Cell cortex regulation by the planar cell polarity protein Prickle1

Yunyun Huang and Rudolf Winklbauer

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October 11, 2020

Re: JCB manuscript #202008116

Dr. Rudolf Winklbauer
University of Toronto
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25 Harbord Street
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Canada

Dear Dr. Winklbauer,

We have now received comments from three external reviewers of your manuscript "Cell cortex regulation by diffuse cortical accumulation of the planar cell polarity protein Prickle1." As you will see from the attached reviews, enthusiasm was mixed. While Reviewer #2 was positive and had few comments, reviewers #1 and 3 were more negative, with Reviewer #3 feeling that the work in its current form is not suitable for JCB. For these reasons, we are sorry to have to reject your manuscript.

In brief, we feel that a key point brought up by the reviewers is the lack of convincing evidence for epistasis between Pk and CKII. They suggest several ways to tackle this problem experimentally. There are also issues about "diffuse" Pk and regulation of cortical actin, weak characterization of Pk localization at cell-cell adhesions, other effects of the SMIFH2 inhibitor, and lack of analysis of the specificity of the morpholinos.

We are open to a re-evaluation of a substantially revised version of the study, but we believe that this would entail a significant amount of additional experimental work. If you would be interested in this possibility, we propose that you submit a revision plan that includes a point-by-point response to each of the comments, and how you would address them.

If you would like to resubmit this work to JCB, please contact the journal office to discuss an appeal of this decision or you may submit an appeal directly through our manuscript submission system. Please note that priority and novelty would be reassessed at resubmission.

Regardless of how you choose to proceed, we hope that the comments below will prove constructive as your work progresses. We would be happy to discuss the reviewer comments further once you've had a chance to consider the points raised in this letter. You can contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for thinking of JCB as an appropriate place to publish your work.

Sincerely,

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

Dan Simon, Ph.D.
Scientific Editor
Journal of Cell Biology

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

This report used isolated, non-adherent Xenopus prechordal mesoderm (PCM) cells to study the antagonistic effects of the core PCP proteins Pk1 and Dvl2 on cell cortex F-actin content and cortical tension. Using Pk1-GFP and Lifeact F-actin reporters, together with morpholinos and chemical inhibitors, the study showed that Pk1 diffusely associates with and positively regulates cortical density and tension, while Dvl2 antagonizes Pk1 and downregulates cortical density and tension through CKII inhibition. By monitoring cortical F-actin associated with membrane blebs, it was suggested that CKII regulates both assembly and disassembly of cortical F-actin.
Overall, this is an interesting study that takes advantage of the high spatial resolution afforded by dissociated PCM cells, and begins to address the significant knowledge gap about the cell autonomous function of PCP genes in the regulation of the actomyosin network. The impact of the manuscript could be improved by digging a bit deeper into the mechanisms underlying

cortical localization of Pk1 and regulation of F-actin assembly by PCP/CKII signaling.

Specific comments:
1. Figure 1 and related text. The cell cortex consists of Formin-mediated unbranched and Arp2/3-mediated branched actin filaments. In addition to SMIFH2 treatment, it would be interesting to compare and contrast the effects of Formin vs. Arp2/3-inhibition (e.g. using CK666) on cortical F-actin organization and Pk1 localization. This would also shed light on the mechanisms underlying cortical localization of Pk1.

2. Related to above, the effect of Formin and Arp2/3-inhibition on cortical density should be quantified and compared with Pk1MO and Dvl2MO. Depending on the outcome of Arp2/3 inhibition, it would also be interesting to analyze the effects of Pk1MO or Dvl2MO combined with Formin or Arp2/3 inhibition on cortical density. This would help shed light on how these PCP genes regulate cortical F-actin.

3. Figure 1D, E and related text. It would be informative to test whether cell-cell adhesion promotes Pk1 puncta formation, which was implied but not experimentally tested. For example, do acute treatment with calcium chelating agents affect Pk1 puncta formation? In addition, does intercellular tension play a role?

4. Figure 2D. The specificity of Dvl2MO should be demonstrated by rescue experiments similar to those of Pk1MO.

5. Page 3, paragraph 3 and Figure 3D. The interpretation of epistasis between CKII and Pk1 was problematic. Specifically, the fact that PK1MO and CKII inhibition singly or together caused similar decrease in cortical density does not indicate that CKII acts downstream of Pk1. For that, one would have to demonstrate CKII activation can rescue Pk1 MO. Moreover, the effect of CKII inhibition on Pk1 cortical localization should also be examined.

6. Figure 3 and related text. To provide additional evidence for the proposed Pk1-Dvl2-CKII module, the effects of Pk1MO and Dvl2MO on bleb formation and retraction should be demonstrated along with CKII inhibition. In addition, the bleb retraction defects should be quantified for each condition.

7. Figure 4A and related text. It should be clarified whether the cortical tension experiments were performed in calcium-free media. Otherwise, cadherin-mediated cell-cell adhesion could be affected by Pk1MO and secondarily influence cortical tension.

Minor comments:

1. Figure 1E. With n=1 each for the "cell clusters" and "explant fractions" categories, it is unclear how statistical values were calculated.

2. Figure 2C. The statistical significance between Pk1 rescue and WT should be indicated.

3. Figure 2E. The statistical significance between M Dvl2MO and H Pk1MO/ M Dvl2MO should be indicated.

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

Using a combination of isolated Xenopus mesodermal cells, cell clusters, and intact tissues, the authors examine the relationship between cortical accumulation of the PCP protein Pk1, the cortical actin cytoskeleton, and cortical tension. From data that are clearly presented and convincing, they conclude that diffuse cortical Pk1 promotes accumulated cortical actin and this is reflected in inferred increased cortical tension. They further conclude that Pk1 and Dvl2 have opposite activity on the cortical actin cytoskeleton, and that they function through the kinase CKII. These observations have important implications for a number of morphogenetic events and will be of considerable interest to the community.

