Review COMMONS

JCB

Adherens junction regulates cryptic lamellipodia formation for epithelial cell migration

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Revision 0

Review #1

1. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Less than 1 month

2. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

The authors present a well designed study in the form of a clearly written manuscript with extensive and strong data. They study is somewhat integrative in nature, bringing together some results that are familiar (the basic phenotype of loss of aE-catenin loss; cryptic lamellipodia) in service of characterizing their model system and then focusing on deepening the molecular understanding of how the AJ relates to these lamellipodia, how these lamellipodia relate to migration, and how they are organized at the molecular level. They are scholarly in their attribution of credit for prior discoveries and very careful in their summary of the results at the end of each section and in the discussion. I think it is a compelling manuscript and should be published in something close to its present form. The major conclusions are all well supported by extensive data and I found several times that what I would have requested in revision was already done in the next figure. I think this will be a landmark paper in terms of thinking about the molecular tradeoffs between strong junctional adhesion and protrusive activity in epithelial cells. **Minor comments:** • I would suggest finding a way to make the images bigger or to make more extensive use of insets. Among the journal options in this network are several that are willing to publish full page figures (e.g. JCB) and so I would give the reader more space for the images; there is a lot of information there. • The Western blots are presented as single blots without the emerging standard of triplicate plus quantification. But, I am not sure this is absolutely necessary here; in each case the necessary result is clear. • The intent is to understand normal cell migration and the cells being studied are adenocarcinoma but this weakness is ameliorated by the fact that these cells are standard for studies of cell

polarity. • I debated a bit in the first half of the paper whether the language of crypic lamellipodia being "under the control of" of "generated by" AJs was too strong when it seemed likely that mature/stable AJs were repressing lamellipodia and they were initiated "at AJs" because of disassembly. I liked the way it was described in the last couple pages of the results plus the discussion better. There is nothing incorrect but I encourage the authors to reread for this issue and see if they want to fine tune how they present this issue early. • Page 10: I think "ever alter the sever" is supposed to be "even after the severe".

3. Significance:

Significance (Required)

It is well known that cryptic lamellipodia form and that there is some level of tradeoff between actin in bundles attached to the AJ and in dynamic form contributing to these protrusions. I think this manuscript takes the analysis of the molecular basis of these phenomena further than anything in the published literature. It is popular to want to see everything in 3D models now but I don't think they could have done this work at this level of resolution in a more complex system. I think this manuscript will be of broad interest to cell, developmental, and cancer biology researchers, especially those studying collective cell migration.

Review #2

1. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Cannot tell / Not applicable

2. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

Summary: This manuscript by Ozawa et al. investigates the role and regulation of cryptic lamellipodia (c-lamellipodia) in the in vitro collective migration of

epithelial cells, and shed an important light on the role of cell-cell adhesion in this process. As the authors refer to in their manuscript, E-Cadherin-based cell-cell adhesion is already shown to be crucial for coordinated movement during collective cell migration. Here the authors further demonstrate this requirement, and more importantly, provide a novel mechanism by which junctional integrity contributes to such coordination, namely through the regulation of c-lamellipodia, i.e. basal protrusions extended by marginal and submarginal cells underneath the neighboring cells ahead. The authors provide a structural and compositional description of these c-lamelipodia and show that i) their presence is polarized in wildtype cells, ii) they seemingly generate from AJ-linked actin cables, iii) they originate from multicellular junctions, and iv) protrusions in follower cells can grow both underneath the cell ahead, and upwards. These observations can provide novel insights on c-lamellipodia formation. The authors show that loss of α-ECatenin, which disrupts junctional integrity by preventing the binding of E-Cadherin to actin, leads to uncoordinated c-lamellipodia growth, and that this is accompanied by uncoordinated cell migration. They demonstrate that the formation of c-lamellipodia requires components of the actin regulatory complexes WRC and Arp2/3, and provide evidence that in the absence of c-lamellipodia, cells lose their capacity to migrate as marginal or submarginal cells. Finally, the authors show that the loss of junctional integrity and the subsequent migratory defects in E-cadherin- or α-ECatenin-deficient cells is dependent on MyoII activity. Altogether, Ozawa et al's work provide support for a role of Cadherin-based junctional integrity in regulating protrusive activity in marginal and submarginal follower cells in migrating cell collectives. The data presented here also suggests that this regulation may be partly dependent on mechanical properties of the junctions, controlled by MyoII contractility. **Major comments:** 1.In Figure 5D, why is the data for migration distance shown in arbitrary values? It would be informative to be able to compare the deficiency in migration for these WRC- or Arp2/3-defective cells to that of α-Ecat KO cells shown in Figure 1. The difference in units used makes this impossible. If the units can't be standardized could the authors at least comment on how these phenotypes compare? 2.The authors convincingly demonstrate that MyoII activity is increased in the disrupted junctions of ECadherin- or α-Ecat-deficient cells, and that this activity contributes to the loss of junctional integrity and increased protrusive activity in these cells. The authors also generate and test constitutively active MLC2 mutants. However, they test the effect of this activation only in α-Ecat-deficient cells. It would be interesting to see the effect of such constitutive activation in α-Ecat wildtype cells, especially as the α-Ecat-deficient cells already show defects in junctional integrity and migration that do not seem to be impacted by increased MyoII activity, and the loss of MyoII activity does not seem to have an impact on wildtype cells. In addition, to evaluate the recovery of migration phenotypes in MyoII mutants (Figure 7A) better, it would be helpful to have a comparison to migration distance in wildtype cells.

