Micron-scale supramolecular myosin arrays mediate cytoskeletal assembly at mature adherens junctions

Hui-Chia Yu-Kemp, Rachel Szymanski, Daniel Cortes, Nicole Gadda, Madeline Lillich, Amy Maddox, and Mark Peifer

Corresponding Author(s): Mark Peifer, UNC-Chapel Hill

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April 26, 2021

Re: JCB manuscript #202103074

Dr. Mark Peifer
UNC-Chapel Hill
Department of Biology University of North Carolina at Chapel Hill CB#3280; Coker Hall
Chapel Hill, NC 27599-3280

Dear Dr. Peifer,

Thank you for submitting your manuscript entitled "A role for a micron-scale supramolecular myosin array in adherens junction cytoskeletal assembly". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

You will see that the reviewers were overall enthusiastic about the paper, but they have each raised a number of concerns that will need to be addressed before the paper would be deemed appropriate for publication in JCB. In particular and given that the proposed role of the myosin's motor activity as the driving force behind junctional actin assembly at adherens junctions is one of the major claims of this study, we hope that you add, as requested by reviewer #1, a different cellular model that does not rely on an enhanced ROCK signaling to firmly establish the mechanism underlying the condensation of myosin II stacks at the apical junctions. Although the impact of the study would be superior by a deeper mechanistic investigation on how myosin II stacks reorganize F-actin, as suggested by reviewer #1, and by demonstrating that assembly of junctions takes place in the same manner that proposed here upon different conditions than removal of calcium, as noted by reviewer #3, we acknowledge these experimental analyses are not strictly needed to support the main conclusions of the paper so we will not require such experiments for resubmission. We hope that you will be able to address each of these concerns in full, including substantial new data to support the main conclusions of the study.

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

GENERAL GUIDELINES:

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

Figures: Articles may have up to 10 main text figures. Figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, https://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

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

Supplemental information: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of 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)):

This manuscript tackles the complicated task of understanding how a contractile cytoskeleton is assembled at cadherin-based cell-cell adhesions. We now understand a good deal about how the minimal molecular machinery of the cadherin-catenin complex interacts with actin filaments. But understanding cell and tissue biology carries the super-added challenges of working out how the cell builds and regulates micron-scale interactions between adhesion and actomyosin cytoskeleton. The molecular understanding of the minimal complex is not enough for this.

Now, Yu-Kemp et al extend our knowledge by making the following observations: 1) Junctional actin assembly is not attributable to a single apparatus, but is likely to involve contributions from Arp2/3,
In its early phase the junctional cytoskeleton is distinguished by a large network of Myosin II stacks, which seem to be present at the apical cortex, near the presumptive junction - and which then condense into the apical junctions in a ROCK-dependent fashion. 3) Myosin II activity may contribute to organizing and condensing F-actin as the AJ assemble.

I think that these are informative observations, which are founded on data of superb quality. But I am not sure that they currently yield the depth of conceptual or mechanistic advance that one might expect of a JCB paper.

Specific points

1) Multiple nucleators. While this is not something that had previously been addressed by direct comparison, as Yu-Kemp and her colleagues now do, it is not surprising that multiple nucleators contribute to the AJ cytoskeleton. As the authors point out, inhibition of individual pathways did not abolish junctional F-actin in earlier studies. And a role for multiple actin nucleators and regulators is a concept that has been well-established for other systems, e.g. filopodia. Indeed, the Gallop lab’s recent JCB paper is an interesting example of how one might pursue the concept of overlapping nucleator contributions.

2) A role for Myosin II in organizing F-actin also has a precedent especially in reconstitution studies (e.g. Gardel, Lenart). It is good to know that it may also operate at AJ, but the current analysis is based on the correlation of Myosin and F-actin "co-condensation) and the fact that Myosin II drug inhibitors compromises actin organization. I think we need a bit more mechanistic insight, although I appreciate that these are challenging and potentially beyond the scope of the present paper. For example, is the condensation of Myosin II stacks responsible for reorganizing actin? (The current data say that Myosin II activity is necessary.) Some clues to guide experiments might be found in the work of Bershadsky (actinins in stack biogenesis) or by targeting tropomyosins.

3) The authors use ZO-1/2 KD cells for their studies because they make beautiful Myosin II arrays. But this is arguably a gain-of-function system. The Peifer lab’s earlier work indicated that it is likely to reflect an enhanced ROCK system (which would possibly explain why ROCK inhibition affected the Myosin arrays, but not MLCK inhibition). And it is also difficult to exclude other effects on the cytoskeleton associated with loss of ZOs (e.g. the recent paper from Dan Fletcher’s group identifying a novel actin-binding sequence). While the ZO KD cells are a useful test of principle, it would be good to confirm some of their conclusions in another (less manipulated) cell line.