I have one modest point that should be corrected. The conclusion that CKII is downstream of Dvl2 is strong, but the data are equally consistent with CKII being downstream or in parallel to Pk1. To conclude the proposed epistasis, the authors would have to show that overexpressing Pk1 increases cortical actin and that this is blocked by CKII knockdown.

It would also be of interest to have the authors speculate on the potential that similar effects on actin might occur when the PCP proteins are clustered, and what biophysical implications this might have.

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

Huang and Winklbauer report on the possible regulation of the actin cortex by the core PCP proteins Pk and Dvl. Pk and Dvl form antagonistic complexes during PCP function, and disrupting the function of these proteins show opposite effects on overall cortical actin intensities. The authors suggest that Pk and Dvl may regulate Casein Kinase II function to control cortical actin, although the mechanism of this regulation (both upstream and downstream) is unclear. The authors conclude the manuscript by inferring potential membrane tension from measurement of cellular contact angles. Unfortunately, I find this to be a very preliminary (and, at times, cursory) study. The logic, as presented, was not very clear and compelling. My apologies to the authors, but there appear to be many issues - a few are listed below:

1) One of the central issues is teasing apart the mechanism by which potential cortical actin regulation occurs. Given the known antagonistic interactions between Dvl and Pk, can the authors satisfactorily conclude their model of a mutual co-regulation of CKII by Dvl and PK? Is this regulation direct or indirect (ex., through regulation of PCP proteins)? Also, their model puts CKII downstream of both Pk and Dvl, but I don't believe this can be concluded from the data - just because CKII disruption in the Pk disrupted background does not show a further depletion of actin does not mean it is downstream of Pk. This simply suggests that CKII and Pk are (potentially) in the same genetic pathway, such that further disruption of the pathway does not produce a greater effect. Classic arguments on epistasis usually employ an over-active version of one protein that cannot rescue the disruption of a downstream component (for example).

2) Much of the manuscript focuses on "diffuse" Pk regulation of cortical actin (and "diffuse" is also in the manuscript title), but the study has no way of differentiating between (or targeting) the different types of Pk localization. The disruptions are simply global morpholino-based disruptions of function. Single morpholinos were injected, and (to my knowledge) the specificity and effect of morpholino function was not established. This is a further experimental concern.

3) I am not sure what the characterization of Pk changes at sites of cell-cell adhesion establishes other than a rough developmental correlation between Pk and actin levels. This correlation is not further tested, and the paragraph ends with two speculative statements suggesting that (perhaps) diffuse actin correlates with actin cortex regulation while punctate Pk may correlate with PCP signaling, rather than a true summary statement of the tested data.

4) The paper starts with an examination of possible Formin function by inhibiting actin with the SMIFH2 inhibitor, which strangely causes an increase (!) in cortical actin. This is odd, and is not remarked on, other than to observe a correlation with an increase in Pk localization. Are the relative contributions of Arp2/3 and Formin proteins known for the cortex at these stages? These data suggests a possible stabilization of Pk by actin, although the remainder of the manuscript then examines how Pk may regulate cortical actin. The authors also note that although actin and Pk levels increase, they did not observe matched increases in the same cellular regions.

5) Along the above lines, SMIFH2 has been shown to also disrupt Myosin function. What does Myosin look like in these backgrounds? Does this explain differences in actin intensities? The authors use "data not shown/unpublished data" (one of two places this is used, which most journals are moving away from) to say that Myosin is present in the cortex.

6) I did not see a Methods section that adequately explained the statistical methods (and significance test) used.

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Rudolf Winklbauer, PhD
Professor

January 18th, 2022

Response to reviewer comments for APPEALED manuscript #202008116

We thank the editors for permitting us to submit an appealed manuscript for publication in the Journal of Cell Biology, and the reviewers for their insightful and stimulating comments. As requested, we rewrote the paper as a full article instead of a short report. We added new data which expanded the scope of the work. Previously, we had exclusively studied the role of diffuse Pk1 in single cells. We now added new Figures 1, S1 and 7 to analyze the effects of Pk1 knockdown at the tissue and embryo level, to put our single cell results into an organismal context. A second main addition consists of data on the induction and possible role of Pk1 puncta. This was prompted by numerous comments from the reviewers. It provides an instructive contrast to the role of diffuse Pk1 which remains the focus of the study. Former Figures 1D and S1 were combined and expanded to a new Figure 2, and Figures 8, 9 and 10 were added to provide data on Pk1 puncta formation and function. As mentioned in our appeal, all our frogs were euthanized at the beginning of the Covid-19 lockdown. We were able to purchase a few new frogs recently (there is presently a shortage of commercially available Xenopus frogs), which were of moderate quality though and often gave poor quality embryos that could not be used. This forced us to strictly prioritize experiments and we could not pursue all the interesting aspect suggested by the previous short-report version of the manuscript.

Point-by-point responses to the reviewer comments.

Reviewer #1 (Comments to the Authors (Required)):
This report used isolated, non-adherent Xenopus prechordal mesoderm (PCM) cells to study the antagonistic effects of the core PCP proteins Pk1 and Dvl2 on cell cortex F-actin content and cortical tension. Using Pk1-GFP and Lifeact F-actin reporters, together with morpholinos and chemical inhibitors, the study showed that Pk1 diffusely associates with and positively regulates cortical density and tension, while Dvl2 antagonizes Pk1 and downregulates cortical density and tension through CKII inhibition. By monitoring cortical F-actin associated with membrane blebs, it was suggested that CKII regulates both assembly and disassembly of cortical F-actin.
Overall, this is an interesting study that takes advantage of the high spatial resolution afforded by dissociated PCM cells, and begins to address the significant knowledge gap about the cell autonomous function of PCP genes in the regulation of the actomyosin network. The impact of the manuscript could be improved by digging a bit deeper into the mechanisms underlying cortical localization of Pk1 and regulation of F-actin assembly by PCP/CKII signaling.