Minor comments. 1.The authors demonstrate that loss of aEcat causes a delay in cell migration, accompanied by a loss of directionality. They propose the latter as the cause of the former. While this is a reasonable suggestion, it is also possible that the loss of aEcat may cause a reduction in cell movement in addition to, and independently of the loss of directionality. If the authors could quantify the speed at which wildtype and aEcat KO cells move, this would clarify whether aEcat KO cells have reduced speed overall, or they can actually maintain movement at similar speed but in randomized directions. It should be feasible to quantify this from the existing data, and would be informative to better characterize the role of cell-cell adhesion and c-lamellipodia in cell migration. 2 In conclusion to the results shown in Figure 1A and B, the authors remark 'These results indicate that the epithelial cells used here require the cadherin adhesion system for their efficient migration, as shown for other cell types'. At this point, the data provided by the authors only demonstrate a requirement for aEcat, hence this conclusion on the requirement for the cadherin adhesion system appears somewhat unsubstantiated. To what extent does loss of aEcat inhibit Cadherin-mediated adhesion? The authors actually do answer this question later in the paper, and information on this can also be found in literature. If the authors could refer to this data here, it would help the reader, and better support their conclusion. 3.The authors describe the morphology of marginal and submarginal cells shown in Figure 2A, as being flatter than the internal cells. While it is clear from the figure that these cells are more spread, their flatness is harder to infer from the xy-plane view shown in the image. A cross section in the z-plane would be more helpful. 3.Similarly, for Figure 2B, a cross section might be more helpful in visualizing the 'slanted' nature of the lateral cell-cell contacts (LCs). It is understandable if the flatness of the cells makes it difficult to identify the LCs in cross-sections. In that case, the authors should better define their criteria for distinguishing LCs from protrusions. This gets to be even more important later, when the authors discuss conversion of protrusions to stable LCs (Figure 5). 4.In reference to Figure 2A, the authors comment:'These changes, induced by aEcatenin loss, were observed throughout a cell sheet (Fig. 2A)'. This figure only shows actin distribution and cell morphology throughout the sheet, while the authors talk about many other phenotypes prior to this sentence. As the figure does not show that all of these changes occur throughout the sheet, it would be better to clarify the sentence, or provide the data. 5.In Figure 2C, the gap between the actin cables the authors are referring to is difficult to see in the given images. Though the quantification of signal intensity given is helpful in identifying this gap, it would also be useful to provide a single channel image of the actin signal alone. Are the authors really observing two parallel bundles with a gap in between, or just a wider region of multiple parallel bundles? In this aspect, it is also somewhat confusing that they observe the gaps to contain amorphous F-actin networks. 6. The opening and closing of junctions that the authors mention in the text in reference to Figure 2F is not that easy to identify in the figure. Could the authors point this out with arrows or

some other marking? 7.At multiple points the authors claim that the protrusions emerge from AJs or that the AJs function as 'a site to generate' protrusions. While it is true that the protrusions appear to emerge from the same site as the AJs, this can easily be explained without a functional link. Protrusions, as expected, emerge from the cell periphery, which in the xy-plane views provided, coincides with the AJs and associated actin cables. Thus, the colocalization shown is not sufficient evidence for the conclusion that protrusions emerge from AJs. For example, how can the authors be sure that they do not emerge from the LCs instead? Given the flat morphology of the cells, it may not be feasible to investigate the colocalization of AJs and protrusions along the apical-basal axis, which is understandable. And the authors do provide enough support for a scenario where the AJ-associated actin cables and protrusions are generated from a common source of actin and actin regulators. Overall, this issue can be resolved with more accurate wording of the conclusions when it comes to the link of AJs and protrusions. 8.Likewise, the authors claim that 'the AJ begins to control cell motility at the two-cell stage,'. While the data they discuss here show that the cells start showing a difference in cell motility starting at the 2-cell stage, i.e. upon forming a shared junction, they do not directly demonstrate that it is the AJ that controls cell motility. This issue can again be resolved by rephrasing. 9.Based on the observed correlation between the appearance/disappearance of protrusions and opening/closing of junctions, shown in Figure 2, the authors write 'the observed membrane dynamics can be controlled by simple mechanical cell-cell contacts in the absence of aE-catenin.'. However, the authors show earlier that the 'open' junctions do not lose contact (as is shown with the continuous presence of ECadherin at the boundary in Fig. 2C). Thus, this conclusion on control by cell-cell contact should be clarified further. 10. In Figure 3, magenta asterisks within images are very hard to see, and actually helpful in identifying the edge. Perhaps the authors would consider changing the color if the asterisk marks? 11.In reference to Figure S4C, the authors say that 'Abi1-positive protrusions disappeared' upon treatment. While this is technically not incorrect, it would be more accurate/clear to say Abi1 disappeared from (or was reduced in) the protrusions, as the protrusions are still present. Also in this panel, it would be nice to see a side-by-side comparison of untreated cells. 12.The authors convincingly demonstrate that the cells that are deficient in WRC or Arp2/3 components lag behind when migrating along wildtype cells. Is this lag because they are slower overall or because they are excluded specifically from marginal and/or submarginal cell pools? In this context, does the term 'follower' refer to all cells that are not leaders (i.e. include submarginal cells with c-lamellipodia), or does it only refer to the interior, trailing group of cells with close to no c-lamellipodia activity to begin with? Clarifying these distinctions would be important to conclude on the requirement for these actin regulators and for c-lamellipodia for collective migration. Also helpful would be actual speed quantifications for Nap1- or p34-KD cells vs non-KD cells in different zones. Supposedly the KD cells can still migrate but at lower