Minor points

1. Is there any ENA/VASP protein left at AJ/tricellular junctions in the FP4-Mito cells?

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

The manuscript by Yu-Kemp et al describes the formation and function of a supramolecular array of myosin filaments in adherens junction assembly. The study is of significant interest to the entire cytoskeletal community. It is thorough and quantitative. The model system are ZO-knockdown MDCK cells which lose their junctions and tend to round up when deprived of calcium, but recover their structure and junctions upon readdition of calcium over a 3 hr period. The conclusions are that the myosin assembly creates forces along cell junctions that straighten the edges and allow for
polarization of the cytoskeleton. Studies with drugs that are known to perturb various actin assembly pathways had little effect, but inhibition of myosin globally with blebbestatin and, perhaps, more locally with ROCK inhibitors disrupted the process. The paper is well organized, well written and the figures are organized in a logical fashion. I have only minor criticisms.

The authors focus on nonmuscle myosin 2B in the paper, but do acknowledge the existence of nonmuscle myosins 2A and 2C. As mentioned, these myosins can form heterotypic filaments. Given that Conti et al described a likely role for NM2A in adhesion it would be interesting to at least show one panel of staining with NM2A antibodies before and after calcium manipulation.

Is a larger phenotype observed if cells are treated with inhibitors of both MLCK and ROCK? Similarly, can cells recover after blebbistatin wash-out if the ROCK inhibitor is still present?

The speculation on the role of myosin-18 is intriguing and it would be interesting to see how knockdown of this would affect the myosin assemblies, but this may be too much to ask for in revision at this time.

Minor Point:
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Yu-Kemp et al. present a comprehensive description of the steps of contractile system assembly in MDCK monolayers during the recovery from calcium depletion. Recovery from calcium depletion is a decades old experimental paradigm and the work presented in this manuscript demonstrates that revisiting previous results with modern techniques can reveal interesting insights. The data presented here is well controlled for with the exception of the formin inhibition experiments, which I believe is not something that needs to be addressed during revision (see minor point 4 below). My only concern stems from whether the recovery from calcium depletion represents how the assembly of junctions occurs in monolayer with perturbation. Do these steps-particularly the formation of an enlarged myosin stack-filled contractile apparatus condensing down into a relatively thinner sarcomere-like arrangement-occur using any other experimental paradigm? For example, MDCK cells that are freshly plated at high density are also rounded before the assembly of junctions. Is it possible to use such a system to show that a large array of myosin stacks are assembled at early time points with a thinner array later on? To be clear, I am not asking for the authors to repeat any of the perturbations presented using their recovery model with a different condition.

Minor points:
1. The authors state that they are using a "well-characterized calcium switch assay". While this statement is correct, I do not think that single reference to a paper from 1986 is enough to guide readers to explore this assay and judge from themselves. I would like to see more references to this assay and the underlying biology. I believe the Methods section would be a good place to add references. While foundational, Gumbiner and Simons, 1986 does not-in and of itself-support this assay as being "well-characterized".

2. The authors cite three papers that investigate myosin stacks using super-resolution microscopy (Beach et al., 2017; Fenix et al., 2016; Hu et al., 2017). By reading the text, one would get the
impression that these were the first to show stack formation using SIM. However, the Lippincott-Schwartz lab demonstrated stack assembly in 2014. Why was this particular paper omitted?

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Response to Reviewers: JCB manuscript #202103074.

We are very grateful to the editor and reviewers for their generally supportive response to our initial manuscript and their very helpful feedback on our manuscript. Prompted by their suggestions, we carried out new experiments including those suggested by the Editor and each of the Reviewers to strengthen the mechanistic insights, and also substantially revised the text and Figures to address concerns of all three Reviewers. The new experiments include verification that the role of myosin seen in our original cell line is also seen in another, non-mutant cell line (Caco-2 cells; New Figures 9 and 10; S6 and S7), use of Cytosim based modeling to ask what role myosin arrays might have in organizing actin (New Figure 5), exploration of the effect in inhibiting both the Arp2/3 complex and formins (Revised Fig S3), asking if the spiky actin induced by blebbstatin treatment was driven by formins (Fig 8I-K), exploration of the effect of combining treatment with the ROCK inhibitor and blebbistatin (Revised Fig S5), addition of the localization of the actin cross linker alpha-actinin in the myosin arrays (Revised Figure 2), addition of the localization of myosin-2A (Revised Fig S1R,S), and assessment of whether myosin arrays were caused by the Ca switch (Revised FigS1T). This significant body of new data is included in new Figures 5, 9 and 10, new Supplemental Figures S3, S6 and S7, and Revised Figures 1, 2, S1, S2, S5 and S8. We have also substantially modified the text in response to their suggestions, clarifying and softening points as they suggested. All of the text additions and changes are noted in an attached “MarkedUp” version of the text, with new text and changes highlighted in red. These changes significantly strengthened the manuscript. Below we outline our specific responses to each Reviewer concern.