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Specific comments:

1. Figure 1 and related text. The cell cortex consists of Formin-mediated unbranched and Arp2/3-mediated branched actin filaments. In addition to SMIFH2 treatment, it would be interesting to compare and contrast the effects of Formin vs. Arp2/3-inhibition (e.g. using CK666) on cortical F-actin organization and Pk1 localization. This would also shed light on the mechanisms underlying cortical localization of Pk1.

Figure 9B has been added to show the effects of CK666 inhibition on the cortex. Cortical localization of diffuse Pk1 has been reinterpreted, based on Figures 2C,H,M, 5D and S2, S3. We think that Pk1 is ubiquitous in the cytoplasm and appears more concentrated in the cortical zone because it is not "diluted" there by organelles like yolk platelets and the ER. However, we describe a striking effect of CK666 inhibition on the punctate form of Pk1 in Figure 9B.

2. Related to above, the effect of Formin and Arp2/3-inhibition on cortical density should be quantified and compared with Pk1MO and Dvl2MO. Depending on the outcome of Arp2/3 inhibition, it would also be interesting to analyze the effects of Pk1MO or Dvl2MO combined with Formin or Arp2/3 inhibition on cortical density. This would help shed light on how these PCP genes regulate cortical F-actin.

Inhibition with CK666 generates an extremely inhomogeneous cortex (Fig.9B), and we did not quantify and compare it to that produced by Pk1MO or Dvl2MO. We agree, it would be very interesting to combine Pk1MO and Dvl2MO with formin and Arp2/3 inhibition, but we had to leave the whole issue of the molecular control of F-actin sub-structures by Pk1 and Dvl2 unexplored at present. This would be a major but separate topic, and instead of adding a few partial results we decided to focus our efforts on issues more aligned with the present work.

3. Figure 1D, E and related text. It would be informative to test whether cell-cell adhesion promotes Pk1 puncta formation, which was implied but not experimentally tested. For example, do acute treatment with calcium chelating agents affect Pk1 puncta formation? In addition, does intercellular tension play a role?

We added data analysing puncta formation in response to cell contact and tensions in the revised Figure 2, and in the new Figures 8A,B, 9A and 10.

4. Figure 2D. The specificity of Dvl2MO should be demonstrated by rescue experiments similar to those of Pk1MO.

We used previously characterized and validated Dvl2 and Pk1 morpholinos, as referenced in the Methods section.

5. Page 3, paragraph 3 and Figure 3D. The interpretation of epistasis between CKII and Pk1 was problematic. Specifically, the fact that PK1MO and CKII inhibition singly or together caused similar decrease in cortical density does not indicate that CKII acts downstream of Pk1. For that, one would have to demonstrate CKII activation can rescue Pk1 MO.

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Instead, and as also requested by reviewer #2, we showed that overexpressing Pk1 increases cortical actin and that this is blocked by CKII inhibition (Figure 4F).

Moreover, the effect of CKII inhibition on Pk1 cortical localization should also be examined.

Our interpretation of the cortical localization of Pk1 has changed, please refer to our response to point 1 above. This renders the question of a physical localization of Pk1 to the cortex by CKII obsolete. We propose instead a "functional" localization of Pk1 activity, not Pk1 protein, to the membrane/cortex (first paragraph of the Discussion section).

6. Figure 3 and related text. To provide additional evidence for the proposed Pk1-Dvl2-CKII module, the effects of Pk1MO and Dvl2MO on bleb formation and retraction should be demonstrated along with CKII inhibition. In addition, the bleb retraction defects should be quantified for each condition.

Acute inhibition with TBB allowed us to discern immediate before/after differences in cortex behavior, which is not possible with morpholino inhibition. We also noted that blebs retract even when no obvious new cortex forms at the bleb membrane (Fig.4B'). We do not understand this effect but had to refrain from following up this sidetrack into bleb mechanics.

7. Figure 4A and related text. It should be clarified whether the cortical tension experiments were performed in calcium-free media. Otherwise, cadherin-mediated cell-cell adhesion could be affected by Pk1MO and secondarily influence cortical tension.

The cortical tension experiments (now Figure 6) were performed in calcium-containing MBS, the normal culture medium, as mentioned now in the Methods section.

Minor comments:

1. Figure 1E. With n=1 each for the "cell clusters" and "explant fractions" categories, it is unclear how statistical values were calculated.

This typographical error was corrected.

2. Figure 2C. The statistical significance between Pk1 rescue and WT should be indicated.

Done (now in Figure 4C).

3. Figure 2E. The statistical significance between M Dvl2MO and H Pk1MO/ M Dvl2MO should be indicated.

Done (now in Figure 4E).

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Reviewer #2 (Comments to the Authors (Required)):
Using a combination of isolated Xenopus mesodermal cells, cell clusters, and intact tissues, the authors examine the relationship between cortical accumulation of the PCP protein Pk1, the cortical actin cytoskeleton, and cortical tension. From data that are clearly presented and convincing, they conclude that diffuse cortical Pk1 promotes accumulated cortical actin and this is reflected in inferred increased cortical tension. They further conclude that Pk1 and Dvl2 have opposite activity on the cortical actin cytoskeleton, and that they function through the kinase CKII. These observations have important implications for a number of morphogenetic events and will be of considerable interest to the community.

