JCB

DAPLE orchestrates apical actomyosin assembly from junctional polarity complexes

Arthur Marivin, Rachel Xi-Yeen Ho, and Mikel Garcia-Marcos

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December 14, 2021

Re: JCB manuscript #202111002

Prof. Mikel Garcia-Marcos
Boston University School of Medicine
72 E. Concord St K206
Boston, MA 02118

Dear Mikel,

Your manuscript "DAPLE orchestrates apical actomyosin assembly from junctional polarity complexes" has now been evaluated by two external reviewers with expertise in this area. I am pleased let you know that both reviewers considered the study to provide new insights into the role of DAPLE in organizing assembly of the apical actin cortex, and that the work is solid and well-executed. However, both reviewers also noted several issues that will need to be addressed prior to further consideration for publication by JCB. We are happy, therefore, to consider a suitably revised version, based on their comments and our notes below.

Reviewer #1 felt that the work is interesting but was concerned that loss of DAPLE produces a different phenotype than does the loss of PAR3 (Figure 2F vs Figure 2B) which suggests that Par-3 is required for the *defects* observed when DAPLE is lost (e.g. DAPLE is repressing Par-3) or that something is seriously wrong. Along similar lines, the reviewer states that very little is done to confirm that loss of Par-3 phenocopies DAPLE loss in other respects. It would strengthen the paper if it is possible to demonstrate that Par-3 loss causes similar defects to DAPLE loss for example by examining whether the apical actomyosin network defects are the same or similar in each condition.

Reviewer #2 considered the study to be compelling and important, and has only two relatively minor points. First, can DAPLE bind CD2AP and PAR3 at the same time, or are these separate complexes? And second they suggest that Figure S4 provides valuable information and be moved into the main figures.

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.

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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.

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

As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of social distancing and shelter in place measures that limit spread of COVID-19 also pose challenges to scientific researchers. Lab closures especially are preventing scientists from conducting experiments to further their research. Therefore, JCB has waived the revision time limit. We recommend that you reach out to the editors once your lab has reopened 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.

Sincerely,

Ian Macara, Ph.D.
Editor
The Journal of Cell Biology

Lucia Morgado-Palacin, PhD
Scientific Editor
Journal of Cell Biology

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

Marvin et al report on the role of the DAPLE protein in epithelial junction function, building on a recent discovery that DAPLE binds the Par-3 polarity protein. The study predominantly uses cultured cells as a model system but also examines the consequences of DAPLE loss in the Xenopus embryonic epidermis. In MDCK cells they find that there are no defects in DAPLE depleted cells at early time points but cells ultimately become more deformable with the junctional protein E-cadherin localizing to a more diffuse area than the ZO-1 counterpart. This is accompanied by morphological changes as assessed by the linearity of the plasma membrane between tricellular junctions leading the authors to conclude that DAPLE is required to maintain the normal mechanical properties of the junctions. This role seems to be supported by recruitment to junctional Par-3 (via the DAPLE PBM and Par-3 PDZ3), and at least in part by DAPLE's subsequent recruitment of the actin stabilizing protein CD2AP. These conclusions come from a series of well executed and clearly communicated experimental results. However, as described below there are some apparently conflicting observations relating to the connection between DAPLE and polarity that should be addressed before publication.

As laid out by the authors in the introduction and discussion, much of the significance of the paper comes from the possible connection DAPLE makes between cortical polarity (through Par-3) and the actin cytoskeleton. In regards to this issue, however, some clarification is in order before publication. According to figure 2, loss of DAPLE causes severe defects in junctional linearity that are rescued by WT DAPLE but not DAPLE lacking the PBM. However, loss of Par-3 does not appear to cause the same phenotype (Figure 2F vs Figure 2B) which suggests that Par-3 is required for the *defects* observed when DAPLE is lost (e.g. DAPLE is repressing Par-3) or that something is seriously wrong.