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We agree that exploring roles for multiple actin regulators acting in parallel is important. We added new experiments in which we treated cells simultaneously with CK666 plus SMIFH2 during recovery from the calcium switch. Intriguingly, this did not prevent ZA actomyosin assembly, nor was there an obvious delay in the process. Cells treated with CK666 plus SMIFH2 reassembled a sarcomeric myosin array with tightly bundled actin, with timing that was parallel to the controls (new Figure S3; representative of three experiments). Quantification confirmed the tight bundling of actin. We did note some cell toxicity, similar to that observed after SMIFH2 treatment alone. These data suggest our cells can reassemble the ZA without either
Arp2/3 or formin activity. Of course, more complex overlapping combinations of the Arp2/3 complex, formins and Ena/VASP proteins might act in this system and we now note this explicitly in the Discussion.

2) A role for Myosin II in organizing F-actin also has a precedent especially in reconstitution studies (e.g. Gardel, Lenart). It is good to know that it may also operate at AJ, but the current analysis is based on the correlation of Myosin and F-actin “co-condensation) and the fact that Myosin II drug inhibitors compromises actin organization. I think we need a bit more mechanistic insight, although I appreciate that these are challenging and potentially beyond the scope of the present paper. For example, is the condensation of Myosin II stacks responsible for reorganizing actin? (The current data say that Myosin II activity is necessary.) Some clues to guide experiments might be found in the work of Bershadsky (actinin in stack biogenesis) or by targeting tropomyosins.

As the Reviewer notes, further mechanistic insights into how myosin contributes to actin bundling are of interest, and will potentially extend beyond the scope of the manuscript. To begin to address this issue, we initiated a collaboration with Daniel Cortes and Amy Maddox to use Daniel’s agent-based modeling approach to explore potential mechanisms by which myosin “could” act. These suggest myosin may act by moving existing actin filaments toward the junctional cadherin-catenin complexes—these data are in new Figure 5. With regard to the second question, we have localized alpha-actinin, finding it co-localized with the myosin tail in both the final ZA actomyosin sarcomeric array, and in the micron-scale myosin stacks seen during recovery (revised Figure 2 J-M). We agree that studying its function would be of value, and now suggest this in the Discussion, but we feel functional analysis of alpha-actinin’s role extends beyond the current manuscript.

3) The authors use ZO-1/2 KD cells for their studies because they make beautiful Myosin II arrays. But this is arguably a gain-of-function system. The Peifer lab’s earlier work indicated that it is likely to reflect an enhanced ROCK system (which would possibly explain why ROCK inhibition affected the Myosin arrays, but not MLCK inhibition). And it is also difficult to exclude other effects on the cytoskeleton associated with loss of ZOs (e.g. the recent paper from Dan Fletcher’s group identifying a novel actin-binding sequence). While the ZO KD cells are a useful test of principle, it would be good to confirm some of their conclusions in another (less manipulated) cell line.

This was a very important point, as noted by the Reviewers and the Editor. We chose Caco-2 cells as a less manipulated model, because earlier work from the Yap and Gardel labs had suggested they also assemble a robust and contractile actomyosin array at the ZA. This proved very interesting. As we now document, these cells also assemble micron-scale myosin arrays during junctional re-assembly after calcium switch. Strikingly, junctional re-assembly is not affected by inhibitors of the Arp2/3 or formin actin nucleators. However, Caco-2 cells are even more susceptible to myosin inhibition, with dramatic effects on reassembly at tricellular junctions. These data are in new Figures 9 and 10 and new Figures S6 and S7. We’re grateful to the Reviewers and Editor for suggesting this work, as it broadens the impact of our student.
1. Is there any ENA/VASP protein left at AJ/tricellular junctions in the FP4-Mito cells?