speed. Are they slower than interior cells as well? Or losing c-lamellipodia bring them to the speed of interior cells? The latter would be an interesting observation as it would solidify that interior cells have another mechanism of migration, while the marginal and submarginal follower cells actively participate in migration using c-lamellipodia. 13.In Figure 6D and E, some of the phenotypes discussed by authors are difficult to detect in the images. This could probably be fixed by better presentation of the data. In Figure 6D, right-most panels, ppMLC2 staining appears to be shown in magenta for controls but in cyan for Blebbistatin-treatment, which makes it very difficult to compare the two. For Figure 6E, it would again help to see the actin signal shown alone in a single channel image to be able to assess the junctional opening/closure better. Lastly, Actin and Abi not only seem to be reduced, but also further enhanced in multicellular junctions in the Blebbistatin-treated junctions shown here. This could be something the authors might consider commenting, as it can further support a model where a common pool of actin and its regulators shift between junctions and protrusions depending on permissibility or regulation during stationary vs migratory states. 14.Throughout the manuscript, the authors cite a lot of review articles on collective cell migration and its regulation, which is good. But it would be nice to also cite some of the original work that provided the data discussed in those reviews. It would also help their cause to more clearly cite and explain what is already known on the role of AJs on collective cell migration (ex: Seddiki et al, 2017; Menko et al, 2018), as it would highlight the novel aspects of their work.

3. Significance:

Significance (Required)

The work presented by Ozawa et al. in this manuscript include novel observations on the formation and regulation of c-lamellipodia, and insight on the role of cell-cell junctions in migrating cell collectives. Thus, this study would conceptually advance the field of collective cell migration, a morphogenetic process of key importance both in normal development and pathogenic contexts such as cancer. While the role and regulation of lamellipodia in cell migration quite well characterized, less is known about c-lamellipodia, even though they have been first described over a decade ago (Farooqui and Fenteany, 2005). The study at hand fills an important gap in this sense. Previous studies on the role of Cadherin-based cell-cell junctions in collective cell migration (reviewed in Ladoux and Mege, 2017) demonstrated that they were important for coordinated movement of cells, and linked their role to processes such as contact inhibition of locomotion, or maintenance of contact stability and cohesiveness within cell collectives. Here Ozawa et al. adds an additional aspect to the role of cell-cell adhesions in collective cell migration by showing that junctions among follower cells contribute to the regulation of protrusive activity at these sites.

Furthermore, they provide evidence suggesting that this regulation is linked to mechanical stability of the junctions. Altogether these findings can open interesting new directions for studies on c-lamellipodia and the contribution of submarginal cells to collective migration, the inter-dependent regulation of protrusive activity, junctional stability and the role of junctional mechanics in these processes. Our exprtise in epithelial tissue polarity an dynamics.

Review #3

1. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Cannot tell / Not applicable

2. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

In this work, it is shown that adherence junctions are the site where cryptic lamellipodia are extended to propel collective and orderly cell migration. The authors confirm the recently identified role of the WAVE complex and ARP2/3 axis in the process. They further provide evidence that disruption of AJ by alphaE-catenin genetic interference results in uncontrolled cryptic lamellipodia which have the tendency to form at mechanically-weak junctional sites. The work is careful executed and technically sound. Its innovative content, however, is limited particularly in light of the fact that there are a set of important papers that are not cited nor discussed. It would be critical to present this set of finding on the context of the current literature. Specifically: 1.In the work by J. Spatz group (Das et al., 2015; Vishwakarma et al., 2018), the authors identified the tumour suppressor protein, merlin, as a critical determinant that coordinates collective migration of tens of cells, by acting as a mechanochemical transducer. Merlin was shown to localized at cell-cell junction by intercellular pulling forces of cryptic lamellipodia and to depend on actomyosin contractility. Merlin was further shown to modulate in a spatially controlled fashion RAC1 activation, and RAC1-dependent cryptic lamellipodia formation. Hence it would be essential to take this finding into consideration in the cellular context used

