TRIP6 is required for tension at adherens junctions

Srividya Venkatramanan, Consuelo Ibar and Kenneth Irvine
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Original submission

First decision letter

MS ID#: JOCES/2020/247866

MS TITLE: TRIP6 is required for tension at adherens junctions

AUTHORS: Srividya Venkatramanan, Maria Consuelo Ibar Valenzuela, and Kenneth Irvine
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 are enthusiastic about the study but raise a number of substantial criticisms that prevent me from accepting the paper at this stage. There are concerns about the presentation and quantification of the data and also suggestions for new experiments to deepen the level of mechanistic insight and also to allow the present results to be better integrated with what is already known about these signaling pathways. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. 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 currently be unable to access the lab to undertake experimental revisions. 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 this manuscript by Venkatramanan and colleagues, the authors study the relationships between TRIP6 (a zyxin family member), LATS, and LIMD1 in being recruited to E-cadherin-based junctional complexes. They combine a series of imaging, knockdown, and pharmacological treatments to discern the relationships.

Comments for the author

Specific comments:
1. Can you please add an opening diagram with the hypothesis of how you think the proteins are interacting and each protein's domain structures? Since the figures all look so similar and the protein names are all acronyms, this will allow the reader to refer back to this to help keep the proteins straight.

2. The data are dependent on being able to see the details of the images.
While I appreciate that colored images are prettier, it is more difficult to make out the specifics. I recommend presenting the single colors in gray scale because you get a wider dynamic range that is visible to the eye. Then, you can show a single overlay for the "pretty picture".

3. Also, so much is riding on the co-localization of the proteins, yet I do not see any validation of the antibodies. I think at the very least a panel of singly labeled cells should be provided in the supplement to ensure what the patterns look like on their own. Then, upon confirming patterns, then you can combine. I recognize that the secondary Abs chosen should be far enough apart in their emission spectra, but shocking things happen. Presumably the authors did this

4. Why was p-MLC staining done over the weekend? If stainings were performed over multiple experiments, couldn't at least one have been done with the standard protocol?

5. The authors conclude that by activating Rho that they increase myosin II-mediated tension. Actually, if you activate Rho, you can only conclude that you have activated Rho. It is much better to be specific. This is an ongoing problem in the literature where the role of myosin II is enormously over-simplified to a fault. It is true that increasing Rho activity can lead to increased ROCK activity, which can activate formins, inhibit MYPT1, which has many substrates, and activate myosin II. But, which of these activities are changing the state of the cell. Even if you could conclude that you had only activated myosin II, myosin II contributes to a battery of activities, of which tension is just one. And even then, which tension do you mean – cortical tension or line tension (different parameters – I think you mean line tension).

Further to this point, from the images, it looks like the apical cross-sectional area is actually greater in the control than in the TRIP6 knockdown which would seem contrary to a purely tension-dependent mechanism.

Please just be strict in your description of your results so as to avoid being misleading throughout your Results section. Then, speculate away on what it might mean in the Discussion.

6. When comparing apical vs. basal, I suspect some x-z and y-z images would be useful. Also, how does the z-dimension change when depleting TRIP6?

7. Scale bars need to be provided throughout and when a single scale bar is presented, it needs to be confirmed in the figure legend that it applies to all panels, assuming it does (but it does not seem like it does – e.g. Fig. S1I).

8. In the Discussion, the authors spend a fair amount of time rightly discussing discrepancies in the literature. However, I did not see any real proposal for how these results can be reconciled. I think some thought on this would greatly increase the value of this paper.

9. Figure layouts are a bit clunky. You should put a little extra space in between panel groupings so that they do not run together so much.

Reviewer 2

Advance summary and potential significance to field

This paper is part of complex body of studies addressing the complex question of tension-dependent activation of Yap/TAZ pathway, the role of cadherin-mediated contacts in this process and as well the related question of the impact of tension on adherens junction formation. The authors focus on two Ajuba family proteins, TRP6 and LIMD1. In a previous paper, they addressed the role of LMD1 in Yap/TAZ pathway regulation (which is tension-dependent) (Ibar et al 2018). They refer to another paper addressing similarly the role of TRP6 in the regulation of the pathway (Dutta et al., 2018). Both protein inhibits the pathway. Both molecules were shown to be recruited in junction submitted to high tension (low density cells) allowing YAP activation via LATS inhibition, and release in the cytoplasm at low junctional tension (high density cells) inhibiting YAP.
In this paper, the authors do not analyze further the functional interaction of theTRP6 and LIMD1 in LATS and YAP activations but rather their potential role for Â“tension at adherens junctionsÂ” as said in the title.