We have amplified on this discussion. We cannot, of course, completely rule out whether a small amount of VASP remains at junctions, but have added to Fig S2L an image taken at the level of the apical junctions that shows no detectable VASP in FP4 expressing cells, when it is readily apparent in apical junctions of neighbors, and b) a picture of a more basal section (1.8 µm basal) where the mitochondria localize) with the signal enhanced to make clearer that most or all VASP is gone from the cortex. We also slightly softened the text: “We confirmed that FP4mito sequestered VASP at mitochondria in our cell line, while AP4 did not (Fig S2K,L)—VASP was no longer detectable at apical junctions and enhancing the signal more basally suggested most or all VASP was lost from the cortex (Fig S2L).”

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

The manuscript by Yu-Kemp et al describes the formation and function of a supramolecular array of myosin filaments in adherens junction assembly. The study is of significant interest to the entire cytoskeletal community. It is thorough and quantitative. The model system are ZO-knockdown MDCK cells which lose their junctions and tend to round up when deprived of calcium, but recover their structure and junctions upon readdition of calcium over a 3 hr period. The conclusions are that the myosin assembly creates forces along cell junctions that straighten the edges and allow for polarization of the cytoskeleton. Studies with drugs that are known to perturb various actin assembly pathways had little effect, but inhibition of myosin globally with blebbestatin and, perhaps, more locally with ROCK inhibitors disrupted the process. The paper is well organized, well written and the figures are organized in a logical fashion. I have only minor criticisms.

The authors focus on nomuscle myosin 2B in the paper, but do acknowledge the existence of nonmuscle myosins 2A and 2C. As mentioned, these myosins can form heterotypic filaments. Given that Conti et al described a likely role for NM2A in adhesion it would be interesting to at least show one panel of staining with NM2A antibodies before and after calcium manipulation.

This was a good suggestion. Myosin 2A and 2B both colocalize in the sarcomeric arrays at the ZA in unperturbed cells and both localize to the expanded myosin stacks seen during recovery. We have added this to Fig S1R-S and briefly describe it in the text.

Is a larger phenotype observed if cells are treated with inhibitors of both MLCK and ROCK? Similarly, can cells recover after blebbistatin wash-out if the ROCK inhibitor is still present?

These are interesting questions, and are among many such double drug combinations that are possible. We chose to try what we thought would be the most substantial reduction in myosin function, combining ROCK inhibition and Blebbistatin (Revised Figure S5). These results of this were intriguing. The effect on myosin assembly was similar to that seen after ROCK inhibition
alone, with strongly reduced cortical myosin and total loss of the myosin arrays. At the level of the apical junctions, the effect on actin organization also resembled that of ROCK inhibition alone—actin accumulated in broad disorganized cortical arrays rather than the tightly bundled actin seen in controls. The spiky actin protrusions seen at the level of the apical junctions after blebbistatin treatment were absent. However, when we focused on the apical surface of the cells, spiky protrusions were seen there similar to those seen at the level of the cell junctions in cells treated with blebbistatin alone. Perhaps most interesting, combining ROCK inhibition and Blebbistatin did not prevent cells from re-establishing cadherin-based junctions and zipping up, but the apical enrichment of cadherin at the ZA was strongly reduced or abolished.

The speculation on the role of myosin-18 is intriguing and it would be interesting to see how knockdown of this would affect the myosin assemblies, but this may be too much to ask for in revision at this time.

We agree that this is an important future direction, but given the other requested experiments we confined the change to an additional sentence in the Discussion as follows: “Perhaps Myosin-18 isoforms are present in our system, helping anchor myosin stacks to the membrane and further assist stack formation. Knockdown of myosin-18 in these cells would provide an interesting future test of this possibility.” In light of our new data on alpha-actinin localization in arrays, we also added this to the Discussion: “Given our localization of alpha-action in the myosin arrays in our cells, it would be interesting in the future to knock it down in our cell type.”

Minor Point:
p.11 Reference(s) is/are needed for the statement starting with "Myo2A has the highest rate...."

Good point—we added a reference to a Sellers review

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

Yu-Kemp et al. present a comprehensive description of the steps of contractile system assembly in MDCK monolayers during the recovery from calcium depletion. Recovery from calcium depletion is a decades old experimental paradigm and the work presented in this manuscript demonstrates that revisiting previous results with modern techniques can reveal interesting insights. The data presented here is well controlled for with the exception of the formin inhibition experiments, which I believe is not something that needs to be addressed during revision (see minor point 4 below). My only concern stems from whether the recovery from calcium depletion represents how the assembly of junctions occurs in monolayer with perturbation. Do these steps-particularly the formation of an enlarged myosin stack-filled contractile apparatus condensing down into a relatively thinner sarcomere-like arrangement—occur using any other experimental paradigm? For example, MDCK cells that are freshly plated at high density are also rounded before the assembly of junctions. Is it possible to use such a system to show that a large array of myosin stacks are assembled at early time points with a
thinner array later on? To be clear, I am not asking for the authors to repeat any of the perturbations presented using their recovery model with a different condition.