Similarly, given the focus on the connection to Par-3, very little is done to confirm that loss of Par-3 phenocopies DAPLE loss in other respects. The authors should demonstrate that Par-3 loss causes similar defects to DAPLE loss for example by examining whether the apical actomyosin network defects are the same or similar in each condition.

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

The authors have made a compelling case that DAPLE is necessary for the assembly of the apical actin cortex and the actin cytoskeleton at apical adhesive junctions. This is important work that will be of lasting value to several different research communities. DAPLE is a non-canonical regulator of heterotrimeric G-proteins. Previous work showed that it accelerates nucleotide exchange of some G-alphas while inhibiting it on others. Additional published work has recently implicated DAPLE as

an important organizer of epithelial cells where it contributes to Wnt signaling by binding to disheveled, and it also helps organize microtubules. The current study extends this work to show that DAPLE specifically controls apical actin networks, which are know to be important for the formation and stability of epithelial cell-cell junctions. New biochemical data in this study shows that DAPLE binds to both the PAR polarity complex through PAR3, and DAPLE binds to CD2AP. Structure-function analysis is used to identify the binding sites in DAPLE for these partners. Knockdown rescue experiments with wildtype versus mutated DAPLE constructs shows that the binding interactions are likely necessary for DAPLE to organize the apical actin cytoskeleton. From the combined data, the authors propose a reasonable model in which DAPLE recruits CD2AP to apical junctions to build/maintain the cytoskeleton and also triggers Rho signaling to activate myosin contractility, which are both important determinants of epithelial structure and function. I thought the experiments were logically presented and that the data were of high quality. Quantification of results is on par with expectations for JCB. The figures are gorgeous, and I really liked the way the authors organized the panels within each figure along with the accompanying diagrams that made it possible to understand the figure just by looking at them. It's a nice style that I am tempted to steal, assuming I can duplicate the artistic flair. I also thought the paper was well written, especially the introduction, which achieved a nice balance of providing encyclopedic background information while leading the reader up to the question at hand.

In general, this study makes an important contribution to the emerging view of the complexity of actomyosin regulation in epithelial cells that could not have been predicted from classic studies in yeast or fibroblasts or the tidy biochemistry of formin and Arp2/3 activation (all of which was very important). The unexpectedly elaborate machinery used to control actin in epithelial cells, which now includes DAPLE, underscores the importance of these actin networks in epithelial biology despite the fact that the cells are not moving.

I only have two comments:

1. Can DAPLE bind CD2AP and PAR3 at the same time, or are these separate complexes?

2. Figure S4 shows that "DAPLE binds to an SH3 domain in CD2AP but not to many other SH3 domains." I think this is valuable information. I vote for moving it out of the supplement and cramming it into figure 4.

RESPONSE TO REVIEWERS

EDITORIAL DECISION

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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.

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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.

Sincerely,

Ian Macara, Ph.D.
Editor
The Journal of Cell Biology

Lucia Morgado-Palacin, PhD
Scientific Editor
Journal of Cell Biology

Response to the Editorial Decision: We are glad that the manuscript was well received by the reviewers and the editor, who seem to agree that the work was done well and that it provides valuable new information. We have also taken very seriously the issues raised by the reviewers and tried our best to address them. To do so, we have performed additional experiments, provided further explanations, or reorganized the manuscript as needed. We have uploaded a MARKED version with significant changes indicated in yellow highlight.

We have also paid attention to the Editorial guidelines to format the resubmission. Most notably, we have assembled the "Source Data" files for the gels and Western Blots presented in our figures.

We are excited about the prospects of seeing this work eventually published in the *Journal of Cell Biology*, and appreciate the invitation to submit a revised version. We hope that the Editor and reviewers find it suitable for publication.