by the authors and explain what are the novel insights that the present work provide since as such it appears as an interesting and solid study, that only marginally advance our knowledge. It would be relevant also to take into consideration some of the key finding depicted in (Das et al., 2015) and a subsequent follow up (Vishwakarma et al., 2018) where the mechanical interaction between follower and leader has been neatly and extensivenly explored. Specifically what is the role of Merlin in their systems. Can Merlin explain the anetire set of present findings? 2.In a recent manuscript, it has been shown that epithelial cell ensemble acquire collective motion by extending cryptic lamellipodia in an coordinated and oriented fashion (Malinverno et al., 2017). In a follow up paper (Palamidessi et al., 2019) by the same group, these protrusions were shown to be dependent on the WAVE2 phosphorylation and be inhibited following NAP1 silencing (that aborgate the etire WAVE2 complex). These finding are in line with what is shown in the present manuscript, albeit the upstream molecular signals controlling the activity of the NPF might be different. In this respect, It would be critical to provide some molecular clues as to how weakened junctions trigger WAVE/Arp2/3 activation, whether this pathway is stritcly RAC1 dependent and, if so how RAC1 becomes activated at weakened junctions. 3.Recently, Jen S. et al Nature Physics 2020, using MDCK cells on circular patterns showed that "cells follow coordinated rotational movements after establishing directed Rac1-dependent polarity over the entire monolayer". They further demonstrated that "the maintenance of coordinated migration requires the acquisition of a front-rear polarity within each single cell but does not require the maintenance of cell-cell junctions". The apparent difference of this set of findings from what is reported in the present manuscript might be ascribed to stirking diverse geometric arrangments of the cells. Yet it would be paramound to assess whether the integrity of AJ is required just for the onset of cryptic lamellipodia, but once they are fomed as proposed by the Benoit's work, this requirment is no longer needed. 4.In Figure, 1 using DLD1 and Caco-2, the authors provide convincing evidence that removal of alphaE-catenin impairs wound closure, mainly by disrupting directional motility. This phenotype is shown to be an emergent property of cell collective as individual cell migration is marginally affected by alphaEcatenin removal. It would be critical to determine the status and thedynamics of lamellipodia at the leading edge under these conditions. It is critical to determine whether the removal of alphaE-catenin impact on the epithelial vs mesenchymal state of these cells, the morphology of which appear significantly more elongated with respect to control cells. The experiment showing that a full EMT as induced by SNAIL expression in MDCK or TGFbeta stimulation in A549 is not sufficient to rule out the possibility that a partial or plastic EMT is induced by the removal of alphaECatenin. It would also be important to determine the direction of motion not only of the leader cells, but also of the followers. Is the motion and directionality of cells within the wounded epithelia affected by the removal of alphaE-catenin. Cell tracking using nuclear markers or PIV analysis would seem necessary to

describe the collective behavior of these cells. 5.In Figure 2, the analysis of AJ in control vs alphaECatenin null cells is provided. These experiments are neat and clear. It would be important to restore the expression of alphaECatenin and show that the phenotype is indeed formally dependent on it. The process of generating KO requires cloning, which might display spurious effects due to genetic drifts or unwanted genetic alterations. 6.In Video 6 it is shown the dynamics of Life-Act expressing single and dual cells. From the analysis, of these movies, the authors conclude that:" Thus, the AJ begins to control cell motility at the two-cell stage, but it requires more cells to organize a polarized cell sheet in order to conduct directed migration." This is a neat observation but would require to be supported by the analysis of cell protrusion and their orientation as a function of the number of cells. 7.The experiments depicted in figure 3 to 5 are largely confirmatory of previous data (Das et al., 2015; Malinverno et al., 2017; Palamidessi et al., 2019) and fully consistent with a RAC1/WAVE2 complex/ARP2/3 axis operative in cryptic lamellipodia formation. This should be openly acknowledged. 8.Fig. 6 and 7 depict some interesting set of finding, supporting the notion that disruption of junctional strength may lead to altered of RHOA and RHOA mediated actomyosin contractility, which in turn correlate with cryptic lamellipodia formation. This is fine, but not particularly novel albeit the images and experiments in support of this contention are convincing. References Das, T., K. Safferling, S. Rausch, N. Grabe, H. Boehm, and J.P. Spatz. 2015. A molecular mechanotransduction pathway regulates the collective migration of epithelial cells. Nat Cell Biol. 17:276-287. Malinverno, C., S. Corallino, F. Giavazzi, M. Bergert, Q. Li, M. Leoni, A. Disanza, E. Frittoli, A. Oldani, E. Martini, T. Lendenmann, G. Deflorian, G.V. Beznoussenko, D. Poulikakos, K.H. Ong, M. Uroz, X. Trepat, D. Parazzoli, P. Maiuri, W. Yu, A. Ferrari, R. Cerbino, and G. Scita. 2017. Endocytic reawakening of motility in jammed epithelia. Nature materials. 16:587-596. Palamidessi, A., C. Malinverno, E. Frittoli, S. Corallino, E. Barbieri, S. Sigismund, G.V. Beznoussenko, E. Martini, M. Garre, I. Ferrara, C. Tripodo, F. Ascione, E.A. Cavalcanti-Adam, Q. Li, P.P. Di Fiore, D. Parazzoli, F. Giavazzi, R. Cerbino, and G. Scita. 2019. Unjamming overcomes kinetic and proliferation arrest in terminally differentiated cells and promotes collective motility of carcinoma. Nature materials. 18:1252-1263. Vishwakarma, M., J. Di Russo, D. Probst, U.S. Schwarz, T. Das, and J.P. Spatz. 2018. Mechanical interactions among followers determine the emergence of leaders in migrating epithelial cell collectives. Nature communications. 9:3469.

