Twinfilin1 controls lamellipodial protrusive activity and actin turnover during vertebrate gastrulation

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Original submission

First decision letter

MS ID#: JOCES/2020/254011

MS TITLE: Twinfilin1 controls lamellipodial protrusive activity and actin turnover during vertebrate gastrulation

AUTHORS: John Wallingford, CAitlin Devitt, Chanjae Lee, Rachael Cox, Ophelia Papoulas, Jose Alvarado, and Edward Marcotte
ARTICLE TYPE: Short Report

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. In addition to the detailed issues they raise, there is a broader sense that greater mechanistic insight is achievable with a limited set of new experiments (they made suggestions) and that there is room to improve the manuscript by more clearly tying the results together conceptually both within the manuscript and in reference to what is already known. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

In their manuscript, Devitt et al, perform an in vivo analysis of role of Twinfilin1 (Twf1) in lamellipodial dynamics during convergent extension in the Xenopus embryo. This work builds on previous findings in single cells and Drosophila embryos which has shown that this actin monomer binding protein plays an important role in a variety of actin-based cellular processes. The experiments are well performed, the analysis is sound and the imaging data is of a very high quality. However, the findings are somewhat incremental and lacking in mechanistic insight. The work would be significantly strengthened by addressing this lack of mechanism in a revised manuscript.

Comments for the author

Major points:
1. The authors originally pull Twf1 out of a screen for Cofilin (Cfl) interactors using affinity purification mass spectrometry but never return to investigate this interaction. As they state, one possibility is that they are simply enriching for actin binding proteins, but this is not explored. The paper would be strengthened by investigating how Twf1's function in the lamellipodium is assisted by/related to actin regulation provided by other actin-binding proteins. A sensible start would be to explore links to Cfl, the bait in the APMS and Cap, which was pulled out in the APMS.

For example:
• Do Twf1, Cfl and Cap localisation domains overlap? The fluorescence intensity plots in Figure 2 suggest they do, but this should be confirmed using colocalization.
• How does Twf1 KD impact the localization of Cfl2 and Cap1?
• Can overexpression of Cfl2 or Cap1 rescue the phenotypes described for Twf1?
• Do the functions of Twf1 sit downstream of Rac and/or Rho? In their Introduction, the authors mention roles for Rac and Rho in CE previously identified in Xenopus, how does the actin regulation provided by Twf1 fit into these roles?

2. The difference in speckle lifetime between control and Twf1 MO seems relatively small. This result would be strengthened by demonstrating a similar finding using FRAP as an alternative technique.

Minor points:
1. In the Introduction "DMZ" is not defined (this is only done in the Results section).
2. On page 5, second paragraph, Fig 4D-I is referred to, I think this should be Fig3D-I.
3. I would like more detail on the stats used in the paper – were normality tests used to check for normal distributions before parametric tests (t-tests and ANOVA) were applied? Some of the data (e.g. Twf1 MO in Fig 3F and Twf1 CRISPR in Fig3I) do not look normally distributed by eye.

Reviewer 2

Advance summary and potential significance to field

This report speaks to the large question of asking how cytoskeletal regulators influence morphogenetic processes. This is an enormously challenging issue for the field because we have a

plethora of molecular players, yet the functional impact plays out on much longer length and time scales. Here Devitt et al provide useful information to begin to dissect this problem: they identify interactors of cofilin 2, and focus on twinfilin, one of the least studied.
They show that twinfilin supports convergent extension and link this to polarization of lamellipodial activity, a key determinant of planar-polarized cell intercalation.