I have one modest point that should be corrected. The conclusion that CKII is downstream of Dvl2 is strong, but the data are equally consistent with CKII being downstream or in parallel to Pk1. To conclude the proposed epistasis, the authors would have to show that overexpressing Pk1 increases cortical actin and that this is blocked by CKII knockdown.

We added the results of this experiment as Figure 4F.

It would also be of interest to have the authors speculate on the potential that similar effects on actin might occur when the PCP proteins are clustered, and what biophysical implications this might have.

We added data which show that unexpectedly, the effect of Pk1 puncta is opposite to that of diffuse Pk1 (Figure 9) and speculate on the function of this effect during cell separation.

Reviewer #3 (Comments to the Authors (Required)):
Huang and Winklbauer report on the possible regulation of the actin cortex by the core PCP proteins Pk and Dvl. Pk and Dvl form antagonistic complexes during PCP function, and disrupting the function of these proteins show opposite effects on overall cortical actin intensities. The authors suggest that Pk and Dvl may regulate Casein Kinase II function to control cortical actin, although the mechanism of this regulation (both upstream and downstream) is unclear. The authors conclude the manuscript by inferring potential membrane tension from measurement of cellular contact angles. Unfortunately, I find this to be a very preliminary (and, at times, cursory) study. The logic, as presented, was not very clear and compelling. My apologies to the authors, but there appear to be many issues - a few are listed below:

1) One of the central issues is teasing apart the mechanism by which potential cortical actin regulation occurs. Given the known antagonistic interactions between Dvl and Pk, can the authors satisfactorily conclude their model of a mutual co-regulation of CKII by Dvl and PK? Is this regulation direct or indirect (ex., through regulation of PCP proteins)? Also, their model puts CKII downstream of both Pk and Dvl, but I don't believe this can be concluded from the data - just because CKII disruption in the Pk disrupted background does not show a further depletion of actin does not mean it is downstream of Pk. This simply suggests that CKII and Pk are (potentially) in the same genetic pathway, such that further disruption of the pathway does not produce a greater effect. Classic arguments on epistasis usually employ an over-active

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version of one protein that cannot rescue the disruption of a downstream component (for example).

As also requested by reviewer #2, we show now that overexpressing Pk1 increases cortical actin and that this is blocked by CKII inhibition (Figure 4F).

2) Much of the manuscript focuses on "diffuse" Pk regulation of cortical actin (and "diffuse" is also in the manuscript title), but the study has no way of differentiating between (or targeting) the different types of Pk localization. The disruptions are simply global morpholino-based disruptions of function.

We use the fact that single PCM cells do not form Pk1 punctca to study the role of diffuse Pk1. The results shown in Figures 3, 4, 5, 6A-C are all obtained with single cells. In Figure 10 puncta are artificially induced in single cells. To contrast the effects of diffuse Pk1, we added now also data on Pk1 puncta in Figures 2, 8 and 9. Puncta which are present in multicellular tissues could indeed not be specifically disrupted in the tissue context, but only correlated with putative effects, e.g. with cortical F-actin downregulation. We think that these observations provide nevertheless significant insights, complementing the results on diffuse Pk1, our main topic.

Single morpholinos were injected, and (to my knowledge) the specificity and effect of morpholino function was not established. This is a further experimental concern.

We used previously characterized and validated Dvl2 and Pk1 morpholinos, as referenced in the Methods section.

3) I am not sure what the characterization of Pk changes at sites of cell-cell adhesion establishes other than a rough developmental correlation between Pk and actin levels. This correlation is not further tested, and the paragraph ends with two speculative statements suggesting that (perhaps) diffuse actin correlates with actin cortex regulation while punctate Pk may correlate with PCP signaling, rather than a true summary statement of the tested data.

We assume this point refers to the previous Figure 1D,E. This is now replaced by the expanded new Figure 2 and the added Figure S3. The correlation between Pk1 and actin levels at cell-cell contacts is no longer referred to in the light of our new interpretation of Pk1 localization. We propose that Pk1 is ubiquitous in the cytoplasm and simply appears more concentrated in the cortical zone because it is not partially excluded there by organelles like yolk platelets and the ER. There is no need for a specific mechanism that localizes Pk1 to the cortex, e.g. by binding to F-actin. We also add now data on punctate Pk1, in part replacing our speculative statements.

4) The paper starts with an examination of possible Formin function by inhibiting actin with the SMIFH2 inhibitor, which strangely causes an increase (!) in cortical actin. This is odd, and is not remarked on, other than to observe a correlation with an increase in Pk localization. Are the relative contributions of Arp2/3 and Formin proteins known for the cortex at these stages? These data suggests a possible stabilization of Pk by actin, although the remainder of the manuscript then examines how Pk may regulate cortical actin.

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The relative contributions of Formin and Arp2/3 proteins to the PCM cell cortex is not known, to our knowledge. We added data on the inhibition of Arp2/3 with CK666 (Figure 9B). But we cite now Chesarone and Goode (2009) who discuss instances of cross-talk between F-actin assembly factors which could explain how SMIFH2 slightly increased total cortical F-actin (first paragraph of Discussion section).

The authors also note that although actin and Pk levels increase, they did not observe matched increases in the same cellular regions.

We wanted to suggest that Pk1 is not localized to the cortex by binding to F-actin as Pk1 density does not parallel F-actin density. This is consistent with our notion of a ubiquitous localization of diffuse, cytoplasmic Pk1 protein which simply overlaps with the cortex (in a "cortex zone"; see comments above, point 3).

5) Along the above lines, SMIFH2 has been shown to also disrupt Myosin function. What does Myosin look like in these backgrounds? Does this explain differences in actin intensities? The authors use "data not shown/unpublished data" (one of two places this is used, which most journals are moving away from) to say that Myosin is present in the cortex.