3. Significance:

Significance (Required)

What is missing from all these experiments is the mechanisms through which the RAC1/WAVE2/ARP2/3 axis becomes aberrantly elevated following junctional

disruption and actomyosin contractility perturbation. In the absence of new insights, the manuscript is solid but advances marginally our knowledge of collective motion and cryptic lamellipodia formation.

Authors' response to the reviews for the manuscript 'Review Commons Refereed Preprint #RC-2020-00257'

We are thankful to all three reviewers, who have carefully evaluated our work. Our responses to each comment by the reviewers (in italic) are explained below. In the revised text, major changes are shown with blue-colored letters. Figures were also updated to incorporate these changes. We have also changed the title of the manuscript, considering the comments from the Reviewers #1 and #2.

Reviewer #1:

I think this will be a landmark paper in terms of thinking about the molecular tradeoffs between strong junctional adhesion and protrusive activity in epithelial cells.

Response: We appreciate very supportive comments on our work from this reviewer.

• I would suggest finding a way to make the images bigger or to make more extensive use of insets. Among the journal options in this network are several that are willing to publish full page figures (e.g. JCB) and so I would give the reader more space for the images; there is a lot of information there.

Response: The figures provided to reviewers may not have retained the original quality and resolution of each image, causing some difficulty for them to detect details. We will try to present high-resolution images upon publication of the manuscript.

• The Western blots are presented as single blots without the emerging standard of triplicate plus quantification. But, I am not sure this is absolutely necessary here; in each case the necessary result is clear.

Response: We always confirmed reproductivity of Western blot data by multiple experiments. This has now been commented at the newly added section of 'Western blotting' in the Materials and Methods.

• The intent is to understand normal cell migration and the cells being studied are adenocarcinoma but this weakness is ameliorated by the fact that these cells are standard for studies of cell polarity.

Response: Thanks for this commitment. One reason why we chose adenocarcinoma cells was that we are interested in understanding carcinoma cell behavior, which is related to their invasiveness.

• I debated a bit in the first half of the paper whether the language of crypic lamellipodia being "under the control of" of "generated by" AJs was too strong when it seemed likely that mature/stable AJs were repressing lamellipodia and they were initiated "at AJs" because of

disassembly. I liked the way it was described in the last couple pages of the results plus the discussion better. There is nothing incorrect but I encourage the authors to reread for this issue and see if they want to fine tune how they present this issue early.

Response: We agree that the structural and functional relations of AJs to cryptic lamellipodia formation had better to describe with more accuracy. We therefore rephrased the applicable parts of the text, and also changed the title of the manuscript.

• *Page 10: I think "ever alter the sever" is supposed to be "even after the severe".*

Response: this typo was corrected.

Reviewer #2:

General response to this reviewer from the authors: This reviewer provided a number of very constructive comments to improve the manuscript. We have attempted to faithfully address these comments.

Major comments:

1.In Figure 5D, why is the data for migration distance shown in arbitrary values? It would be informative to be able to compare the deficiency in migration for these WRC- or Arp2/3-defective cells to that of α-Ecat KO cells shown in Figure 1. The difference in units used makes this impossible. If the units can't be standardized could the authors at least comment on how these phenotypes compare?

Response: We re-analyzed the data of cell migration which were used for Figure 5D, and replaced the arbitrary values with the actual migration distances.

2.The authors convincingly demonstrate that MyoII activity is increased in the disrupted junctions of ECadherin- or α-Ecat-deficient cells, and that this activity contributes to the loss of junctional integrity and increased protrusive activity in these cells. The authors also generate and test constitutively active MLC2 mutants. However, they test the effect of this activation only in α-Ecat-deficient cells. It would be interesting to see the effect of such constitutive activation in α-Ecat wildtype cells, especially as the α-Ecat-deficient cells already show defects in junctional integrity and migration that do not seem to be impacted by increased MyoII activity, and the loss of MyoII activity does not seem to have an impact on wildtype cells. In addition, to evaluate the recovery of migration phenotypes in MyoII mutants (Figure 7A) better, it would be helpful to have a comparison to migration distance in wildtype cells.

Response: To address this comment, we have added new data of wild-type cells transfected with MCL2 mutants to the revised manuscript (Figure S7), as these cells were already isolated before. Ectopic expression of the MCL2 mutants did not show any effect on junctional integrity or cell migration, which suggests that MLC2 activation gives such drastic

effects on cell junctions only in the absence of αE-catenin. We have also added the migration distance of wild-type cells to Figure 7A, as this datum was already available.

Minor comments.

1.The authors demonstrate that loss of aEcat causes a delay in cell migration, accompanied by a loss of directionality. They propose the latter as the cause of the former. While this is a reasonable suggestion, it is also possible that the loss of aEcat may cause a reduction in cell movement in addition to, and independently of the loss of directionality. If the authors could quantify the speed at which wildtype and aEcat KO cells move, this would clarify whether aEcat KO cells have reduced speed overall, or they can actually maintain movement at similar speed but in randomized directions. It should be feasible to quantify this from the existing data, and would be informative to better characterize the role of cell-cell adhesion and c-lamellipodia in cell migration.

Response: As recommended, we have measured the speed of individual cells and also quantified the directionality of cell migration, finding that only directionality was affected by αE-catenin loss. These results are now shown in Figure 1C, along with re-editing of the figure.

