

Karyopherin compensation fortifies the nuclear pore complex against nucleocytoplasmic leakage

Joanna Kalita, Larisa Kapinos, Tiantian Zheng, Chantal Rencurel, Anton Zilman, and Roderick Lim

Corresponding Author(s): Roderick Lim, University of Basel

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October 8, 2021

Re: JCB manuscript #202108107

Prof. Roderick Lim
University of Basel
Biozentrum
Spitalstrasse 41
Basel CH4056
Switzerland

Dear Prof. Lim,

Thank you for submitting your manuscript entitled "Karyopherin compensation fortifies the nuclear pore complex against nucleocytoplasmic leakage." 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.

You will see that overall the reviewers appreciate the detailed quantitative assessment of the roles of karyopherins on NPC permeability. Reviewers #1 & 2 have fairly minor comments that we believe can be easily addressed by text revisions. However, Reviewer #3 points out that the effects of Kap β 1 depletion are subtle and that different Kap β 1 siRNA's seem to have opposite effects and requests that you test effects of other siRNAs and add rescue experiments. We agree and ask that you address these comments in full. This reviewer also feels that the claim that "Kap β 1 and CRM1 engage in a balancing act to reinforce NPC barrier function" is somewhat overstated since depletion of CRM1 did not have a significant effect and its role was not tested in all assays. If you have data in hand or can do the experiments to clarify the role of CRM1 in a reasonable timeframe, we strongly encourage you to do so as it would substantially enhance the paper. If this is not possible then please tone down claims regarding CRM1.

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 an Article is < 40,000, 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: Articles may have up to 10 main text figures. Figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, <https://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.***

Please note that JCB now requires authors to submit Source Data used to generate figures containing gels and Western blots with all revised manuscripts. This Source Data consists of fully uncropped and unprocessed images for each gel/blot displayed in the main and supplemental figures. Since your paper includes cropped gel and/or blot images, please be sure to provide one Source Data file for each figure that contains gels and/or blots along with your revised manuscript files. File names for Source Data figures should be alphanumeric without any spaces or special characters (i.e., SourceDataF#, where F# refers to the associated main figure number or SourceDataFS# for those associated with Supplementary figures). The lanes of the gels/blots should be labeled as they are in the associated figure, the place where cropping was applied should be marked (with a box), and molecular weight/size standards should be labeled wherever possible. Source Data files will be made available to reviewers during evaluation of revised manuscripts and, if your paper is eventually published in JCB, the files will be directly linked to specific figures in the published article.

Source Data Figures should be provided as individual PDF files (one file per figure). Authors should endeavor to retain a minimum resolution of 300 dpi or pixels per inch. Please review our instructions for export from Photoshop, Illustrator, and PowerPoint here: <https://rupress.org/jcb/pages/submission-guidelines#revised>

Supplemental information: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 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.

As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of measures that limit spread of COVID-19 also pose challenges to scientific researchers. Therefore, JCB has waived the revision time limit. We recommend that you reach out to the editors to decide on an appropriate time frame for resubmission. 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,

Larry Gerace, PhD
Monitoring Editor
Journal of Cell Biology

Dan Simon, PhD
Scientific Editor
Journal of Cell Biology

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

Nuclear pore complexes (NPCs) are enriched with nuclear transport receptors (NTRs, or Kaps). This paper asks how their presence impacts on the NPC barrier. They assess importin β 1/karyopherin β 1 (Kap β 1), Importin-5 and exportin 1/chromosomal maintenance 1 (CRM1). The authors use permeabilized cells and replace the natively present NTRs for fluorescently-labeled NTRs allowing them to quantify the amount of replaced NTRs dependent on the concentration provided alone or in competition with a second NTR. They report that Kap β 1, CRM1, and Imp5 bind to NPCs in a concentration-dependent manner and that exoKap β 1 exhibits a higher propensity to outcompete against exoImp5 than exoCRM1, which is comparable to the competition between exoImp5 and exoCRM1. The paper shows that CRM1 can compensate for the loss of Kap β 1. They complement these studies with SPR measurements of the binding affinities of Crm1 and Imp5 with a panel of FG-Nup domains (Nup62, Nup98, Nup153 and Nup214) showing that Imp5 exhibits a faster koff in the slow phase than Kap β 1 and CRM1 consistent with its low enrichment in NPCs in vivo. Following this, the authors use a theoretical model which matched the experimental data showing that the occupancy of NTRs depends on their sizes, abundances and affinities. Finally a set of studies assess the effect of siRNA knockdown of Kap β 1 and Crm1 measuring if other NTRs replace the lost NTR at the NPC and measuring the impact on the permeability barrier using xGFP-NES xGFP-NLS or xGFP and measuring steady state localizations and FRAP. These experiments confirm that Kap enrichment fortifies the NPC permeability barrier in vivo. The final figure is very useful to sum up the insights.