We explain now in the Methods section that we used SMIFH2 at such a low concentration that according to the reference cited it should inhibit myosin function by only 10% or less. Apart from this, the conclusions drawn from the experiment – concerning the relationship between diffuse Pk1 and a widened and inhomogeneous cortex – do not depend on SMIFH2 specificity. Also, since cortical F-actin content and cortical tension were well correlated, we felt no need to obtain myosin data which could have explained discrepancies between the two parameters if any would have been present.

6) I did not see a Methods section that adequately explained the statistical methods (and significance test) used.

Statistical methods have been added at the end of the Methods section.

We thank again the reviewers for sharing their much appreciated expertise and insight.

Yours sincerely,

Rudi Winklbauer

February 18, 2022

Re: JCB manuscript #202008116R-A

Prof. Rudolf Winklbauer
University of Toronto
Department of Cell and Systems Biology
25 Harbord Street
Toronto M5S 3G5
Canada

Dear Rudi,

Thank you for your revised manuscript on "Cell cortex regulation by the planar cell polarity protein Prickle1," which has now been evaluated by the three original referees. As you will see from the appended comments, two of the referees are enthusiastic about the work and feel that it is potentially acceptable for publication by the journal after additional revisions. The third reviewer remains negative. We feel that the work has been significantly improved, but that additional revisions are required. We hope that a suitably revised version could be assessed editorially without further evaluation by the external referees, assuming that you are able to address their comments satisfactorily.

Reviewer #1 suggests minor revisions, particularly that you use an Arp2/3 inhibitor to compare with Formin inhibition. Experiments were included with CK666 Arp2/3 inhibitor but in PCM explants whereas the Formin inhibition studies were done on isolated PCM cells. The Reviewer asks that you test CK666 on isolated PCM cells in order to directly compare with effects of Formin inhibition. We do not think their request to assess the role of diffuse Pk1 in cell motility/migration in isolated PCM cells plated on FN is necessary, but agree that you should quantify frequency of cell intercalation in control vs MOs.

Reviewer #2 is very supportive but comments that the distribution of Pk1 would be more convincing if dextran labeling was used to quantitatively normalize the Pk1 distribution across the cell. Other comments, such as those about the Discussion, just need modifications to the text.

Reviewer #3 feels that the ability to determine something concrete about a specific mechanism of cortical actin regulation by Pk and Dvl function, and especially that of "diffuse" or "punctate" Pk subpopulations is deeply compromised by the experimental approaches (tissue fragments, global morpholino disruptions, and low-resolution imaging). It seems the cells/tissues being examined are in very different physiological states (for example, adherent vs non-adherent cells), and it is difficult to know if a host of factors beyond the two PCP proteins they examine might also be changed by this altered physiology (and there are no controls presented to examine this possibility). They also comment that the writing is also challenging to follow - at times the reader has to guess at the meaning of some sentences or potential connections. We disagree with the reviewer about the suitability of your manuscript for publication by JCB, but do agree that there are issues with the presentation and the limitations of the study that need to be addressed.

Our general policy is that papers are considered through only one revision cycle; however, given that the suggested changes are relatively minor we are open to one additional short round of revision. If you decide to resubmit a suitably revised version, we would need a point-by-point response to each of the reviewer comments.

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

Sincerely,

Ian Macara, PhD
Monitoring Editor
Journal of Cell Biology

Dan Simon, PhD
Scientific Editor
Journal of Cell Biology

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

In the revised manuscript by Huang et al., the authors have addressed some of the issues raised previously and provided additional data not included in the previous submission. Overall, the experiments using isolated PCM cells support a positive role of Pk1-CKII in cortical actin regulation, while analysis of PCM explants suggest an opposite role of Pk1 membrane plaques in cortical actin regulation. Although the precise downstream mechanisms remain to be determined, these findings are still novel and significant.

I suggest several minor revisions:

1. It is unclear why the effect of CK666 was only shown in PCM explants (Figure 9B) but not in isolated PCM cells, which would be informative for comparison with Formin-inhibited cells shown in Figure 3.

2. MO gastrula phenotypes are caused by lack of both diffuse and punctate Pk1. If feasible, it would be informative to assess the role of diffuse Pk1 in cell motility/migration in isolated PCM cells plated on FN.

3. Figure 1A. The frequency of cell intercalation in control and morphants should be quantified.

4. P.3 first paragraph. "Pk1puncta" should be "Pk1 puncta".

5. P.3 last paragraph. "...bundles actin filaments" should be "bundled actin filaments".

6. P. 6 first paragraph. For better clarity, "the cortex became wider" should be "the cortex became thicker".

7. P.10 middle paragraph. For better clarity, "where diffuse Pk1 is present only" should be "where only diffuse Pk1 is present".

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

This heavily revised manuscript was a pleasure to read. I asked for speculation about the role of Pk1 puncta and instead got an abundance of data! The expanded presentation now makes a much fuller story. The reinterpretation of diffuse Pk1 is welcome, though some additional documentation would be helpful. The exclusively diffuse distribution in isolated cells vs diffuse plus puncta in tissue is a fortunate and useful way to get at the specific function of the diffuse population; the problem of assigning function to distinct pools is always a challenging problem in cell biology.

The authors now claim that the diffuse Pk1 is uniformly distributed in the cytoplasm, but appears more concentrated at the cortex due to less exclusion by various organelles relative to the more interior regions of the cell. This would be more convincing if they used their dextran labeling to quantitatively compare to Pk1 distribution across the cell. Their discussion of Pk condensates of specific conformers potentially responding to cortical tension at least vaguely implies the possibility of a physical interaction that could run counter to the claim of uniformity.