Silencing either TRIP 6 or LMD1, the authors show that TRIP6 is required for junctional recruitment of LMD1 but not the reverse. TRIP6 is required for vinculin and VASP recruitment at junction and its silencing changes interestingly the redistribution of actin from cell-cell junction to focal adhesion which are increased. It affect also activated MLC distribution.
These are interesting data that are not correctly quantified and not sufficiently worked out to produce a clear picture of the mechanistic relation between the two proteins and their place in the adherens junction-cell contractility-Yap/TAZ pathway.

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However, tension at adherens junctions” is not a well defined notion. What are the parameters allowing to measure tension at adherens junctions? The authors use a simple indirect proxy which is vinculin recruitment. Why authors did not follow on the functional role of the Ajuba protein in YAP regulation? Are these two proteins interacting, etc… would have been very interesting avenues to pursue. The problem of the chosen object of the study is that since YAP activation has been shown both to depend on cell-substratum adhesion and contractility and affect also these two biological responses, nothing allow to support that the effect of the two proteins on vinculin protein complex interactions at junctions. It could as well be due to a feedback implicating YAP regulations.

The illustrations of the manuscript are good, but most of the data presented are microscopy images and a few western blots relating changes in localization or degree of recruitment without any quantification. RNA silencing lack rescues controls, and the levels of expression of the various proteins are only partially checked biochemically. These flaws preclude any publication of this work at this point.
Silencing either TRIP 6 or LMD1, the authors show that TRIP6 is required for junctional recruitment of LMD1 but not the reverse. TRIP6 is required for vinculin and VASP recruitment at junction and its silencing changes interestingly the redistribution of actin from cell-cell junction to focal adhesion which are increased. It affect also activated MLC distribution.
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More specific points:
What allow to affirm that low cell density condition promotes junctional tension beside vinculin recruitment at junction (that is never quantified by the way).
The paper relies entirely of junctional localization or colocalisation without any quantification. It is need to apply quantification everywhere (line scan on multiple junctions, segmentation of junctional areas,…). TRIP6, LMD1 vinculin, VASP, LATS proteins junctional accumulations need to be quantified for each experimental condition before saying there is more, less or similar levels…
Moreover, the junctions not only recruit more or less of the proteins or actin but they often change from serrate or punctated to linear junctions as defined in many papers in the field. This is never discussed and quantify. This may have a big impact of the recruitment impression and is an important parameter by itself, in particular in relation to actin relocalization and reorganization.
It is difficult to see the rational of the experimental activation of Rho.
What it gives more than culturing at low density. It would have been better to show a point by point comparison of localization at high/low density or at high density with and without Rho activation.
A first question that one may ask is whether the two proteins interact directly together and with vinculin or alpha-catenin. This could be checked by co-IP and pull-down experiments.
The fact that the authors noticed a divergence of their result to the one of Dutta (2018) relating to Vinc recruitment further advocates for more quantitative analysis and production of more accurate controls (rescue after siRNA).
The characterization of actin fiber distribution and redistribution is insufficient and misleading, the authors only stress out evident actin fibers within the cells. They do not quantify the junctional actin (actin belt at adherens junctions), they do not take in account the change in junctional morphology.
A more systematic biochemical analysis (western blot) should be provided to show not only the relative expression of the two Ajuba proteisn but also in the clones used, but also of E-cadherin, vinculin and the others proteins the localization of which is analysed.
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More than phosphorylated light chain, it would be good to have the localization of Myosin IIA and myosin IIB following recent reports by either Yap, Ladoux/Mège and Svitkina labs.

Minor points
Differences are very low in Fig 5E. What defines strong and weak nuclear Yap staining in Fig 5J.

First revision

Author response to reviewers' comments

A formatted version of the response is also included as a supplement

Response to Reviewers

We thank both reviewers for their comments and suggestions.