2. In conclusion to the results shown in Figure 1A and B, the authors remark 'These results indicate that the epithelial cells used here require the cadherin adhesion system for their efficient migration, as shown for other cell types'. At this point, the data provided by the authors only demonstrate a requirement for aEcat, hence this conclusion on the requirement for the cadherin adhesion system appears somewhat unsubstantiated. To what extent does loss of aEcat inhibit Cadherin-mediated adhesion? The authors actually do answer this question later in the paper, and information on this can also be found in literature. If the authors could refer to this data here, it would help the reader, and better support their conclusion.

Response: Thanks for this comment. We rewrote this sentence, inserting additional references, as follows:

‘These results suggest that the epithelial cells used here require the cadherin-based AJs, whose formation is dependent on αE-catenin (Watabe et al., 1994; Watabe-Uchida et al., 1998), for their efficient migration, as shown for other cell types (Mayor and Etienne-Manneville, 2016).’

3.The authors describe the morphology of marginal and submarginal cells shown in Figure 2A, as being flatter than the internal cells. While it is clear from the figure that these cells are more spread, their flatness is harder to infer from the xy-plane view shown in the image. A cross section in the z-plane would be more helpful.

Response: We agree with this comment. As recommended by the reviewer, we now use the word ‘spread’, instead of ‘flat’.

3.Similarly, for Figure 2B, a cross section might be more helpful in visualizing the 'slanted' nature of the lateral cell-cell contacts (LCs). It is understandable if the flatness of the cells makes it difficult to identify the LCs in cross-sections. In that case, the authors should better define their criteria for distinguishing LCs from protrusions. This gets to be even more important later, when the authors discuss conversion of protrusions to stable LCs (Figure 5).

Response: We understand this problem. However, confocal microscopy along the vertical axis does not give any clear images to demonstrate the configuration of E-cadherin-positive LCs, due to the extreme flatness of the cells, as the reviewer suspected. We therefore chose the option to describe LCs and protrusions more carefully. For example:

1) We have cited previous works which reported that LCs are detected as 'slanted' structures in monolayer cultures of epithelial cell lines, as below:

'E-cadherin also distributed to lateral cell-cell contacts (LCs), which were generally slanted toward either side of the junction, exhibiting a strand-like or dotted pattern (Fig. 2B, arrows), as reported previously (Kametani and Takeichi, 2007; Nishimura et al., 2016; Otani et al., 2006).'

2) We rephrased the sentences to interpret the effect of Abi1 removal on E-cadherin distribution at the first paragraph of the result section 'WRC-Arp2/3 system is required for collective migration of epithelial cells', as follows:

'Notably, the E-cadherin-positive areas which extend below the AJ dramatically increased in these cells (Figs. 5A and 5C), which suggests that static LCs form in place of c-lamellipodia when the WRC-Arp2/3 system is inactive.'

2) We have also added the following discussion on the relation between LCs and protrusions to the Discussion section:

'c-Lamellipodia were morphologically similar to slanted LCs, as larger c-lamellipodia actually contained E-cadherin. Of note, silencing of WRC or Arp2/3 resulted in an increase of E-cadherin-positive LCs, along with suppression of dynamic protrusions. These suggest that the lateral plasma membranes normally organize into static LCs, but, when cells move as a collective, the membranes acquire motile functions to become 'protrusions', responding to activation of the WRC–Arp2/3 system.'

4.In reference to Figure 2A, the authors comment:'These changes, induced by aEcatenin loss, were observed throughout a cell sheet (Fig. 2A)'. This figure only shows actin distribution and cell morphology throughout the sheet, while the authors talk about many other phenotypes prior to this sentence. As the figure does not show that all of these changes occur throughout the sheet, it would be better to clarify the sentence, or provide the data.

Response: This is a reasonable comment. We deleted this sentence, instead added the following sentence:

'The gaps are often filled with irregular F-actin networks, and such actin reorganization occurred throughout the cell sheet (Fig. 2A).'

5.In Figure 2C, the gap between the actin cables the authors are referring to is difficult to see in the given images. Though the quantification of signal intensity given is helpful in identifying this gap, it would also be useful to provide a single channel image of the actin signal alone. Are the authors really observing two parallel bundles with a gap in between, or just a wider region of multiple parallel bundles? In this aspect, it is also somewhat confusing that they observe the gaps to contain amorphous F-actin networks.

Response: To respond to this comment, we chose another junction to enlarge in Figure 2C, which show two separate actin cables more clearly than before even in the double-stained image.

6. The opening and closing of junctions that the authors mention in the text in reference to Figure 2F is not that easy to identify in the figure. Could the authors point this out with arrows or some other marking?

Response: To address this point, we now show time-lapse images with a longer time span, also updating Video 9. I believe that the opening and closing of junctions are more clearly visible in the revised Figure 10F.