This was a good suggestion. To assess this, we re-plated cells without a calcium switch and asked how the ZA reformed de novo between cells within colonies, at early time points. These cells also formed similar myosin arrays during junction establishment (Fig S1T)

Minor points:
1. The authors state that they are using a "well-characterized calcium switch assay". While this statement is correct, I do not think that single reference to a paper from 1986 is enough to guide readers to explore this assay and judge from themselves. I would like to see more references to this assay and the underlying biology. I believe the Methods section would be a good place to add references. While foundational, Gumbiner and Simons, 1986 does not-in and of itself-support this assay as being "well-characterized".

Excellent point. We re-phrased what we said in the main text as follows ("To study AJ re-assembly, we used the calcium switch assay (Gumbiner and Simons, 1986), which relies on the calcium-dependence of classic cadherin structure and function (Shapiro et al., 1995; Takeichi, 1988)," and added several additional references to the relevant section of the Methods (p. 15), illustrating its use in multiple epithelial cell types.

2. The authors cite three papers that investigate myosin stacks using super-resolution microscopy (Beach et al., 2017; Fenix et al., 2016; Hu et al., 2017). By reading the text, one would get the impression that these were the first to show stack formation using SIM. However, the Lippincott-Schwartz lab demonstrated stack assembly in 2014. Why was this particular paper was omitted?

Good point—can’t believe we missed this paper! Added!

3. I appreciate that the authors provided visual demonstrations of how quantifications were made in the supplement. Combined with the description in the Quantification section of the Methods, I believe others could replicate the measurements.

We are pleased you found those helpful

4. The authors use SMIFH2 as the sole perturbation of formins. There is increasing evidence that SMIFH2 has off target effects (e.g., inhibiting myosin II). However, SMIFH2 does inhibit formins and the authors report mainly negative results, which are different than when they inhibit myosin II with blebbistatin. If there were more major phenotypic changes during recovery in SMIFH2, then other controls would be warranted.

Thank you for these thoughts—we tried to be careful with the caveats and our conclusions
October 4, 2021

RE: JCB Manuscript #202103074R

Dr. Mark Peifer
UNC-Chapel Hill
Department of Biology University of North Carolina at Chapel Hill CB#3280; Coker Hall
Chapel Hill, NC 27599-3280

Dear Mark:

Thank you for submitting your revised manuscript entitled "A role for a micron-scale supramolecular myosin array in adherens junction cytoskeletal assembly". We have now assessed your revised manuscript and we would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

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

A. MANUSCRIPT FORMATTING:

Full guidelines are available on our Instructions for Authors page, http://jcb.rupress.org/site/misc/ifora.xhtml. Submission of a paper that exceeds these limits without prior discussion with the journal office will delay scheduling of your manuscript for publication.

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.

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 figures 1B'-B', 2J-M', 4C-F (insets), 6C', 6D', 8A-B (insets), 8B-K, and supplementary figures 1R-T, 4E-F (insets), 6E (inset), 6G (inset), 6I (inset), 6K (inset).

*** Also, please avoid pairing red and green for those images in which separate channels or quantification graphs are not shown to ensure legibility for color-blind readers. Please change the color scheme of main figures 1D, 2H-I, 3A-I, 4A-B, 6A-B, and supplementary figures 1F-J, 1M-Q, 1V-X, 2F, 2H-L, 3A-J, 5E-F, 6A-C, 8D.

4) Statistical analysis:
Error bars on graphic representations of numerical data must be clearly described in the figure legend. *** We are aware that you indicated in the methods under the "Quantification" section that
"Scatter plots presents all the quantified data points, with mean {plus minus} SD.", but we would appreciate it if you could please describe error bars also in the corresponding figure legends, where appropriate.

*** 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. Apologies if I missed this information but I cannot find it in the methods nor in the figure legends.

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, 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) 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. *** Although your title is fine, we suggest something a bit more specific like "Micron-scale supramolecular myosin arrays support cytoskeletal assembly at mature adherens junctions".

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."

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 reconstructions, 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:
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