In sum this carefully constructed and executed study which allows for a well-controlled quantitative assessment of NTRs in NPCs and how this impacts transport. The level of detail is much higher than in previous studies and this is the main merit of the paper. Overall the paper is a bit less strong in terms of revealing novel insight. The authors also write so them selves "This validates previous studies in permeabilized cells which showed that depleting endoKap β 1 abrogated NPC barrier function whereas adding back exoKap β 1 rescued it (Kapinos et al., 2017). Likewise, adding Kap β 1 (Lowe et al., 2015) and transportin (Mohr et al., 2009) further reduced NPC permeability against passive cargoes."

I have no concerns related to the experimental methods or interpretation of the data. I would only suggest that the authors comment more on the finding in figure 1 where the localization of exoImp5 is dissimilar from what is seen in-vivo. A reader may wonder if this may mean that the permeabilized cell assay has major problems (as for 1 out of 3 NTRs the localization of the endo-kap is different from that of the exo-kap).

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

The internal workings of the nuclear pore complex with multiple transport factors is resistant to many experimental approaches. Here the Lim lab concentrates on how different karyopherins can cross interact with each other. They use a combination of

fluorescent tagging on permeabilized cells, surface plasmon resonance, and siRNA knockdowns to establish differing levels and cross interference between three transport factors.

The data certainly fully support the cross talk between this set of factors and illustrate an important approach to the problem. In the discussion, the observations are not clearly linked to the extensive discussion of aging and disease states, and the authors should consider tightening up their logical flow in this area.

In addition, the authors should consider in any revision...

- a. p2 | 9. 'fortify' I suggest 'strengthen' is more appropriate or even 'maintain'
- b. p 2 | 12 'balancing act' this seems an extreme analogy. It seems obvious that Kap concentrations will effect the occupancy of the FG Nup sites and passively inhibit each other.
- c. p 3 | 10. 'also show to regulate' not clear
- d. p 4 | 4-6. Authors should be clear that the numbers are their estimates. The papers cited do not provide the information.
- e. p 4 | 24. It would be better to provide references to the presence of Kaps in the NPC, including the authors own, and {Kim, 2018, 29539637}
- f. p 16 | 13 on tp p17 . Links of the results obtained and the pathologies described should be made clearer (if any).
- g. p 20 | 11. It's bad to describe a molecule solely by its atomic composition. And less than 1% of JCB readers will identify triethylene glycol mono-11-mercatooundecyl ether.

I complement the authors on the experimental detail.

Reviewer #3 (Comments to the Authors (Required)):

This paper by Kalita et al characterizes the nature of the selectivity barrier of the nuclear pore complex (NPC). The central finding is that nuclear transport factors (NTFs) of the karyopherin family play an important role in the establishment of the permeability barrier of the NPC and work in conjunction with FG nucleoporins. Earlier papers from, e.g., the Rout and Weis groups had already suggested this (Jovanovic-Talisman et al. 2009; Lowe et al., 2015) and also a previous paper from the Lim group (Kapinos et al., 2017) came to this conclusion. Whereas these prior manuscript mainly relied on in vitro assays (either reconstituted in vitro systems or assays with permeabilized cells), the strength of this papers is that it addresses the role of NTFs in tissue culture cells using siRNA knock-downs. The key findings of the manuscript are reported in Figure 7 and 8. Unfortunately, some of these results are not very convincing.