Reviewer 1 Advance summary and potential significance to field
In this manuscript by Venkatramanan and colleagues, the authors study the relationships between TRIP6 (a zyxin family member), LATS, and LIMD1 in being recruited to E-cadherin-based junctional complexes. They combine a series of imaging, knockdown, and pharmacological treatments to discern the relationships.

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Response: We have added the requested schematics to Figure 1.

2.The data are dependent on being able to see the details of the images. While I appreciate that colored images are prettier, it is more difficult to make out the specifics. I recommend presenting the single colors in gray scale because you get a wider dynamic range that is visible to the eye. Then, you can show a single overlay for the "pretty picture".

Response: We have made the requested change to the figures, converting all single channel images to gray scale.

3.Also, so much is riding on the co-localization of the proteins, yet I do not see any validation of the antibodies. I think at the very least a panel of singly labeled cells should be provided in the supplement to ensure what the patterns look like on their own. Then, upon confirming patterns, then you can combine. I recognize that the secondary Abs chosen should be far enough apart in their emission spectra, but shocking things happen. Presumably the authors did this

Response: We have used the secondary antibodies alone and in a variety of combinations so that we know we are not getting detectable cross-over under our conditions. The key antibodies have been validated by siRNA, eg in this manuscript we show examples of loss of Ab signal on images and western blots after siRNA for TRIP6, LIMD1, YAP and VCL. LATS1 Ab was validated by us previously in Ibar et al 2018. We have now also added the requested single channel images for E-cad, VASP, ZO-1, pMLC2, MyoIIb, and PXN to a new supplemental figure (Fig S7).

4.Why was p-MLC staining done over the weekend? If stainings were performed over multiple experiments, couldn't at least one have been done with the standard protocol?

Response: We did also do it with the standard protocol, but the pMLC2 staining is weaker with the standard protocol. We also find this with the same antibody when staining Drosophila tissues, it seems to require a longer incubation time for good staining.

5.The authors conclude that by activating Rho that they increase myosin II-mediated tension. Actually, if you activate Rho, you can only conclude that you have activated Rho. It is much better to be specific. This is an ongoing problem in the literature where the role of myosin II is enormously over-simplified to a fault. It is true that increasing Rho activity can lead to increased ROCK activity, which can activate formins, inhibit MYPT1, which has many substrates, and activate myosin II. But, which of these activities are changing the state of the cell. Even if you could conclude that you had only activated myosin II, myosin II contributes to a battery of activities, of which tension is just one. And even then, which tension do you mean – cortical tension or line tension (different parameters – I think you mean line tension).

Further to this point, from the images, it looks like the apical cross-sectional area is actually greater in the control than in the TRIP6 knockdown, which would seem contrary to a purely tension-dependent mechanism.

Please just be strict in your description of your results so as to avoid being misleading throughout your Results section. Then, speculate away on what it might mean in the Discussion.

Response: We have modified the text to try to clarify the interpretation of the experiments with activated Rho.

We also note that our prior work, and work from others, has established that recruitment of TRIP6 and LIMD1 to adherens junctions depends upon tension experienced by a-catenin at adherens junctions, and that the conditions we are using, including Rho activation, are sufficient to generate this tension. This is further supported by a new experiment using an a-catenin FRET tension sensor that we added to the manuscript.

We do not see a consistent difference in cross-sectional cell area between control and TRIP6 knockdown cells.

6.When comparing apical vs. basal, I suspect some x-z and y-z images would be useful. Also, how does the z-dimension change when depleting TRIP6?

Response: We have added some xz sections to a new supplemental figure (Fig S6). When depleting TRIP6, the z dimension increases slightly (by about 15%)

7.Scale bars need to be provided throughout and when a single scale bar is presented, it needs to be confirmed in the figure legend that it applies to all panels, assuming it does (but it does not seem like it does – e.g. Fig. S1I).

Response: We have added additional scale bars to the figures so that there is no confusion about the scale. In some panels cells appear smaller because they are at high density.

8.In the Discussion, the authors spend a fair amount of time rightly discussing discrepancies in the literature. However, I did not see any real proposal for how these results can be reconciled. I think some thought on this would greatly increase the value of this paper.

Response: We think it's likely to be due to differences in experimental conditions but at this point we are not certain what is responsible so we do not want to make a specific proposal.

9.Figure layouts are a bit clunky. You should put a little extra space in between panel groupings so that they do not run together so much.