Comments for the author

Overall, this report is informative, although it does not yet provide substantial new conceptual insight. It will contribute to the field by identifying twinfilin as a determinant of convergent extension. I have some suggestions for minor additional experiments that might fill out the current picture:
a) How does Twinfilin depletion affect the cellular localization of cofilin?
As Twf depletion appears to stabilize F-actin in the lamellipodium, is it working by controlling the cortical recruitment of cofilin? (Might this explain why there are more actin cables that are less polarized in the Twf morphant – because the off-axis cables are not being turned over?)
b) Do the authors have any evidence to suggest that adhesions (integrin or cadherin-based) might be different in the Twf morphants?
c) Is cell polarization altered in the Twf morphants? Such as organization of the microtubule cytoskeleton or orientation of the golgi apparatus?
Minor-teensy points:
Fig 3Q caption: should be control vs Twf MO, rather than CK666?

Reviewer 3

Advance summary and potential significance to field

Significance: Understanding the role of actin modulators during morphogenesis in vivo is an intrinsically important topic. While we have learned much from studying cultured cells in vitro, there is simply no substitute for analyzing motility in intact (or explanted) embryonic tissues. This is true both because collective migration events add a layer of additional complexity compared to migration of single cells, and because of the much more complicated geometry of three-dimensional embryos vs. flat cultured cells. Thus even when the basic storyline turns out to be the same (as it does here), the result is significant.

Overall assessment: This paper from the Wallingford lab examines twinfilin, an actin severing protein that is not very well understood relative to cofilin. In fact, this work stems from a top-down proteomics screen to identify cofilin interactors in Xenopus embryonic mesoderm, from which twinfilin was pulled out. The basic work here is presented nicely, and makes an advance on our understanding of the role of twinfilin in a metazoan during a key development event: convergent extension in the mesoderm of the Xenopus gastrula. Moreover, the data presentation sets a high standard for studying deep cells of the involuting marginal zone of the Xenopus gastrula, which are notoriously difficult to image.

Positive specifics aspects of this paper include:
* The historical references: these are very rare in today's papers!
* The proteomics here is reported in a way that focuses on the development of the main story nicely.
* The use of TIRF imaging, speckle microscopy, and the microscopy analysis is a strong point of this paper.

This is very appropriate for publication in JCS. I have one major and a few minor suggestions for improving the paper.

Comments for the author

Main suggestion: My main suggestion has to do with properly contextualizing this work. The discussion at the end of the paper seemed to end too abruptly and didn't tie the intellectual threads of this work together as well as I would have hoped. Moreover, I feel that the authors could

help the average reader by pointing out the significance of the present work more clearly for the uninitiated, who are not "Xenopus insiders".

There are at least two main ways in which this work could be contextualized (not mutually exclusively):
(1) Subcellular actin dynamics: One approach is to try to understand how twinfilin affects the complicated interplay of actin regulators that act at various levels (e.g. at the level of monomers, barbed ends, severing proteins, etc.). This turns out to be a very hard problem that involves stoichiometry of many regulators simultaneously (as the classic, likely overused Pollard-esque figures that combine actin regulators into some sort of coherent model of a lamellipodium suggest). The experiments aimed at assessing actin turnover are a move in this direction that are a good first step. However, there seems to be no attempt to tie these subcellular threads together. What is new in terms of he lamellipodium production that has not been learned in cultured cells (e.g., mouse melanoma cells, etc.)? My sense is there may be less here than in (2) below, although the actin turnover data here seem to be interesting. How might the authors highlight their unique results better?

(2) Unique features of rearranging deep cells: To me an even better route is to capitalize on the unique biology of the Xenopus gastrula. The authors cite work from the Keller group about the poorly characterized actomyosin networks in these cells. The preset work seems to show that they are independent of the bipolar lamellipodia that rearranging cells extend. The present work further suggests that lamellipodial extension and these networks are related, especially when it comes to their alignment.
The results further indicate that the relationship must be indirect. I think this is correct, and and important conclusion. How does changing actin turnover rate (presumably via changes in severing rate or monomer interactions) lead to this aggregate phenotype of changes in actomyosin network alignment? Raising some possibilities would tie a bow around this results for the interested reader.