1. The effects of Kapb1 depletion are very subtle and some are barely significant (Fig 7 A, B; Fig 8). Can they be rescued by expression of an siRNA-resistant construct?
2. It is disturbing that the siRNA treatment that has the strongest effect in Fig 7A, B (siRNA 1 55pmol), has no effect in the diffusion assay in Fig 8. Somewhat the opposite is true for siRNA 2 (110pmol). How do the authors explain that? It would be important to add additional experiments with other siRNAs and perform rescue experiments (see point 1)
3. One major conclusion of the paper in the abstract is that "Kap β 1 and CRM1 engage in a balancing act to reinforce NPC barrier function". However, depletion of CRM1 has no significant effects on the barrier (Fig 7C) and wasn't tested in Fig 8, thus this conclusion is not supported by the results.

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

Nuclear pore complexes (NPCs) are enriched with nuclear transport receptors (NTRs, or Kaps). This paper asks how their presence impacts on the NPC barrier. They assess importin β 1/karyopherin β 1 (Kap β 1), Importin-5 and exportin 1/chromosomal maintenance 1 (CRM1). The authors use permeabilized cells and replace the natively present NTRs for fluorescently-labeled NTRs allowing them to quantify the amount of replaced NTRs dependent on the concentration provided alone or in competition with a second NTR. They report that Kap β 1, CRM1, and Imp5 bind to NPCs in a concentration-dependent manner and that exoKap β 1 exhibits a higher propensity to outcompete against exoImp5 than exoCRM1, which is comparable to the competition between exoImp5 and exoCRM1. The paper shows that CRM1 can compensate for the loss of Kap β 1. They complement these studies with SPR measurements of the binding affinities of Crm1 and Imp5 with a panel of FG-Nup domains (Nup62, Nup98, Nup153 and Nup214) showing that Imp5 exhibits a faster koff in the slow phase than Kap β 1 and CRM1 consistent with its low enrichment in NPCs in vivo. Following this, the authors use a theoretical model which matched the experimental data showing that the occupancy of NTRs depends on their sizes, abundances and affinities. Finally a set of studies assess the effect of siRNA knockdown of Kap β 1 and Crm1 measuring if other NTRs replace the lost NTR at the NPC and measuring the impact on the permeability barrier using xGFP-NES xGFP-NLS or xGFP and measuring steady state localizations and FRAP. These experiments confirm that Kap enrichment fortifies the NPC permeability barrier in vivo. The final figure is very useful to sum up the insights.

In sum this carefully constructed and executed study which allows for a well-controlled quantitative assessment of NTRs in NPCs and how this impacts transport. The level of detail is much higher than in previous studies and this is the main merit of the paper. Overall the paper is a bit less strong in terms of revealing novel insight. The authors also write so themselves "This validates previous studies in permeabilized cells which showed that depleting endoKap β 1 abrogated NPC barrier function whereas adding back exoKap β 1 rescued it (Kapinos et al., 2017). Likewise, adding Kap β 1(Lowe et al., 2015) and transportin(Mohr et al., 2009) further reduced NPC permeability against passive cargoes." I have no concerns related to the experimental methods or interpretation of the data. I would only suggest that the authors comment more on the finding in figure 1 where the localization of exoImp5 is dissimilar from what is seen in-vivo. A reader may wonder if this may mean that the permeabilized cell assay has major problems (as for 1 out of 3 NTRs the localization of the endo-kap is different from that of the exo-kap).

Thank you. To clarify, exoImp5 enriches NPCs in permeabilized cells due to the lack of other Kaps following Ran mix treatment (Fig. 2D). In comparison, Imp5 is outcompeted by other Kaps *in vivo* (Fig. 1A and B) and in pairwise binding assays with Kap β 1 (Fig. 3D). We have placed further emphasis on this stating:

P6L20 “We hypothesized that this behavior could arise from the absence of other Kaps which compete with Imp5 *in vivo* (see next section).”

This refers to the following sentences:

P8L4 “Moreover, the qualitative trends suggest that exoKap β 1 exhibits a higher propensity to outcompete against exoImp5 than exoCRM1, which is comparable to the competition between exoImp5 and exoCRM1.”