7.At multiple points the authors claim that the protrusions emerge from AJs or that the AJs function as 'a site to generate' protrusions. While it is true that the protrusions appear to emerge from the same site as the AJs, this can easily be explained without a functional link. Protrusions, as expected, emerge from the cell periphery, which in the xy-plane views provided, coincides with the AJs and associated actin cables. Thus, the colocalization shown is not sufficient evidence for the conclusion that protrusions emerge from AJs. For example, how can the authors be sure that they do not emerge from the LCs instead? Given the flat morphology of the cells, it may not be feasible to investigate the colocalization of AJs and protrusions along the apical-basal axis, which is understandable. And the authors do provide enough support for a scenario where the AJ-associated actin cables and protrusions are generated from a common source of actin and actin regulators. Overall, this issue can be resolved with more accurate wording of the conclusions when it comes to the link of AJs and protrusions.

Responses: We thank the reviewer for this important and reasonable comment. We carefully revised the text related to this issue at every applicable place throughout the manuscript. Please check the revised sentences with blue letters.

8.Likewise, the authors claim that 'the AJ begins to control cell motility at the two-cell stage,'. While the data they discuss here show that the cells start showing a difference in cell motility starting at the 2-cell stage, i.e. upon forming a shared junction, they do not directly demonstrate that it is the AJ that controls cell motility. This issue can again be resolved by rephrasing.

Response: We admit that the original description lacked accuracy. We rephrased this part of the manuscript as follows:

‘Thus, cells begin to remodel the actin cytoskeleton at the two-cell stage through AJ formation, ----'

9.Based on the observed correlation between the appearance/disappearance of protrusions and opening/closing of junctions, shown in Figure 2, the authors write 'the observed membrane dynamics can be controlled by simple mechanical cell-cell contacts in the absence of aE-catenin.'. However, the authors show earlier that the 'open' junctions do not lose contact (as is shown with the continuous presence of ECadherin at the boundary in Fig. 2C). Thus, this conclusion on control by cell-cell contact should be clarified further.

Response: Thanks for pointing out the confusing description. The text was revised as follows:

‘------ the junctional closure resulted in a temporary suppression of membrane ruffling (Fig. 2F), which implies that a certain form of cell-cell contacts are sufficient for suppressing membrane protrusion when αE-catenin is absent.’

10. In Figure 3, magenta asterisks within images are very hard to see, and actually helpful in identifying the edge. Perhaps the authors would consider changing the color if the asterisk marks?

Response: The asterisks have now been colored with white or black throughout the images, which gives a clearer contrast than previous symbols.

11.In reference to Figure S4C, the authors say that 'Abi1-positive protrusions disappeared' upon treatment. While this is technically not incorrect, it would be more accurate/clear to say Abi1 disappeared from (or was reduced in) the protrusions, as the protrusions are still present. Also in this panel, it would be nice to see a side-by-side comparison of untreated cells.

Response: In EHT1864-treated wild-type cells, protrusions entirely disappeared from cell-cell contact zones, as judged by actin staining, although these remain to some extents in αE-catenin KO cells. To present the differences between treated and untreated cells in clearer ways, images of the untreated cells are placed side-by-side, as recommended by the reviewer.

12.The authors convincingly demonstrate that the cells that are deficient in WRC or Arp2/3 components lag behind when migrating along wildtype cells. Is this lag because they are slower overall or because they are excluded specifically from marginal and/or submarginal cell pools? In this context, does the term 'follower' refer to all cells that are not leaders (i.e. include submarginal cells with c-lamellipodia), or does it only refer to the interior, trailing group of cells with close to no c-lamellipodia activity to begin with? Clarifying these distinctions would be important to conclude on the requirement for these actin regulators and for c-lamellipodia for collective migration.

Response: Whether the migration lag observed in WRC or Arp2/3-depleted cells occurs solely due to their slower speed or it involves their exclusion from the wild-type marginal and/or submarginal cells is an intriguing question. We are, however, unable to determine whether the 'active' exclusion is involved in this phenomenon, as their migration speed is intrinsically slower than wild-type cells, as shown in Figure 5D. Definition of 'follower' is not easy, as all 'non-leaders cells' could be categorized as the followers, but their behavior gradually changes from submarginal to more interior zones. Considering this point and the comments raised by the reviewer, we more carefully described 'follower cell' behavior.

Also helpful would be actual speed quantifications for Nap1- or p34-KD cells vs non-KD cells in different zones. Supposedly the KD cells can still migrate but at lower speed. Are they slower than interior cells as well? Or losing c-lamellipodia bring them to the speed of interior cells? The latter would be an interesting observation as it would solidify that interior cells have another mechanism of migration, while the marginal and submarginal follower cells actively participate in migration using c-lamellipodia.

Response: This is an important suggestion. Collection of such data would allow us to describe more precisely the role of c-lamellipodia in collective migration of epithelial cells. To perform the suggested experiments, however, we need to prepare another line of cells stably transfected with some fluorescence markers to distinguish between KD and non-KO cells in their live imaging. In our current circumstances, it is difficult to prepare them within a short period. Therefore, we would like to leave this experiment for future tests. We believe that, without the suggested data, we are still able to conclude the importance of WAVE-Arp2/3 dependent c-lamellipodia formation in the collective migration of epithelial cells.

13.In Figure 6D and E, some of the phenotypes discussed by authors are difficult to detect in the images. This could probably be fixed by better presentation of the data. In Figure 6D, right-most panels, ppMLC2 staining appears to be shown in magenta for controls but in cyan for Blebbistatin-treatment, which makes it very difficult to compare the two. For Figure 6E, it would again help to see the actin signal shown alone in a single channel image to be able to assess the junctional opening/closure better. Lastly, Actin and Abi not only seem to be reduced, but also further enhanced in multicellular junctions in the Blebbistatin-treated junctions shown here. This could be something the authors might consider commenting, as it can further support a model where a common pool of actin and its regulators shift between junctions and protrusions depending on permissibility or regulation during stationary vs migratory states.