Response: Where possible we have made some adjustments to the spacing as suggested.

Reviewer 2 Advance summary and potential significance to field

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Response: This research was motivated by the question of the relationship between TRIP6 and LIMD1 raised by the prior studies noted by the reviewer. In the course of this work we discovered the striking effects of TRIP6 on F-actin organization, and decided to focus on that observation. While it could be of interest to conduct further studies on the functional role of Ajuba proteins in YAP regulation, that is not the focus of this work.

What we mean by tension at adherens junctions is tension experienced by a-catenin, which leads to conformational changes that allow recruitment of LIMD1 and VCL. The inference that TRIP6 influences this is further supported by new experiments added to the manuscript assaying an a-catenin FRET tension sensor (added to Fig. 3).

The reviewer also appears to suggest that the effects of TRIP6 on F-actin could be an indirect consequence of effects on YAP activity. This would not be expected because we don’t see significant effects on YAP with TRIP6 knockdown, and LIMD1 knockdown, which does reduce YAP activity, does not share the effects on F-actin observed with TRIP6 knockdown. To further rule this out, we have now characterized the effects of YAP knockdown in these cells, and we do not observe a difference in VCL or F-actin localization (new supplemental figure Fig S5).

The illustrations of the manuscript are good, but most of the data presented are microscopy images and a few western blots relating changes in localization or degree of recruitment without any quantification. RNA silencing lack rescues controls, and the levels of expression of the various proteins are only partially checked biochemically. These flaws preclude any publication of this work at this point.

Response: The original manuscript included quantitation of the influence of TRIP6 knockdown on junctional levels of four key proteins. As requested, we have now added additional quantitation of images, and also additional western blots and quantitation of western blots. We have also now added rescue experiments for the TRIP6 knockdown.

Silencing either TRIP 6 or LMD1, the authors show that TRIP6 is required for junctional recruitment of LMD1 but not the reverse. TRIP6 is required for vinculin and VASP recruitment at junction and its silencing changes interestingly the redistribution of actin from cell-cell junction to focal adhesion which are increased. It affect also activated MLC distribution. These are interesting data that are not correctly quantified and not sufficiently worked out to produce a clear picture of the mechanistic relation between the two proteins and their place in the adherens junction-cell contractility-Yap/TAZ pathway.

More specific points:
What allow to affirm that low cell density condition promotes junctional tension beside vinculin recruitment at junction (that is never quantified by the way).

Response: Previously published studies from our lab and others have established that tension at adherens junctions is affected by cell density, and that in the low cell density conditions we used, tension-dependent recruitment of Vinculin, Lats, and Ajuba family proteins is observed (eg see Ibar et al 2018, Dutta et al 2018). In this manuscript, we are simply using these low cell density conditions to investigate the relationship between TRIP6 and LIMD1. We have revised the text to try to make this clear.

The paper relies entirely of junctional localization or colocalisation without any quantification. It is need to apply quantification everywhere (line scan on multiple junctions, segmentation of junctional areas,…). TRIP6, LMD1, vinculin, VASP, LATS proteins junctional accumulations need to be quantified for each experimental condition before saying there is more, less or similar levels…

Response: We did include quantitation of key results, including the impact of TRIP6 knockdown on junctional localization of LIMD1, LATS1, VCL, and VASP. The quantitation is based on segmentation of junctional volumes, as defined by E-cad staining. We have revised the text to make this clearer. As requested we have now added additional quantitation of junctional localization, including quantitation under activated-Rho as well as low density conditions, and quantitation of junctional protein levels examined under LIMD1 knock-down conditions, which we had not included before. We note that in cases where we don't have a good marker for segmentation we have not quantified the images, as we think it would give mis-leading results. We have also added additional western blots examining protein levels, and quantitation of these blots. We also added examples of line scans along junctions to Figure 1.

Moreover, the junctions not only recruit more or less of the proteins or actin but they often change from serrate or punctated to linear junctions as defined in many papers in the field. This is never discussed and quantify. This may have a big impact of the recruitment impression and is an important parameter by itself, in particular in relation to actin relocalization and reorganization.

Response: We incorporated changes in junctions into our quantitation by using E-cad staining to define the junctions and by normalizing the amounts of analyzed protein at junctions to E-cad levels. The changes in morphology of the junctions when TRIP6 is knocked down are presumably a consequence of the reduction in tension and changes in F-actin organization, as this type of effect has been described in the literature previously, and we now make note of this in the revised text.