Reviewer #1

[General comments] Marvin et al report on the role of the DAPLE protein in epithelial junction function, building on a recent discovery that DAPLE binds the Par-3 polarity protein. The study predominantly uses cultured cells as a model system but also examines the consequences of DAPLE loss in the Xenopus embryonic epidermis. In MDCK cells they find that there are no defects in DAPLE depleted cells at early time points but cells ultimately become more deformable with the junctional protein E-cadherin localizing to a more diffuse area than the ZO-1 counterpart. This is accompanied by morphological changes as assessed by the linearity of the plasma membrane between tricellular junctions leading the authors to conclude that DAPLE is required to maintain the normal mechanical properties of the junctions. This role seems to be supported by recruitment to junctional Par-3 (via the DAPLE PBM and Par-3 PDZ3), and at least in part by DAPLE's subsequent recruitment of the actin stabilizing protein CD2AP. These conclusions come from a series of well executed and clearly communicated experimental results. However, as described below there are some apparently conflicting observations relating to the connection between DAPLE and polarity that should be addressed before publication.

Response to the General comments: We appreciate the detailed and accurate description of the main findings in the manuscript. We were obviously pleased to read the positive general assessment that our "*conclusions come from a series of well executed and clearly communicated experimental results.*" As for the reviewer's concerns about apparently conflicting observations in the manuscript, we hope that they will be mitigated by the clarifications and additional data described below.

[Specific comments] As laid out by the authors in the introduction and discussion, much of the significance of the paper comes from the possible connection DAPLE makes between cortical polarity (through Par-3) and the actin cytoskeleton. In regards to this issue, however, some clarification is in order before publication. According to figure 2, loss of DAPLE causes severe defects in junctional linearity that are rescued by WT DAPLE but not DAPLE lacking the PBM. However, loss of Par-3 does not appear to cause the same phenotype (Figure 2F vs Figure 2B) which suggests that Par-3 is required for the *defects* observed when DAPLE is lost (e.g. DAPLE is repressing Par-3) or that something is seriously wrong.

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Response to the Specific comments: The reviewer raises two points that are interrelated. One is that, in Figure 2, loss of Par-3 does not phenocopy the morphological defects observed in the same figure upon loss of DAPLE, and the second one is that further experiments to show that loss of Par-3 phenocopies loss of DAPLE would be desirable. We believe that both points can be clearly addressed together as we explain below.

It is true that loss of Par-3 in Fig. 2F does not phenocopy the defects observed upon loss of DAPLE in Fig. 2B, but the comparison is not meaningful because the experimental conditions are very different in each situation. The reason why the experimental conditions are very different is that it is not possible to analyze Par-3 depleted cells in the same way we analyzed DAPLE depleted cells. We will elaborate next on the details to support this idea, and the reasons why our conclusions are fully consistent with the results.

In Figure 2B, we investigated the effect of DAPLE depletion by generating stable cell lines and analyzing their phenotypes after 8 days of culture to allow the formation of "mature" epithelial monolayers with a fully developed apical actomyosin network. However, using the same approach with Par-3 would not be meaningful in this system because its loss disrupts apico-basal polarity, which in turn prevents the formation of apical cell junctions (Chen and Macara, 2005; Horikoshi et al., 2009; Sfakianos et al., 2007). The experiments in Figure 2F were carried out upon acute depletion of Par-3 and by culturing cells for shorter times than in Figure 2B for DAPLE-depleted cells (4 versus 8 days and starting at half the cell density). Under these conditions, loss of Par-3 has not led to the disruption of apical cell junctions yet, which allowed us to appropriately assess the relationship between the absence of Par-3 and the junctional localization of DAPLE (i.e., DAPLE junctional localization requires Par-3, but not indirectly because apical junctions have been disassembled).

We did try to generate Par-3 depleted cells and to analyze them exactly under the same conditions as we analyzed DAPLE-depleted cells. The key observations were that most of the surviving cells after antibiotic selection still expressed Par-3, suggesting that clones with poor knock-down had a fitness advantage, and that those patches of cells that did not express Par-3 had an overt disruption of apical junctions as determined by the loss of ZO-1. Not surprisingly, DAPLE was also lost from junctions in Par-3 depleted cells, but this could be an indirect consequence of the disassembly of apical junctions. These results are presented in a new supplementary figure, **Fig. S2**.