Minor suggestions:
In several spots I would recommend a bit more comparison to other systems. Admittedly, little to nothing has been done on twinfilin, but there might be a a few other relevant comparators. Citing details from the Anderson lab's work in mice (see Mahaffey et al 2013. Development 140:1262-71; Grego-Bessa et al., 2015. Development 142:1305-14), the Habas lab's work on profilins in Xenopus (Khadka et al., 2009. Dev. Bio. 332:396–406), the Davidson lab's recent work on actin networks (Shawky et al, 2018. Development 145:dev161281.), or, as an invertebrate outgroup, C. elegans dorsal epidermal cells (Walck-Shannon, 2015.

Development 142:3549-3560) could flesh out the significance of the current results. The last shows a connection between actin regulators and polarity (though a much less dramatic effect than the work reported here).

A few other specific suggestions:
Page: 3 An example of the "insider" language is the reference to the "deep-superficial axis" and "superficial lamelliform protrusions" (p. 3). How would anyone outside of the Keller, Davidson, or Wallingford groups know what these are and the relevance of these fine points? Please help the reader decode these kinds of references throughout.
Page: 4 The differences in localization of cofilin, twinfilin, and Cap are interesting. What do the author think is important about this?

Page: 5 The authors say that "Curiously, the robust elongation defect in embryos lacking Twf1 was not associated with the severe dorsal flexion commonly observed following disruption of convergent extension by manipulations of PCP signaling". I agree this is curious, and might be important. Why do the authors think that the difference arises? One reason might be that the actomyosin networks can no longer exert forces well in the former, but can in a misdirected way in the latter. While I don't expect the authors to add force/extension curve analysis in explants, commenting on these sorts of observations in a more mechanistic way would help guide the reader to the most significant conclusions from this work.

Page: 16 Fig 2, PIP3-BD: the signal intensity measurement doesn't seem to be meaningful, because there is signal from two overlapping cells. If the purpose of the intensity measurements is simply to

show that the PH domain marker looks different, this is fine. Can the authors clarify the significance of this measurement in the absence of mosaic situations in which only one cell has the PH domain marker?

First revision

Author response to reviewers' comments

We thank the reviewers for their helpful comments, and we respond to each in kind below.

Reviewer #1:

1. The authors originally pull Twf1 out of a screen for Cofilin (Cfl) interactors using affinity purification mass spectrometry but never return to investigate this interaction. As they state, one possibility is that they are simply enriching for actin binding proteins, but this is not explored. The paper would be strengthened by investigating how Twf1's function in the lamellipodium is assisted by/related to actin regulation provided by other actin-binding proteins. A sensible start would be to explore links to Cfl, the bait in the APMS and Cap, which was pulled out in the APMS. For example:

- Do Twf1, Cfl and Cap localisation domains overlap? The fluorescence intensity plots in Figure 2 suggest they do, but this should be confirmed using colocalization.
- How does Twf1 KD impact the localization of Cfl2 and Cap1?
- Can overexpression of Cfl2 or Cap1 rescue the phenotypes described for Twf1?
- Do the functions of Twf1 sit downstream of Rac and/or Rho? In their Introduction, the authors mention roles for Rac and Rho in CE previously identified in Xenopus, how does the actin regulation provided by Twf1 fit into these roles?

While some of the several questions posed here fall beyond the scope of this work, we agree with the reviewer on the need for more mechanistic analysis in this manuscript. We have addressed this need with the addition of a substantial amount new data as well as a substantial revision of the Discussion in which we have now integrated our in vivo findings with the more extensive previously published in vitro data on these proteins. Specifically, we:

A. Added new data showing that Twf1 KD disrupts the normal localization of Cap1 [figure 6E-H; blue text in pg. 6-7 of revision].

B. Added an analysis of the orientation of the cellular long axis [Fig. 3D-G] to link our data on lamellipodial polarization to the data on polarization of cytoplasmic actin cables.

B. We discuss the interplay of cfl and twf1 in vitro and how our data provide new insights [page 4, blue text; Page 6 blue text].

2. The difference in speckle lifetime between control and Twf1 MO seems relatively small. This result would be strengthened by demonstrating a similar finding using FRAP as an alternative technique.