It is difficult to see the rational of the experimental activation of Rho. What it gives more than culturing at low density. It would have been better to show a point by point comparison of localization at high/low density or at high density with and without Rho activation.

Response: We included activation of Rho in order to examine two distinct conditions under which tension at junctions is high (low cell density, and Rho activation). We have already previously compared localization of VCL, Lats and Ajuba proteins under low vs high density, and under high density with and without Rho activation (Ibar et al, 2018), and Dutta et al (2018) already published TRIP6 localization under low vs high density conditions. Since there is almost no protein detectable at junctions for TRIP6, VCL, LIMD1, and LATS1 under high cell density without Rho activation, there is nothing for us to examine under these conditions. We have revised the text to make sure the already published observations are more clearly presented.

A first question that one may ask is whether the two proteins interact directly together and with vinculin or alpha-catenin. This could be checked by co-IP and pull-down experiments.

Response: We have added new results to the manuscript showing that we can detect co-IP of TRIP6 with LIMD1 (supplemental figure S3). Dutta et al (2018) reported that TRIP6 could co-IP VCL. We have not been able to obtain convincing co-IP of TRIP6 or LIMD1 with a-catenin, but we think this is

because they only interact with a high-tension, open conformation that is not maintained under our lysis conditions. Further evaluation of this is outside the scope of this manuscript.

The fact that the authors noticed a divergence of their result to the one of Dutta (2018) relating to Vinc recruitment further advocates for more quantitative analysis and production of more accurate controls (rescue after siRNA).

Response: We include quantitation of the loss of VCL at junctions upon TRIP6 knockdown. While we have a difference form the Dutta et al paper here, we note that the loss of VCL we observe is fully consistent with the changes in F-actin and myosin that we observe, as recruitment of VCL to AJ requires cytoskeletal tension at junctions to expose the VCL binding site on a-catenin. We have also now added examples of rescue experiments.

The characterization of actin fiber distribution and redistribution is insufficient and misleading, the authors only stress out evident actin fibers within the cells. They do not quantify the junctional actin (actin belt at adherens junctions), they do not take in account the change in junctional morphology.

Response: We quantified actin stress fibers because our observations suggested that the shift in localization of actin stress fibers from apical to basal is a key consequence of TRIP6 knockdown. As requested we have now also added quantitation of junctional F-actin (added to Figure 5). This quantitation is based on segmentation of images with E-cad, so it takes changes in junctional morphology into account.

A more systematic biochemical analysis (western blot) should be provided to show not only the relative expression of the two Ajuba proteisn but also in the clones used, but also of E-cadherin, vinculin and the others proteins the localization of which is analysed.

Response: As requested we have now added examples of western blots and quantitation of western blots showing how the levels of proteins do or don’t change under TRIP6 or LIMD1 knock-down conditions.

Focal adhesion formation and distribution are not sufficiently characterized: use of another marker (integrin, paxillin..), quantification.

Response: We have now added Paxillin (PXN) stains to our analysis (supplemental figure S6). We have quantified total protein levels for VCL, VASP, and PXN by western blot, however, as we don't have an accurate marker for segmentation of "basal" under control and siRNA condition, we elected not to include this.

We do not see what brings the use of Vinculin deficient cells.

Response: We included this as we think it is of interest to compare the consequences of loss of Vinculin to loss of TRIP6, in terms of their effects on LIMD1 and F-actin organization, particularly as TRIP6 and Vinculin affect each other and have been reported to physically interact (Dutta et al , 2018).

More than phosphorylated light chain, it would be good to have the localization of Myosin IIA and myosin IIB following recent reports by either Yap, Ladoux/Mège and Svitkina labs.

Response: We have added experiments showing MyoIIB localization and levels, which is affected similarly to pMLC. We were not able to obtain a good antibody for analysis of MyoIIA.

Minor points
Differences are very low in Fig 5E.

Response: agreed, but this is what was detected

What defines strong and weak nuclear Yap staining in Fig 5J.

Response: In the original manuscript, this was based on visual scoring. For the revised manuscript, we segmented the images and quantified mean nuclear and cytoplasmic YAP intensities, and divided into two categories based on the ratio of nuclear:cytoplasmic signal.

