Myosin II proteins are required for organization of calcium-induced actin networks upstream of mitochondrial division

Frieda Kage, Miguel Vicente-Manzanares, Brennan McEwan, Arminja Kettenbach, and Henry Higgs

Corresponding author(s): Henry Higgs, Geisel School of Medicine at Dartmouth

Review Timeline:

| Event                  | Date     |
|------------------------|----------|
| Submission Date        | 2022-01-11|
| Editorial Decision     | 2022-02-21|
| Revision Received      | 2022-03-10|
| Accepted               | 2022-04-04|

Editor-in-Chief: Matthew Welch

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)
RE: Manuscript #E22-01-0005
TITLE: Myosin II proteins are required for organization of calcium-induced actin networks upstream of mitochondrial division

Dear Harry,

You will see that both reviewers found your study very interesting. While reviewer #1 has only minor comments, reviewer #2 has two important comments that you should address regarding actin quantification and mitochondrial connectivity measurement.

Finally, reviewer #2 has a key question regarding MIIC KO.

Sincerely,

Laurent Blanchoin
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Higgs,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

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Reviewer #1 (Remarks to the Author):

[Review comments from reviewer #1]

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Kage et al. investigate the role of non-muscle myosin in the process of organizing calcium induced actin (CIA). By performing a series of knockdowns and knockouts of the non-muscle myosin II paralogs, myosin IIA, myosin IIB, and myosin IIC, they were able to show the importance of these proteins in organizing but not polymerizing the actin filaments. CIA filaments are found to be enriched with these paralogs which indicates a role for these proteins in a CIA network. They also investigate the downstream effects of the myosin II perturbations by examining the change in mitochondria morphology and mitochondrial calcium influx. In doing so, they found that there is a significant decrease in calcium influx in myosin II knockouts, and more specifically myosin IIA seems to have the most profound effect on mitochondria. Overall, this paper does an excellent job of characterizing the interaction between NMII and CIA while also putting this in the context of a more functional role concerning mitochondria.

Overall, the authors do an excellent job of providing clear controls for their experiments to ensure confidence in their data. The setup for their experiments are meticulously crafted, and it provides for a clearer picture of their results.

The investigators take a broad view of the effects of their knockdowns/knockouts by looking at their question from a multi-organelle perspective. This approach allows for a greater understanding beyond cytoskeletal impact and a more in depth look at the relationship of the myosin paralogs to critical cellular processes.

Minor Comment:
The comparison between the perinuclear and peripheral mitochondria could use further support. Given that the perinuclear region is not as flat as the peripheral region, it is hard to justify that a single plane view of the mitochondria is reliable enough to draw conclusions. Also, it may serve the authors better to use one metric of either length or diameter of mitochondria, for it is difficult to discern one metric from the other given how they sampled.

Reviewer #2 (Remarks to the Author):

Overall this is a very nice study with lots of great data. It should be of broad interest to the readership of MBc. The paper is also exceptionally well written. I only have three concerns that should be addressed before publication.

(1) My main concern is what I see as a significant disconnect between Figures 4 and 5, which impacts one of their major conclusions (that MII does not influence the assembly of the CIA, just its organization). The imaging data in Figure 4 shows clearly that the actin signal per unit area is less in the mutants, arguing that they make less CIA. This result cannot be explained away due to a change in the "appearance" of the actin. The intensity signals they measured are independent of whether the actin is bundled or not. So to me this data says very clearly that the myosin mutants make less CIA actin. In Figure 5, they use a biochemical assay to argue that the effects reported in Figure 4 are due to a change in actin organization (less bundled/organized) rather than actin assembly. First, I think quantitative morphometric analyses of the images in Figure 4 would be much better at proving that the organization of actin is altered than grinding up the cells and doing triton extractions. But even more importantly, differences in the organization of actin cannot explain away the intensity measurements in Figure 4 that show less CIA is being made in the MII mutants.

(2) My second concern has to do with the mitochondrial connectivity measurements made using a PA matrix marker in Figure 9. It's not clear to me how one can compare experimental and control samples using this method without normalizing for the initial area of matrix photoactivated.

(3) My third concern has to do with how many independent KO clones they scored in their various phenotypic assays. While I see they made independent KO lines (Figure 2), as best as I can tell they used only one KO line for each isofrom in their numerous phenotype assays (all the figures simply refer to "KO" rather than to specific clones). If I am correct, this is dicey, especially as they did not do rescue experiments (unless I missed that). Obviously it's too much to ask the authors to do the whole study over with additional KO clones, but if my comment is right, they should include a caveat about this (or add any evidence they have that independent clones behaved similarly). As for rescue, how such a tiny amount of MIIC can have such a big effect is really odd. This key result would benefit a lot from a rescue experiment or by demonstrating that independent MIIC KO clones have the same phenotype.
We thank the reviewers for their comments, and feel that the resulting changes have definitely improved the manuscript. We address all individual comments below.