While the effect is small, we note that it is a) statistically significant and b) of a similar magnitude to that reported by Iwasa & Mullins using fluorescent speckle imaging in S2 cells in culture and 3) is consistent with the trends shown recently using FRAP in mouse melanoma cells in culture. Finally, as a practical matter, the TIRF scope we have access to in our core facility is not capable of FRAP analysis, so more in-depth quantification is not possible at this time. We hope the reviewers will be understanding.

Minor points:

1. In the Introduction "DMZ" is not defined (this is only done in the Results section).

Fixed, thank you! [changed pg. 3]

2. On page 5, second paragraph, Fig 4D-I is referred to, I think this should be Fig3D-

Fixed, thank you! [changed pg. 5]

3. I would like more detail on the stats used in the paper – were normality tests used to check for normal distributions before parametric tests (t-tests and ANOVA) were applied? Some of the data (e.g. Twf1 MO in Fig 3F and Twf1 CRISPR in Fig3I) do not look normally distributed by eye.

Thank you for pointing this out. After testing for normality, we observed several groups did not have normal distribution and/or had a relatively low n-number. We have thus updated the statistical analysis and have used non-parametric tests throughout the manuscript for consistency. We have used Mann Whitney tests, Kruskal-Wallis tests, or Kolmogorov–Smirnov tests for statistical comparisons of two groups, multiple groups, or distributions, respectively. This has been updated in the figure legends and in the methods section. [page 10, image analysis description, with specific methods also indicated in the revised figure legends].

Reviewer 2:

Overall, this report is informative, although it does not yet provide substantial new conceptual insight. It will contribute to the field by identifying twinfilin as a determinant of convergent extension. I have some suggestions for minor additional experiments that might fill out the current picture:

a) How does Twinfilin depletion affect the cellular localization of cofilin? As Twf depletion appears to stabilize F-actin in the lamellipodium, is it working by controlling the cortical recruitment of cofilin? (Might this explain why there are more actin cables that are less polarized in the Twf morphant – because the off-axis cables are not being turned over?)

Thanks for the invitation to more thoroughly discuss this important point. We have added a discussion about the interplay between protrusion actin and cortical actin populations [page 7]. Moreover, based on the clear localization of Cap at the distal edge of the cell, along with a known interaction between Twf and Cap in vitro (Johnson et al, 2015), we chose to look at the Cap localization changes upon Twf1 knockdown. These data an extensive discussion of the result and how they relate to prior in vitro data has been added to the MS. [figure 6E-H; blue text in pg. 6-7 of revision].

b) Do the authors have any evidence to suggest that adhesions (integrin or cadherin-based) might be different in the Twf morphants?

Previous work from the Davidson and DeSimone groups among others, has shown quite complex patterns of cadherin and integrin adhesions in the cells of the DMZ. Thus, while this is an excellent question, even a cursory examination of the issue would require careful quantification, so we feel this is beyond the scope of this study.

c) Is cell polarization altered in the Twf morphants? Such as organization of the microtubule cytoskeleton or orientation of the golgi apparatus?

We regret not being clear enough in the original manuscript, but unlike migrating cells in culture, there is no evidence of golgi polarization during CE in the DMZ. Rather, the dominant readout of cell polarity in DMZ cells during CE is the position of the lamellipodia, which our data in Fig. 3H-K show is significantly disrupted after Twf1 loss. We further show that the normal polarization of actin cables within these cells is also disrupted after Twf1 loss (Fig. 3L-N).

To reinforce these findings, we have now added new data examining another commonly used readout, the orientation of the cellular long axis (Fig 3D-G). This metric, too, reveals a defect in cell polarization. Thus, three established readouts of polarity in the DMZ converge on a common finding: Twf1 is required for cell polarization in the DMZ.

Minor-teensy points:
Fig 3Q caption: should be control vs Twf MO, rather than CK666?