Second decision letter

MS ID#: JOCES/2020/247866

MS TITLE: TRIP6 is required for tension at adherens junctions

AUTHORS: Srividya Venkatramanan, Maria Consuelo Ibar Valenzuela, and Kenneth Irvine
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 reviewer one raised a question about one of the statistical analyses. Please perform the requested check and address either way (reporting that it remains significant or amending the manuscript given the lack of statistical significance of the result). There is also a need to read carefully for typos and a few labeling issues. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

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

The authors have been quite responsive to the reviewers' suggestions. The addition of the tension sensor has helped clarify the observations. The other additions are helpful too.

Comments for the author

I appreciate the addition of the alpha-catenin strain sensor. However, I doubt that the data distribution justifies parametric analysis (ANOVA). Please repeat the analysis using non-parametric analysis (e.g. Mann Whitney) as the data distributions are somewhat skewed - this is particularly a question for alpha-cat TS + Y27632. As a result, my suspicion is that this sample is not necessarily statistically significantly different from the corresponding (-)Y27632 control.

If it turns out that the distributions are not significantly different, you will need to modify your interpretations, for example, along the lines of what was suggested after the first review. Similar questions apply to Fig. 5E and K.

Panel Labels in Fig. 1 need to be corrected. I think you mean C and D when referring to sub-panels in this section. Please comb through the rest of the manuscript for similar issues and there are a few typos that can be fixed.

Reviewer 2

Advance summary and potential significance to field

The authors made a great job taking in accound both reviewers comments

Comments for the author

I support publication of the manuscript under its present form.

Second revision

Author response to reviewers' comments

Response to Reviewers

Reviewer 1 Advance summary and potential significance to field
The authors have been quite responsive to the reviewers' suggestions. The addition of the tension sensor has helped clarify the observations. The other additions are helpful too.

Reviewer 1 Comments for the author
I appreciate the addition of the alpha-catenin strain sensor. However, I doubt that the data distribution justifies parametric analysis (ANOVA). Please repeat the analysis using non-parametric analysis (e.g. Mann Whitney) as the data distributions are somewhat skewed - this is particularly a question for alpha-cat TS + Y27632. As a result, my suspicion is that this sample is not necessarily statistically significantly different from the corresponding (-)Y27632 control. If it turns out that the distributions are not significantly different, you will need to modify your interpretations, for example, along the lines of what was suggested after the first review. Similar questions apply to Fig. 5E and K.

Response: Thank-you for pointing this out. In response to this query, we evaluated the FRET data (Fig 3) and the data for Fig. 5E and K with a normality test (using GraphPad Prism). For Fig. 5E all data sets have a normal distribution, so the parametric test is appropriate, but two of the FRET data sets, and one of the data sets for Fig 5K, did not have a normal distribution, so we have instead now applied non-parametric statistical tests. For Fig. 5K, a pair-wise comparison, we used a Mann-Whitney test, and the differences between samples remains significant when assessed by this non-parametric test. For the FRET data, as we are making comparisons amongst multiple samples, a Kruskal-Wallis test was used (Mann-Whitney is for pair-wise comparisons). We also repeated the experiment one additional time, and combined the new results with the previous replicates. This revised analysis supports the conclusions that we had in terms of the statistical significance of differences observed, so our conclusions stand. We have modified the text to note the use of non-parametric statistical tests.

Panel Labels in Fig. 1 need to be corrected. I think you mean C and D when referring to sub-panels in this section. Please comb through the rest of the manuscript for similar issues and there are a few typos that can be fixed.

Response: We have corrected the Fig 1 legend, and gone through the manuscript again for typos.

Reviewer 2 Advance summary and potential significance to field
The authors made a great job taking in accound both reviewers comments

Reviewer 2 Comments for the author
I support publication of the manuscript under its present form.

Response: Thank-you

Third decision letter

MS ID#: JOCES/2020/247866

MS TITLE: TRIP6 is required for tension at adherens junctions

AUTHORS: Srividya Venkatramanan, Maria Consuelo Ibar Valenzuela, and Kenneth Irvine
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

The authors have satisfactorily addressed the latest suggestions.

Comments for the author

The authors have satisfactorily addressed the latest suggestions.