Based on the above, we believe that pursuing further experiments to compare DAPLE and Par-3 depleted cells to establish whether they phenocopy each other, as suggested by the reviewer, would not be informative. Observations by us and others clearly indicate that the effects of loss of Par-3 are more pleiotropic than those caused by loss of DAPLE. Phenotypes associated with loss of DAPLE are milder than those observed upon loss of Par-3, e.g. loss of DAPLE does not prevent the establishment of apico-basal polarity or the formation of apical junctions (**Fig. S1**). This indicates that DAPLE is controlled by Par-3, rather than the other way around, which is consistent with our conclusions in the manuscript.

Nevertheless, we understand why the reviewer saw an apparent conflict in the data presented, and we realize now that the use of and rationale for different experimental conditions to analyze Par-3 depleted cells in **Fig. 2F** were not clear. To address this, we have now included a new Supplemental Figure to show the overt disruption of apical junctions upon prolonged knock-down of Par-3 (**Fig. S2**), which serves to provide a explicit rationale for the different conditions used in **Fig. 2F** to specifically assess DAPLE junctional localization. Text in lines 165 to 181 has been added/ changed to clarify this point.

Reviewer #2

[General comments] The authors have made a compelling case that DAPLE is necessary for the assembly of the apical actin cortex and the actin cytoskeleton at apical adhesive junctions. This is important work that will be of lasting value to several different research communities. DAPLE is a non-canonical regulator of heterotrimeric G-proteins. Previous work showed that it accelerates nucleotide exchange of some G-alphas while inhibiting it on others. Additional published work has recently implicated DAPLE as an important organizer of epithelial cells where it contributes to Wnt signaling by binding to disheveled, and it also helps organize microtubules. The current study extends this work to show that DAPLE specifically controls apical actin networks, which are know to be important for the formation and stability of epithelial cell-cell junctions. New biochemical data in this study shows that DAPLE binds to both the PAR polarity complex through PAR3, and DAPLE binds to CD2AP. Structure-function analysis is used to identify the binding sites in DAPLE for these partners. Knockdown rescue experiments with wildtype versus mutated DAPLE constructs shows that the binding interactions are likely necessary for DAPLE to organize the apical actin cytoskeleton. From the combined data, the authors propose a reasonable model in which DAPLE recruits CD2AP to apical junctions to build/maintain the cytoskeleton and also triggers Rho signaling to activate myosin contractility, which are both important determinants of epithelial structure and function. I thought the experiments were logically presented and that the data were of high quality. Quantification of results is on par with expectations for JCB. The figures are gorgeous, and I really liked the way the authors organized the panels within each figure along with the accompanying diagrams that made it possible to understand the figure just by looking at them. It's a nice style that I am tempted to steal, assuming I can duplicate the artistic flair. I also thought the paper was well written, especially the introduction, which achieved a nice balance of providing encyclopedic background information while leading the reader up to the question at hand.

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Response to the General comments: We were truly blown away by the enthusiastic evaluation of our manuscript by this reviewer. We really appreciate that the reviewer took the time to write such a detailed evaluation of the positive aspects of the work. Unfortunately, it is more common to see reviews that elaborate extensively on perceived weaknesses but brush off the strengths with succinct comments, so reading this report was a pleasant breath of fresh air.

I only have two comments:

Specific comment 1. Can DAPLE bind CD2AP and PAR3 at the same time, or are these separate complexes?

Response to the Specific comment 1: We have performed an additional experiment to answer this question. We purified a new MBP-fused PAR3 construct to investigate the potential formation of a tri-partite CD2AP-DAPLE-PAR3 construct. Our results indicate that the three proteins can exist as part of the same complex, in which DAPLE serves as a bridge connecting CD2AP and PAR3. These results are included in **Fig. S5G**.

Specific comment 2. Figure S4 shows that "DAPLE binds to an SH3 domain in CD2AP but not to many other SH3 domains." I think this is valuable information. I vote for moving it out of the supplement and cramming it into figure 4.