Thank you for noting this, it has been updated in Figure 3 legend.

Reviewer 3:

Main suggestion: My main suggestion has to do with properly contextualizing this work. The discussion at the end of the paper seemed to end too abruptly and didn't tie the intellectual threads of this work together as well as I would have hoped. Moreover, I feel that the authors could help the average reader by pointing out the significance of the present work more clearly for the uninitiated, who are not "Xenopus insiders".

Text has been substantially updated throughout to address this concern, and we added a new supplementary fig 1 to help orient the reader to this specific cell type/tissue.

There are at least two main ways in which this work could be contextualized (not mutually exclusively):

(1) Subcellular actin dynamics: One approach is to try to understand how twinfilin affects the complicated interplay of actin regulators that act at various levels (e.g. at the level of monomers, barbed ends, severing proteins, etc.). This turns out to be a very hard problem that involves stoichiometry of many regulators simultaneously (as the classic, likely overused Pollard-esque figures that combine actin regulators into some sort of coherent model of a lamellipodium suggest). The experiments aimed at assessing actin turnover are a move in this direction that are a good first step. However, there seems to be no attempt to tie these subcellular threads together. What is new in terms of the lamellipodium production that has not been learned in cultured cells (e.g., mouse melanoma cells, etc.)? My sense is there may be less here than in (2) below, although the actin turnover data here seem to be interesting. How might the authors highlight their unique results better?

Here again, we have substantially expanded the manuscript to address this concern, with these changes highlighted in blue text in the revision.

(2) Unique features of rearranging deep cells: To me an even better route is to capitalize on the unique biology of the Xenopus gastrula. The authors cite work from the Keller group about the poorly characterized actomyosin networks in these cells. The preset work seems to show that they are independent of the bipolar lamellipodia that rearranging cells extend. The present work further suggests that lamellipodial extension and these networks are related, especially when it comes to their alignment. The results further indicate that the relationship must be indirect. I think this is correct, and an important conclusion. How does changing actin turnover rate (presumably via changes in severing rate or monomer interactions) lead to this aggregate phenotype of changes in actomyosin network alignment? Raising some possibilities would tie a bow around this result for the interested reader.

Thank you for this suggestion! We have added specific hypotheses to the Conclusions session [page 7]

Minor suggestions:
In several spots I would recommend a bit more comparison to other systems. Admittedly, little to nothing has been done on twinfilin, but there might be a few other relevant comparators. Citing details from the Anderson lab's work in mice (see Mahaffey et al 2013. Development 140:1262-71; Grego-Bessa et al., 2015. Development 142:1305-14), the Habas lab's work on profilins in Xenopus (Khadka et al., 2009. Dev. Bio. 332:396– 406), the Davidson lab's recent work on actin networks (Shawky et al, 2018. Development 145:dev161281.), or, as an invertebrate outgroup, C. elegans dorsal epidermal cells (Walck-Shannon, 2015. Development 142:3549-3560) could flesh out the significance of the current results. The last shows a connection between actin regulators and polarity (though a much less dramatic effect than the work reported here).

We have added several additional references to highlight the specific phenotypes and work understanding the role of actin regulators during development, in both the Intro and the Conclusions sections.

A few other specific suggestions:

Page: 3
An example of the "insider" language is the reference to the "deep-superficial axis" and "superficial lamelliform protrusions" (p. 3). How would anyone outside of the Keller, Davidson, or Wallingford groups know what these are and the relevance of these fine points? Please help the reader decode these kinds of references throughout.

We apologize for the oversight. We have added a supplementary figure to highlight these terms and have clarified the language in the text.

Page: 4
The differences in localization of cofilin, twinfilin, and Cap are interesting. What do the author think is important about this?

Please see response to reviewer #1, above. We have added a substantial discussion of this point to the new manuscript.