Reviewer 1:
The comparison between the perinuclear and peripheral mitochondria could use further support. Given that the perinuclear region is not as flat as the peripheral region, it is hard to justify that a single plane view of the mitochondria is reliable enough to draw conclusions. Also, it may serve the authors better to use one metric of either length or diameter of mitochondria, for it is difficult to discern one metric from the other given how they sampled.

These are excellent points, and we have re-organized the Results, to make these distinctions clearer. Also, we have added a paragraph to the Discussion, to address this issue. Indeed, we had neglected the mitochondrial effects completely in the Discussion, so that is an excellent addition.

Reviewer 2:
Overall this is a very nice study with lots of great data. It should be of broad interest to the readership of MBoC. The paper is also exceptionally well written. I only have three concerns that should be addressed before publication.

(1) My main concern is what I see as a significant disconnect between Figures 4 and 5, which impacts one of their major conclusions (that MII does not influence the assembly of the CIA, just its organization). The imaging data in Figure 4 shows clearly that the actin signal per unit area is less in the mutants, arguing that they make less CIA. This result cannot be explained away due to a change in the "appearance" of the actin. The intensity signals they measured are independent of whether the actin is bundled or not. So to me this data says very clearly that the myosin mutants make less CIA actin. In Figure 5, they use a biochemical assay to argue that the effects reported in Figure 4 are due to a change in actin organization (less bundled/organized) rather than actin assembly. First, I think quantitative morphometric analyses of the images in Figure 4 would be much better at proving that the organization of actin is altered than grinding up the cells and doing triton extractions. But even more importantly, differences in the organization of actin cannot explain away the intensity measurements in Figure 4 that show less CIA is being made in the MII mutants.

The reviewer makes an excellent point here, which has prompted us to re-think our logic. We had thought that our analytical method for quantifying TRITC-phalloidin intensity would result in lower readings for less ‘bundled’ actin. However, we do now agree with the reviewer, that the overall TRITC-phalloidin signal should be constant in an ROI, whether the actin is bundled or not. In practice, the signal from single filaments may get lost in the background noise in the cytoplasm, which would cause the bundled actin to be over-represented. However, we have no way of easily knowing whether this is true, so now agree that we should use the strict guidelines that the
fluorescent signal will be the same for one bundle of 10 filaments as it would be for 10 single filaments.

For this reason, we have altered our presentation of the results (which shortened the results) and added a discussion of this discrepancy to the Discussion. We would ask that we be allowed to leave this issue un-resolved at this point.

(2) My second concern has to do with the mitochondrial connectivity measurements made using a PA matrix marker in Figure 9. It's not clear to me how one can compare experimental and control samples using this method without normalizing for the initial area of matrix photoactivated.

We can see how this procedure was unclear in the original manuscript and have now clarified. We now state the exact area (which is fixed as a 2 square micron circle on our microscope) and the fact that at least 90% of the area contained mitochondria in all of the fields in the Methods. We chose peripheral regions for this experiment.

(3) My third concern has to do with how many independent KO clones they scored in their various phenotypic assays. While I see they made independent KO lines (Figure 2), as best as I can tell they used only one KO line for each isoform in their numerous phenotype assays (all the figures simply refer to "KO' rather than to specific clones). If I am correct, this is dicey, especially as they did not do rescue experiments (unless I missed that). Obviously it's too much to ask the authors to do the whole study over with additional KO clones, but if my comment is right, they should include a caveat about this (or add any evidence they have that independent clones behaved similarly). As for rescue, how such a tiny amount of MIIC can have such a big effect is really odd. This key result would benefit a lot from a rescue experiment or by demonstrating that independent MIIC KO clones have the same phenotype.

We totally understand the reviewer's concern. We have put a caveat in the Discussion about potential clonal variation.
2nd Editorial Decision

RE: Manuscript #E22-01-0005R
TITLE: “Myosin II proteins are required for organization of calcium-induced actin networks upstream of mitochondrial division”

Dear Harry

I am happy to congratulate you on your manuscript that is now accepted for publication in MBoC.

Sincerely,
Laurent Blanchoin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Higgs:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #2 (Remarks to the Author):

While I could quibble about one or two explanations/caveats in the author’s rebuttal, I think this paper has more than enough nice data to warrant publication in MBoC in its current form.