Response to the Specific comment 2: We have now removed the original **Fig. S4** to include its content as part of one of the main Figures. Because **Fig. 4** was already very large, we have subdivided it into two new figures. The content previously in **Fig. S4** is now in the new **Fig. 5**.

REFERENCES CITED

Chen, X., and I.G. Macara. 2005. Par-3 controls tight junction assembly through the Rac exchange factor Tiam1. *Nature cell biology*. 7:262-269.

Horikoshi, Y., A. Suzuki, T. Yamanaka, K. Sasaki, K. Mizuno, H. Sawada, S. Yonemura, and S. Ohno. 2009. Interaction between PAR-3 and the aPKC-PAR-6 complex is indispensable for apical domain development of epithelial cells. *Journal of cell science*. 122:1595-1606.

Sfakianos, J., A. Togawa, S. Maday, M. Hull, M. Pypaert, L. Cantley, D. Toomre, and I. Mellman. 2007. Par3 functions in the biogenesis of the primary cilium in polarized epithelial cells. *The Journal of cell biology*. 179:1133-1140.

February 1, 2022

RE: JCB Manuscript #202111002R

Prof. Mikel Garcia-Marcos
Boston University School of Medicine
72 E. Concord St K206
Boston, MA 02118

Dear Prof. Garcia-Marcos:

Thank you for submitting your revised manuscript entitled "DAPLE orchestrates apical actomyosin assembly from junctional polarity complexes". We have now assessed your revised manuscript and we would be happy to publish your paper in JCB pending revisions 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 and Tools 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 and Tools may have up to 10 main text figures.
*** Legends for panels E and G (diagrams) in main Fig 8 seems missing.

3) Figure formatting:
Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.

*** Scale bars must be present on all microscopy images, including inset magnifications. Please add scale bars to main Fig 3D, 3H (inset magnification), 4B (inset magnification), and supplemental Fig 2B.

*** Also, please avoid pairing red and green for images and graphs to ensure legibility for color-blind readers. Please ensure that the particular red and green hues used in main Fig 3A, 3C, 3H, 7B, 8B, 8D and supplemental Fig 1G-H, 3A-B are distinctive with any of the colorblind types. If not, please modify colors accordingly or present separate micrographs for each channel.

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. Please indicate whether n refers to technical or independent replicates in main Fig 1B and 1H.

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.). If you used parametric tests in your study (i.e. t-tests), you should have first determined whether the data was normally distributed before selecting that test. In the stats section of the methods, please indicate how you tested for normality. If you did not test for normality, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

5) Abstract and title: The abstract should be no longer than 160 words and should communicate the significance of the paper for

a general audience. The title should be less than 100 characters including spaces. Make the title concise but accessible to a general readership.

6) 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." Also, the materials and methods should be included with the main manuscript text and not in the supplementary materials.

>>> Thank you for providing such comprehensive "Materials and Methods" section!

7) Please be sure to provide the sequences for all of your primers/oligos and RNAi constructs in the materials and methods.

You must also indicate in the methods the source, species, and catalog numbers (where appropriate) for all of your antibodies.

8) 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.).

9) 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.

10) Supplemental materials:
There are strict limits on the allowable amount of supplemental data. Articles/Tools may have up to 5 supplemental figures. There is no limit for supplemental tables. However, please 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 see any recent JCB paper for an example of this summary).

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) Materials and data sharing:
As a condition of publication, authors must make protocols and unique materials (including, but not limited to, cloned DNAs; antibodies; bacterial, animal, or plant cells; and viruses) described in our published articles freely available upon request by researchers, who may use them in their own laboratory only. All materials must be made available on request and without undue delay.

All datasets included in the manuscript must be available from the date of online publication, and the source code for all custom computational methods, apart from commercial software programs, must be made available either in a publicly available database or as supplemental materials hosted on the journal website. Numerous resources exist for data storage and sharing (see Data Deposition: https://rupress.org/jcb/pages/data-deposition), and you should choose the most appropriate venue based on your data type and/or community standard. If no appropriate specific database exists, please deposit your data to an appropriate publicly available database.

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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