While the manuscript is much enhanced, the new Discussion needs a much more careful presemtation. It contains much hypothesis/speculation, as it should, but throughout, the speculation is not demarcated from direct interpretation and summary of results. Many instances of declarative statements should be stated as hypotheses, and many others that are only imprecisely worded as speculation should be made clearly so. There is great value to speculation and hypothesis generation, but this should not be confused with straight forward interpretation of results.

I'd also like to opine that the comments of the other reviewers have been appropriately addressed.

Minor comments:

In Figures 2 and S2, it would be useful to include membrane and junctional markers.

In Figure 3, the width of the cortical domain will appear wider if a non-equatorial plane is imaged. How did the authors control for this?

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

The resubmission by Huang and Winklbauer reports on the possible regulation of the actin cortex by the core PCP proteins Pk and Dvl, and especially attempts to ascribe specific function to "diffuse" and "punctate" Dvl. The authors characterize global disruption of Pk and observe defects in mesodermal migration and intercalation, and suggest that Pk and Dvl may regulate cortical F-actin through Casein Kinase II function, although the mechanism of this regulation (both upstream and downstream) is

unclear. Unfortunately, I still do not find this to be a particularly clear or compelling study. The logical flow of the manuscript is poor, and much of the study relies on correlations that are performed in very different physiological contexts. Specific tests of causation are generally not performed. Strong connections between the disparate experiments are lacking, or not particularly compelling. The writing is also challenging to follow - at times the reader has to guess at the meaning of some sentences or potential connections. A few detailed comments follow:

1) As mentioned in the first review, much of the manuscript focuses on "diffuse" Pk regulation of cortical actin (and "diffuse" is also in the manuscript title), but the study has no way of differentially targeting the different types of Pk localization at the functional level in a specific way in the same cells. The main way the authors examine this is by looking at tissue fragments that vary in size that show varying amounts of diffuse Pk (or in isolated cells). Why these fragments would have changes in Pk amount/localization is not explained. Indeed, the only way this study has to change diffuse vs punctate distributions of Pk is through an approach that examines tissue fragments/isolated cells that may have very different physiological states (adherent vs non-adherent, for example). I do not think it is possible from this approach to strictly infer relationships between these Pk distributions and F-actin function - how do we know whether the distribution of a host of adhesion and cytoskeletal proteins might be changed in adherent vs non-adherent cells?

2) Pg. 6, beginning of Results section on Pk and F-actin states, "Diffuse Pk1 up-regulates the cortical F-actin level." - this has not been demonstrated. At best, the authors could say there is a correlation between the two, but no causal link has been demonstrated at this point.

3) The data on diffuse and punctate Pk in the retracting tails of cells is again correlative, with no clear causative test.

4) The manuscript is often descriptive, and concrete mechanisms by which PCP regulates F-actin are not detailed. Again, it is strange that inhibiting actin formation with the SMIFH2 inhibitor causes an increase in cortical actin. This manuscript would have been more compelling if it could have offered a direct link to Arp2/3 or Formin function.

Minor comments

a) "It consists of a sub-membrane meshwork of...", pg. 3. "It" in this sentence would refer to Pk, etc as constructed. Line numbers would make referencing items easier.

b) "However, endogenous Pk occur in addition in a less well characterized...", pg. 5. This sentence is unclear, not sure what the meaning is (maybe meant to say "puncta"?).

Dear Ian, Dan,
Thank you for the kind reception of the revised version of our manuscript. We made all changes suggested by you, including the addition of new data. Please find our point-by-point response to the reviewer comments below. We also thank the reviewers for their help in improving the manuscript.

"Thank you for your revised manuscript on "Cell cortex regulation by the planar cell polarity protein Prickle1," which has now been evaluated by the three original referees. As you will see from the appended comments, two of the referees are enthusiastic about the work and feel that it is potentially acceptable for publication by the journal after additional revisions. The third reviewer remains negative. We feel that the work has been significantly improved, but that additional revisions are required. We hope that a suitably revised version could be assessed editorially without further evaluation by the external referees, assuming that you are able to address their comments satisfactorily.

Reviewer #1 suggests minor revisions, particularly that you use an Arp2/3 inhibitor to compare with Formin inhibition. Experiments were included with CK666 Arp2/3 inhibitor but in PCM explants whereas the Formin inhibition studies were done on isolated PCM cells. The Reviewer asks that you test CK666 on isolated PCM cells in order to directly compare with effects of Formin inhibition. We do not think their request to assess the role of diffuse Pk1 in cell motility/migration in isolated PCM cells plated on FN is necessary, but agree that you should quantify frequency of cell intercalation in control vs MOs.

Reviewer #2 is very supportive but comments that the distribution of Pk1 would be more convincing if dextran labeling was used to quantitatively normalize the Pk1 distribution across the cell. Other comments, such as those about the Discussion, just need modifications to the text.

Reviewer #3 feels that the ability to determine something concrete about a specific mechanism of cortical actin regulation by Pk and Dvl function, and especially that of "diffuse" or "punctate" Pk subpopulations is deeply compromised by the experimental approaches (tissue fragments, global morpholino disruptions, and low-resolution imaging). It seems the cells/tissues being examined are in very different physiological states (for example, adherent vs non-adherent cells), and it is difficult to know if a host of factors beyond the two PCP proteins they examine might also be changed by this altered physiology (and there are no controls presented to examine this possibility). They also comment that the writing is also challenging to follow - at times the reader has to guess at the meaning of some sentences or potential connections. We disagree with the reviewer about the suitability of your manuscript for publication by JCB, but do agree that there are issues with the presentation and the limitations

of the study that need to be addressed.