P16L4 “This may explain why Imp5 lacks enrichment *in vivo* (**Fig. 1**) and why more than 80% of its standalone pool is lost when Kap β 1 is present *ex vivo* (**Fig. 3D**).”

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

The internal workings of the nuclear pore complex with multiple transport factors is resistant to many experimental approaches. Here the Lim lab concentrates on how different karyopherins can cross interact with each other. They use a combination of fluorescent tagging on permeabilized cells, surface plasmon resonance, and siRNA knockdowns to establish differing levels and cross interference between three transport factors. The data certainly fully support the cross talk between this set of factors and illustrate an important approach to the problem.

In the discussion, the observations are not clearly linked to the extensive discussion of aging and disease states, and the authors should consider tightening up their logical flow in this area.

In addition, the authors should consider in any revision...

a. P2 I 9. 'fortify' I suggest 'strengthen' is more appropriate or even 'maintain'

'Fortify' and 'strengthen' are synonyms, and we would prefer to keep the wording as it is.

b. P2 I12 'balancing act' this seems an extreme analogy. It seems obvious that Kap concentrations will effect the occupancy of the FG Nup sites and passively inhibit each other.

Agree. We have revised the abstract as follows:

P2L9-13 "Their enrichment at the NPC is sustained by promiscuous binding interactions with the FG Nups, which enable CRM1 to compensate for the loss of Kap β 1 as a means to reinforce NPC barrier function. However, such a compensatory mechanism is constrained by the cellular abundances and different binding kinetics for each respective Kap as evidenced for Importin-5."

c. p 3 I 10. 'also show to regulate' not clear

We have removed this sentence.

d. P 4 I 4-6. Authors should be clear that the numbers are their estimates. The papers cited do not provide the information.

No change. MD simulations by Isgro 2005 had estimated 10 FG-repeat binding pockets on Kap β 1 out of which 4 reproduced experimental observations (Bayliss 2000; Bednenko 2003).

e. P 4 I 24. It would be better to provide references to the presence of Kaps in the NPC, including the authors own, and {Kim, 2018, 29539637}

Done.

f. P 16 I 13 on tp p17 . Links of the results obtained and the pathologies described should be made clearer (if any).

Done. We kindly refer the reviewer to the last two paragraphs of the Discussion that have been reworked.

g. P 20 l 11. It's bad to describe a molecule solely by its atomic composition. And less than 1% of JCB readers will identify triethylene glycol mono-11-mercatoundecyl ether.

Corrected.

P20L3 "Hydroxyl-terminated tri[ethylene glycol] undecane thiol or HS-[CH₂]-[OCH₂CH₂]₃-OH (abbreviated as PUT; Sigma Aldrich Cat. No. 67311) was dissolved in..."

Also, in P20 L19 and 21 we changed "C₁₇H₃₆O₄S" to "PUT".

I complement the authors on the experimental detail.

Thank you for your kind comments and positive feedback.

Reviewer #3 (Comments to the Authors (Required)):

This paper by Kalita et al characterizes the nature of the selectivity barrier of the nuclear pore complex (NPC). The central finding is that nuclear transport factors (NTFs) of the karyopherin family play an important role in the establishment of the permeability barrier of the NPC and work in conjunction with FG nucleoporins. Earlier papers from, e.g., the Rout and Weis groups had already suggested this (Jovanovic-Talisman et al. 2009; Lowe et al., 2015) and also a previous paper from the Lim group (Kapinos et al., 2017) came to this conclusion. Whereas these prior manuscript mainly relied on in vitro assays (either reconstituted in vitro systems or assays with permeabilized cells), the strength of this papers is that it is addresses the role of NTFs in tissue culture cells using siRNA knock-downs.

The key findings of the manuscript are reported in Figure 7 and 8.

Unfortunately, some of these results are not very convincing.

1. The effects of Kap β 1 depletion are very subtle and some are barely significant (Fig 7 A, B; Fig 8). Can they be rescued by expression of an siRNA-resistant construct?

