did not further analyze their purity. Getun et al (EMBO Report 11:555-560, 2010), using the same method, found little cross-contamination between primary and secondary spermatocytes. Although this obviously does not exclude cross contaminations in our experiments, the precise degree of purity of the germ cell populations (Fig. 1f) is not critical for our study. The conclusion from the flow cytometry experiment (Fig. 1f), which is that the abundance of TPCL increases along the maturation path from spermatogonia to spermatids, would still be valid even if the purities of the cell populations were low. Furthermore, the increase of TPCL with increasing maturity is supported by the data in Figs. 1c and 1e.

11. It needs to be clarified whether epididymal sperm evaluated were exclusively from the cauda or the entirety of the epididymal occupying sperm. Significant membrane remodeling, motility acquisition, and many other changes are well-documented through epididymal transit, which could result in high cell-to-cell variation and complicate results interpretation. Was this addressed at all?
We collected sperm from the entire epididymis. This is stated in the revised manuscript. When we performed the experiments, we were not aware of the differences between sperm from different parts of the epididymis, but we appreciate the reviewers comment. It is therefore possible that the concentration of mitochondrial molecules (TPCL and mitochondrial proteins) changes during the passage of sperm through the epididymis. However, we did not address this topic and believe that it should be left to future studies. The results obtained in sperm collected from the epididymis are consistent with the results obtained in spermatids collected from the testes, suggesting that mitochondrial molecules are assembled into the acrosome in spermatids and remain present at least until spermatozoa reach the epididymis. Any effect of further maturation of sperm on the acrosome composition is beyond the scope of this report.

12. Methods:
Specimens: more information needed to define epididymal isolations: How many mice (sample numbers) were used? Were samples from whole epididymis or just tails? This is critical to understanding due to known lipid redistribution associated with plasma membrane maturation during epididymal maturation and transit. Authors must address adequately as this is critical to making conclusions about lipid content and distribution during and following spermiogenesis.
The method of sperm isolation from the epididymis is better described in the revised paper. Sperm were isolated from the whole epididymis (see answer to point 11). Specifically, we used 4

separate animals to collect sperm for Fig. 1f and we used 3 separate animals to collect sperm for Fig. 2e. The images in Fig. 4 and Supplemental Fig. 4 were selected from experimental data obtained in 5 separate animals.

13. Not mentioned in methods are age groups of mice although data is shown and described in Fig legend for Fig1. Need more description of mouse testicular ages and why selected in Methods

Unless stated otherwise (Fig. 1c), mice were 3-4 months old. This is specifically mentioned on p. 9 of the revised manuscript. At this age they have reached full sexual maturity yet are still far removed from senescence. We used a tight age range in order to ensure homogeneity among the collected data.

14. Was the CL synthase assay validated for mouse, or referenced to mouse testis? What was the sensitivity and specificity of the assay used?

The CL synthase assay is a mass spectrometric method, in which the signal intensity of CL 64:2, the species formed in the assay, was measured relative to the intensities of internal standards. The method was validated specifically for mouse testicular tissue in order to ensure that there was no interfering endogenous peak near m/z=1347.9332, the mass/charge ratio of the M-H ion of CL 64:2. In terms of sensitivity, we found the lower limit of detection to be 1-2 pmol/min/mg protein based on the day-to-day noise level in the spectra. In terms of specificity, we measured a mass resolution of >2700 and a mass accuracy of <70 ppm. The latter two parameters are typical for MALDI-TOF instruments. We included a brief statement regarding assay performance on p. 14 of the revised manuscript.

Again, we would like to express our appreciation for the insightful comments provided by the two reviewers.

February 8, 2019

RE: JCB Manuscript #201808131RR

Dr. Michael Schlame
NYU School of Medicine
550 First Avenue
New York, NY 10016

Dear Dr. Schlame:

Thank you for submitting your revised manuscript entitled "Extramitochondrial cardiolipin suggests a novel function of mitochondria in spermatogenesis". We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

- Please provide main and supplementary text as separate, editable .doc or .docx files
- Provide figures as separate, editable files according to the instructions for authors, paying particular attention to the guidelines for preparing blots and images at sufficient resolution for screening and production
- Format references for JCB
- Add scale bar to Figure 3f, 5a?
- Reports have combined results and discussion sections and a limit of 20000 characters - the current format is closer to an Article
- There is a maximum of five supplementary figures for an Article, figures S3 and S4; S5 and S6 could be combined?
- Provide all tables as excel files
- Add conflict of interest statement to Acknowledgements section

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, http://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB's Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure and video files: See our detailed guidelines for preparing your production-ready images, http://jcb.rupress.org/fig-vid-guidelines.

-- Cover images: If you have any striking images related to this story, we would be happy to consider them for inclusion on the journal cover. Submitted images may also be chosen for highlighting on the journal table of contents or JCB homepage carousel. Images should be uploaded as TIFF or EPS files and must be at least 300 dpi resolution.

It is JCB policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.

The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements before choosing the appropriate license.

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days.

Please contact the journal office with any questions, cellbio@rockefeller.edu.

Thank you for this interesting contribution, we look forward to publishing your paper in the Journal of Cell Biology.

Sincerely,

Thomas Langer, Ph.D.
Monitoring Editor

Marie Anne O'Donnell, Ph.D.
Scientific Editor

Journal of Cell Biology