Our general policy is that papers are considered through only one revision cycle; however, given that the suggested changes are relatively minor we are open to one additional short round of revision. If you decide to resubmit a suitably revised version, we would need a point-by-point response to each of the reviewer comments.

We are glad that the manuscript is considered publishable, provided the above-mentioned changes. We added the data requested and modified the text throughout to increase the clarity of presentation and separate more clearly direct interpretation of data from speculation.

Reviewer #1 *(Comments to the Authors (Required)):*
In the revised manuscript by Huang et al., the authors have addressed some of the issues raised previously and provided additional data not included in the previous submission. Overall, the experiments using isolated PCM cells support a positive role of Pk1-CKII in cortical actin regulation, while analysis of PCM explants suggest an opposite role of Pk1 membrane plaques in cortical actin regulation. Although the precise downstream mechanisms remain to be determined, these findings are still novel and significant.
I suggest several minor revisions:

1. It is unclear why the effect of CK666 was only shown in PCM explants (Figure 9B) but not in isolated PCM cells, which would be informative for comparison with Formin-inhibited cells shown in Figure 3.

We show the effects of CK666 inhibition in isolated PCM cells now in Fig.3B,B'.

2. MO gastrula phenotypes are caused by lack of both diffuse and punctate Pk1. If feasible, it would be informative to assess the role of diffuse Pk1 in cell motility/migration in isolated PCM cells plated on FN.

We had these data in Dr. Huang's PhD Thesis but did not include them in the manuscript as it is difficult to interpret the results in the context of the PCM explants examined in the paper. In the explants, cells migrate on each other's surface, are unipolar, and are inhibited by Pk1 knockdown. On the artificial FN substratum in vitro, single cells are bipolar, and although their migration is also inhibited by Pk1-MO injection, this could be due to different reasons. For example, morphant cells are round on FN instead of highly elongated as in explants, which could be due to reduced cell spreading, etc. We were afraid that discussions of this issue would unduly complicate the manuscript without adding substantial insights

into the in situ effects. However, we mention now the inhibitory effect of Pk1MO on single cell migration, citing Dr. Huang's thesis.

3. Figure 1A. The frequency of cell intercalation in control and morphants should be quantified.

We quantify intercalation now in Fig.1A'.

4. P.3 first paragraph. "Pk1puncta" should be "Pk1 puncta".
Done.

5. P.3 last paragraph. "...bundles actin filaments" should be "bundled actin filaments".
Done.

6. P. 6 first paragraph. For better clarity, "the cortex became wider" should be "the cortex became thicker".
Done.

7. P.10 middle paragraph. For better clarity, "where diffuse Pk1 is present only" should be "where only diffuse Pk1 is present".
Done.

Reviewer #2 (*Comments to the Authors (Required)):*
This heavily revised manuscript was a pleasure to read. I asked for speculation about the role of Pk1 puncta and instead got an abundance of data! The expanded presentation now makes a much fuller story. The reinterpretation of diffuse Pk1 is welcome, though some additional documentation would be helpful. The exclusively diffuse distribution in isolated cells vs diffuse plus puncta in tissue is a fortunate and useful way to get at the specific function of the diffuse population; the problem of assigning function to distinct pools is always a challenging problem in cell biology.

The authors now claim that the diffuse Pk1 is uniformly distributed in the cytoplasm, but appears more concentrated at the cortex due to less exclusion by various organelles relative to the more interior regions of the cell. This would be more convincing if they used their dextran labeling to quantitatively compare to Pk1 distribution across the cell. Their discussion of Pk condensates of specific conformers potentially responding to cortical tension at least vaguely implies the possibility of a physical interaction that could run counter to the claim of uniformity.

We co-labelled cells with blue dextran and Pk1-venus (new Fig.5F), and a representative line plot shows co-distribution of both components across cells (new Fig.5F'). To reconcile this with Pk1 a possible protein conformation transition under stress, one could assume that increased cortical stress leads to (direct or indirect) binding of Pk1 to stress-altered cortex molecules, which then secondarily induces a transition between closed and open Pk1 conformations, and protein condensation in the form of puncta.

While the manuscript is much enhanced, the new Discussion needs a much more careful presemtation. It contains much hypothesis/speculation, as it should, but throughout, the speculation is not demarcated from direct interpretation and summary of results. Many instances of declarative statements should be stated as hypotheses, and many others that are only imprecisely worded as speculation should be made clearly so. There is great value to speculation and hypothesis generation, but this should not be confused with straight forward interpretation of results.

Point taken! We modified the text not only in the Discussion, but in all parts of the manuscript according to these suggestions.

I'd also like to opine that the comments of the other reviewers have been appropriately addressed.

Minor comments:
In Figures 2 and S2, it would be useful to include membrane and junctional markers.

We agree in principle, but with rather limited resources presently available to us, we focussed our efforts on the other points raised by the reviewers.

In Figure 3, the width of the cortical domain will appear wider if a non-equatorial plane is imaged. How did the authors control for this?

We manually focussed through cells until the cell diameter was maximal. This was taken as the equatorial plane and used for measurements. A respective explanation has been added to Materials and Methods, p.17, in the section on "Phalloidin staining and cortex density measurements".

Reviewer #3 (Comments to the Authors (Required)):
The resubmission by Huang and Winklbauer reports on the possible regulation of the actin cortex by the core PCP proteins Pk and Dvl, and especially attempts to ascribe specific function to "diffuse" and "punctate" Dvl. The authors characterize global disruption of Pk and observe

defects in mesodermal migration and intercalation, and suggest that Pk and Dvl may regulate cortical F-actin through Casein Kinase II function, although the mechanism of this regulation (both upstream and downstream) is unclear. Unfortunately, I still do not find this to be a particularly clear or compelling study. The logical flow of the manuscript is poor, and much of the study relies on correlations that are performed in very different physiological contexts. Specific tests of causation are generally not performed. Strong connections between the disparate experiments are lacking, or not particularly compelling. The writing is also challenging to follow - at times the reader has to guess at the meaning of some sentences or potential connections. A few detailed comments follow:

We took this criticism to heart. Looking at the manuscript from a distance, we see the shortcomings explained above. We modified the text in all parts of the manuscript to make opaque sentences comprehensible, improve the logical flow of arguments, and separate direct conclusions from data more clearly from speculative interpretations. We have a problem though with always understanding the reviewer's distinction between "causative" and "correlative" (see below).