We respectfully disagree with this comment. Fig. 7A and B clearly show that the N/C ratio of NES-cargoes increases for all three siRNAs. This is significant when comparing each individual siRNA condition against control siRNA-treated cells. This is the result of stringent experimental constraints that we had imposed on our statistical analysis. Namely, only siRNA-treated cells containing Cy5-labeled oligos were considered. Moreover, this was performed using a nonparametric (Kruskal-Wallis) test corrected for False Discovery Rate (Benjamini-Hochberg procedure). Hence, it is clear that the distribution of NES-cargo is affected when Kap β 1 is depleted. Although the measured values might seem modest, their percentage changes are clearly significant (siRNA1 55 pmol ~14%; siRNA2 55 pmol~19%; siRNA2 110 pmol ~14%) (Fig. 7A). Do also bear in mind that CRM1 continues to actively export NES cargoes out of the nucleus after Kap β 1 knockdown thereby mitigating further increases of N/C ratio. On this note, depleting CRM1 itself *only* results in a 29% increase in the N/C ratio of 3xEGFP-NES (Fig. 7B).

In summary, we feel that our experiments involve sufficient controls to verify the validity of the results:

1. Use of control siRNA and three siRNA conditions for silencing Kap β 1.
2. Comparing 2xEGFP-NES and 3xEGFP-NES indicate a “soft barrier” consistent with Timney et al. 2016.
3. N/C ratio of 3xEGFP-NES following CRM1 silencing.
4. FRAP of 2xEGFP non-specific cargoes.

Hence, we disagree with the reviewer’s proposed experiments.

2. It is disturbing that the siRNA treatment that has the strongest effect in Fig 7A, B (siRNA1 55pmol), has no effect in the diffusion assay in Fig 8. Somewhat the opposite is true for siRNA 2 (110pmol). How do the authors explain that? It would be important to add additional experiments with other siRNAs and perform rescue experiments (see point 1)

In both cases, our results indicate a general increase in the permeability of the NPC barrier after Kap β 1 depletion as compared to their respective controls (Fig. 7A,B and Fig 8). However, the data shown in Figs. 7 and 8 make use of different experimental readouts (fluorescence localization/quantification vs. flux) and therefore cannot be quantitatively compared (being inherently different and complex in nature i.e., active transport vs. passive diffusion). Please see comment to pt. 1.

3. One major conclusion of the paper in the abstract is that "Kap β 1 and CRM1 may engage in a balancing act to reinforce NPC barrier function". However, depletion of CRM1 has no significant effects on the barrier (Fig 7C) and wasn't tested in Fig 8, thus this conclusion is not supported by the results.

Agree. We have revised the abstract as follows:

P2L9-13 "Their enrichment at the NPC is sustained by promiscuous binding interactions with the FG Nups, which enable CRM1 to compensate for the loss of Kap β 1 as a means to reinforce NPC barrier function. However, such a compensatory mechanism is constrained by the cellular abundances and different binding kinetics for each respective Kap as evidenced for Importin-5."

December 8, 2021

RE: JCB Manuscript #202108107R

Prof. Roderick Lim
University of Basel
Biozentrum
Spitalstrasse 41
Basel CH4056
Switzerland

Dear Dr. Lim,

Thank you for submitting your revised manuscript entitled "Karyopherin enrichment and compensation fortifies the nuclear pore complex against nucleocytoplasmic leakage." We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

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, <https://jcb.rupress.org/submission-guidelines#revised>.

****Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.****

1) Text limits: Character count for Articles is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, and acknowledgments. Count does not include materials and methods, figure legends, references, tables, or supplemental legends.

2) Figures limits: Articles may have up to 10 main text figures.

3) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.

4) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please also be sure to indicate the statistical tests used in each of your experiments (both in the figure legend itself and in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, if you used parametric tests, please indicate if the data distribution was tested for normality (and if so, how). If not, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

5) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions (at least in brief) in the text for readers who may not have access to referenced manuscripts. The text should not refer to methods "...as previously described."