Page: 5
The authors say that "Curiously, the robust elongation defect in embryos lacking Twf1 was not associated with the severe dorsal flexion commonly observed following disruption of convergent extension by manipulations of PCP signaling". I agree this is curious, and might be important. Why do the authors think that the difference arises? One reason might be that the actomyosin networks can no longer exert forces well in the former, but can in a misdirected way in the latter. While I don't expect the authors to add force/extension curve analysis in explants, commenting on these sorts of observations in a more mechanistic way would help guide the reader to the most significant conclusions from this work.

While we -obviously- agree this is an interesting point, with the substantial expansion of the cell biological aspects in the revised paper, we feel that fitting in additional speculations about the developmental biology may detract from the overall narrative.

Page: 16
Fig 2, PIP3-BD: the signal intensity measurement doesn't seem to be meaningful, because there is signal from two overlapping cells. If the purpose of the intensity measurements is simply to show that the PH domain marker looks different, this is fine. Can the authors clarify the significance of this measurement in the absence of mosaic situations in which only one cell has the PH domain marker?

Yes, the goal of this experiment was to define the region of the lamellipodia and we have updated the language in the text. Clarification has been added to text [page 4, paragraph 1]

Second decision letter

MS ID#: JOCES/2020/254011

MS TITLE: Twinfilin1 controls lamellipodial protrusive activity and actin turnover during vertebrate gastrulation

AUTHORS: Caitlin Devitt, Chanjae Lee, Rachael Cox, Ophelia Papoulas, Jose Alvarado, Shashank Shekhar, Edward Marcotte, and John Wallingford
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but raised a couple of specific points that will require amendments to your manuscript. I hope that you will be able to carry these out because I would like to be able to accept your paper, depending on further comments from reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

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Reviewer 1

Advance summary and potential significance to field

In their revised manuscript, Devitt et al have improved the mechanistic insight of their data, both in terms of new experiments/quantification and a better discussion of their findings in the context of what is known about Twf1 from in vitro studies. The addition of Cap1 localization in Twf1 MO is particularly useful and I found the broadening of the Cap1 domain convincing in both images and quantification. However, I couldnÂ't see a statistical test to confirm this shift in localization, could the authors add this? I would then be happy to support publication of this strengthened manuscript in JCS.

Comments for the author

Minor point: Typo in the Image analysis section of the Methods: 3rd line from bottom: Mann Whitley, should be Mann Whitney.

Reviewer 2

Advance summary and potential significance to field

As per my earlier review.

Comments for the author

I think the authors have reasonably addressed the issues that I raised in my earlier review. I recommend acceptance.

Reviewer 3

Advance summary and potential significance to field

Please see my previous review. This work is significant for several reasons. First, understanding the role of actin modulators during morphogenesis in vivo is an intrinsically important topic, and, there is simply no substitute for analyzing motility in intact (or explanted) embryonic tissues. Second, twinfilin is an actin severing protein that is not very well understood relative to cofilin. This work makes a significant advance on our understanding of the role of twinfilin in a metazoan during a key development event: convergent extension in the mesoderm of the Xenopus gastrula. Third, the data presentation sets a high standard for studying deep cells of the involuting marginal zone of the Xenopus gastrula, which are notoriously difficult to image. The imaging is superb.

Comments for the author

This paper addresses all of my original, very minor concerns. Students of developmental biology reading this paper will be better able to appreciate this work after the revision. My congratulations go to the authors on a very nice paper.

Second revision

Author response to reviewers' comments

These distributions are statistically different. We simply neglected to add the comment to that effect. Sorry for the oversight!

Third decision letter

MS ID#: JOCES/2020/254011

MS TITLE: Twinfilin1 controls lamellipodial protrusive activity and actin turnover during vertebrate gastrulation

AUTHORS: Caitlin Devitt, Chanjae Lee, Rachael Cox, Ophelia Papoulas, Jose Alvarado, Shashank Shekhar, Edward Marcotte, and John Wallingford
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.
Reviewer 1

Advance summary and potential significance to field

See previous.

Comments for the author

I am happy for the paper to be accepted with the stats added to the figure legend of Figure 6.