1) As mentioned in the first review, much of the manuscript focuses on "diffuse" Pk regulation of cortical actin (and "diffuse" is also in the manuscript title), but the study has no way of differentially targeting the different types of Pk localization at the functional level in a specific way in the same cells. The main way the authors examine this is by looking at tissue fragments that vary in size that show varying amounts of diffuse Pk (or in isolated cells). Why these fragments would have changes in Pk amount/localization is not explained.

Given the present focus on Pk or Dvl puncta, tissue fragments of varying sizes were used to confirm that they are indeed present in our experimental system, but their abundance is a flexible parameter. The experimental induction of puncta by cell deformation or osmotic stress also serves this purpose.

Indeed, the only way this study has to change diffuse vs punctate distributions of Pk is through an approach that examines tissue fragments/isolated cells that may have very different physiological states (adherent vs non-adherent, for example). I do not think it is possible from this approach to strictly infer relationships between these Pk distributions and F-actin function - how do we know whether the distribution of a host of adhesion and cytoskeletal proteins might be changed in adherent vs non-adherent cells?

Indeed, we made use of the fact that single cells, having no puncta, can be used to study the role of diffuse cytoplasmic Pk1. The different physiological states of adherent and non-adherent cells seems to be important for puncta formation, but we show that diffuse Pk1 is

present in both conditions, and the parallel changes upon experimental Pk1 manipulations in cortex density in single cells and cortical tension in cell pairs (no puncta) **but also in large cell aggregates** (puncta) argues for the same role of diffuse Pk1 in both physiological states. We point this out now on p.9, end of middle paragraph.

2) Pg. 6, beginning of Results section on Pk and F-actin states, "Diffuse Pk1 up-regulates the cortical F-actin level." - this has not been demonstrated. At best, the authors could say there is a correlation between the two, but no causal link has been demonstrated at this point.

We show that when only diffuse Pk1 is present, knocking down Pk1 lowers cortical F-actin levels and overexpression increases it – we do not see why stating "correlates with" is more appropriate here than "regulates". Below, under point 4), when referring to our formin inhibition experiment, the reviewer himself notes that "the SMIFH2 inhibitor **causes** an increase in cortical actin". We don't see which difference renders an experimental result in the present case correlative and in the one below causative.

3) The data on diffuse and punctate Pk in the retracting tails of cells is again correlative, with no clear causative test.

We had indeed stated that the data are correlative, they are not based on experimental interference. But they strongly suggest nevertheless that Pk1 puncta do have functions in the PCM, which was the purpose here. Alternatively, puncta could have seemed non-functional in all contexts observed, suggesting the possibility that they are irrelevant epiphenomena. Beyond that, the unexpected correlation of puncta with reduced cortical F-actin seems to us interesting and stimulating enough to be mentioned in the paper.

4) The manuscript is often descriptive, and concrete mechanisms by which PCP regulates F-actin are not detailed. Again, it is strange that inhibiting actin formation with the SMIFH2 inhibitor causes an increase in cortical actin. This manuscript would have been more compelling if it could have offered a direct link to Arp2/3 or Formin function.

We agree that establishing a direct link between Pk1 and Arp2/3 or formin function would be a compelling achievement. But this would be a different paper altogether, and one made more likely indeed by the present paper. We think it is worthwhile to first establish the basic function of Pk1 in cortex regulation, which we think we did here, thereby directing further analysis.

Minor comments
a) "It consists of a sub-membrane meshwork of...", pg. 3. "It" in this sentence would refer to Pk,

etc as constructed. Line numbers would make referencing items easier.

Sentence has been rephrased.

b) "However, endogenous Pk occur in addition in a less well characterized...", pg. 5. This sentence is unclear, not sure what the meaning is (maybe meant to say "puncta"?).

Sentence has been rephrased.

March 25, 2022

RE: JCB Manuscript #202008116RR

Prof. Rudolf Winklbauer
University of Toronto
Department of Cell and Systems Biology
25 Harbord Street
Toronto M5S 3G5
Canada

Dear Prof. Winklbauer,

Thank you for submitting your revised manuscript entitled "Cell cortex regulation by the planar cell polarity protein Prickle1." We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

We appreciate the discussion of your data regarding Pk1 role in cell migration. However, JCB policy does not allow for citations of a thesis or unpublished data so we ask that you please remove this part from the text. Additionally, although this is not yet required by the journal, we strongly encourage you to show individual data points from all replicates in histograms.

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. Please add scale bars to Figures 1A, 5B/B', 6E, and S1.

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.

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.

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.

-- 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.

Additionally, JCB encourages authors to submit a short video summary of their work. These videos are intended to convey the main messages of the study to a non-specialist, scientific audience. Think of them as an extended version of your abstract, or a short poster presentation. We encourage first authors to present the results to increase their visibility. The videos will be shared on social media to promote your work. For more detailed guidelines and tips on preparing your video, please visit https://rupress.org/jcb/pages/submission-guidelines#videoSummaries.

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,

Ian Macara, PhD
Monitoring Editor
Journal of Cell Biology

Dan Simon, PhD
Scientific Editor
Journal of Cell Biology