6) For all cell lines, vectors, constructs/cDNAs, etc. - all genetic material: please include database / vendor ID (e.g., Addgene, ATCC, etc.) or if unavailable, please briefly describe their basic genetic features, even if described in other published work or gifted to you by other investigators (and provide references where appropriate). Please be sure to provide the sequences for all of your oligos: primers, si/shRNA, RNAi, gRNAs, etc. in the materials and methods. You must also indicate in the methods the source, species, and catalog numbers/vendor identifiers (where appropriate) for all of your antibodies, including secondary. If antibodies are not commercial please add a reference citation if possible.

7) Microscope image acquisition: The following information must be provided about the acquisition and processing of images:

- a. Make and model of microscope
- b. Type, magnification, and numerical aperture of the objective lenses
- c. Temperature
- d. Imaging medium
- e. Fluorochromes
- f. Camera make and model
- g. Acquisition software

- h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).
- 8) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed. JCB formatting does not allow for a reference list in supplementary materials. Please remove this and add any non-duplicate references to the main reference list.
- 9) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures and 10 videos. Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section. Please include one brief sentence per item.
- 10) Video legends: Should describe what is being shown, the cell type or tissue being viewed (including relevant cell treatments, concentration and duration, or transfection), the imaging method (e.g., time-lapse epifluorescence microscopy), what each color represents, how often frames were collected, the frames/second display rate, and the number of any figure that has related video stills or images.
- 11) eTOC summary: A ~40-50 word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person. It should begin with "First author name(s) et al..." to match our preferred style.
- 12) Conflict of interest statement: JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the following statement: "The authors declare no competing financial interests." If competing interests are declared, please follow your statement of these competing interests with the following statement: "The authors declare no further competing financial interests."
- 13) A separate author contribution section is required following the Acknowledgments in all research manuscripts. All authors should be mentioned and designated by their first and middle initials and full surnames. We encourage use of the CRediT nomenclature (<https://casrai.org/credit/>).
- 14) ORCID IDs: ORCID IDs are unique identifiers allowing researchers to create a record of their various scholarly contributions in a single place. At resubmission of your final files, please consider providing an ORCID ID for as many contributing authors as possible.
- 15) Please note that JCB now requires authors to submit Source Data used to generate figures containing gels and Western blots with all revised manuscripts. This Source Data consists of fully uncropped and unprocessed images for each gel/blot displayed in the main and supplemental figures. Since your paper includes cropped gel and/or blot images, please be sure to provide one Source Data file for each figure that contains gels and/or blots along with your revised manuscript files. File names for Source Data figures should be alphanumeric without any spaces or special characters (i.e., SourceDataF#, where F# refers to the associated main figure number or SourceDataFS# for those associated with Supplementary figures). The lanes of the gels/blots should be labeled as they are in the associated figure, the place where cropping was applied should be marked (with a box), and molecular weight/size standards should be labeled wherever possible. Source Data files will be made available to reviewers during evaluation of revised manuscripts and, if your paper is eventually published in JCB, the files will be directly linked to specific figures in the published article.

Source Data Figures should be provided as individual PDF files (one file per figure). Authors should endeavor to retain a minimum resolution of 300 dpi or pixels per inch. Please review our instructions for export from Photoshop, Illustrator, and PowerPoint here: <https://rupress.org/jcb/pages/submission-guidelines#revised>

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 MP4 video files: See our detailed guidelines for preparing your production-ready images, <https://jcb.rupress.org/fig-vid-guidelines>.

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****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. If complications arising from measures taken to prevent the spread of COVID-19 will prevent you from meeting this deadline (e.g. if you cannot retrieve necessary files from your laboratory, etc.), please let us know and we can work with you to determine a suitable revision period.

Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

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

Sincerely,

Larry Gerace, PhD
Monitoring Editor
Journal of Cell Biology

Dan Simon, PhD
Scientific Editor
Journal of Cell Biology

Reviewer #3 (Comments to the Authors (Required)):

In this revised manuscript, the authors have addressed our point 3 by changing the abstract. They did however not add the requested rescue arguments (our points 1, 2) but provide good arguments why they feel this is not necessary. We are satisfied with this response and given the very positive comments from the other two reviewers recommend publication.