Response: To respond to these helpful suggestions, we have added the panels showing actin alone to Figure 6D (originally 6E). Then, we noticed that actin staining shown in the new Figure 6D is sufficient to document the effect of blebbistatin treatment on junction configuration, and therefore decided to delete the previous Figure 6D that had shown E-cadherin and ppMLC immunostaining. This change also helped us to create a space for adding actin-only images within the figure. Concerning the observation that actin and Abi accumulation at multicellular junctions, we added some comments on this phenomenon within the result section 'AJ disruption induces myosin II activation'.

14.Throughout the manuscript, the authors cite a lot of review articles on collective cell

migration and its regulation, which is good. But it would be nice to also cite some of the original work that provided the data discussed in those reviews. It would also help their cause to more clearly cite and explain what is already known on the role of AJs on collective cell migration (ex: Seddiki et al, 2017; Menko et al, 2018), as it would highlight the novel aspects of their work.

Response: Thanks for this comment. We have cited many more original articles than in the previous version, including Seddiki et al, (2017), although we could not identify the paper 'Menko et al, 2018' which was listed by the reviewer.

Reviewer #3:

The work is careful executed and technically sound. Its innovative content, however, is limited particularly in light of the fact that there are a set of important papers that are not cited nor discussed. It would be critical to present this set of finding on the context of the current literature.

Response: Thanks to this suggestion by the reviewer, we have been able to cite many important papers, which were missing in the original version of the manuscript.

Specifically:
1.In the work by J. Spatz group (Das et al., 2015; Vishwakarma et al., 2018), the authors identified the tumour suppressor protein, merlin, as a critical determinant that coordinates collective migration of tens of cells, by acting as a mechanochemical transducer. Merlin was shown to localized at cell-cell junction by intercellular pulling forces of cryptic lamellipodia and to depend on actomyosin contractility. Merlin was further shown to modulate in a spatially controlled fashion RAC1 activation, and RAC1-dependent cryptic lamellipodia formation. Hence it would be essential to take this finding into consideration in the cellular context used by the authors and explain what are the novel insights that the present work provide since as such it appears as an interesting and solid study, that only marginally advance our knowledge. It would be relevant also to take into consideration some of the key finding depicted in (Das et al., 2015) and a subsequent follow up (Vishwakarma et al., 2018) where the mechanical interaction between follower and leader has been neatly and extensivenly explored. Specifically what is the role of Merlin in their systems. Can Merlin explain the anetire set of present findings?

Response: We appreciate these comments from the reviewer. Considering the importance of the work on merlin by J. Spatz group, we observed merlin distribution in Caco2 cell layers which are moving. However, we could not find any re-localization of merlin to the cytoplasm in correlation with cryptic lamellipodia formation in these cells, unlike the findings by Das *et al*., 2015. Therefore, we could not reason that merlin is involved in cryptic lamellipodia formation at least in the Caco2 cells. These results are explained at the beginning of the section 'AJ disruption induces myosin II activation', using the newly prepared Figure S5.

2.In a recent manuscript, it has been shown that epithelial cell ensemble acquire collective motion by extending cryptic lamellipodia in an coordinated and oriented fashion (Malinverno

et al., 2017). In a follow up paper (Palamidessi et al., 2019) by the same group, these protrusions were shown to be dependent on the WAVE2 phosphorylation and be inhibited following NAP1 silencing (that aborgate the etire WAVE2 complex). These finding are in line with what is shown in the present manuscript, albeit the upstream molecular signals controlling the activity of the NPF might be different. In this respect, It would be critical to provide some molecular clues as to how weakened junctions trigger WAVE/Arp2/3 activation, whether this pathway is stritcly RAC1 dependent and, if so how RAC1 becomes activated at weakened junctions.

Response: We showed that the Rac1 inhibitor EHT1864 suppressed protrusion or cryptic lamellipodia formation in both wild-type and αE-catenin KO cells (Figure S4C), which suggests that Rac1 is active even in wild-type junctions. Actually, we did not obtain any evidence that Rac1 activity differs between normal and disrupted junctions, when we assessed it by using active Rac1 sensors (data not shown). These suggest that Rac1 is unlikely the target of junction weakening. Thus, how junction weakening promotes WAVE/Arp2/39-dependent protrusion formation remains to be elucidated in the future.

3.Recently, Jen S. et al Nature Physics 2020, using MDCK cells on circular patterns showed that "cells follow coordinated rotational movements after establishing directed Rac1-dependent polarity over the entire monolayer". They further demonstrated that "the maintenance of coordinated migration requires the acquisition of a front-rear polarity within each single cell but does not require the maintenance of cell-cell junctions". The apparent difference of this set of findings from what is reported in the present manuscript might be ascribed to stirking diverse geometric arrangments of the cells. Yet it would be paramound to assess whether the integrity of AJ is required just for the onset of cryptic lamellipodia, but once they are fomed as proposed by the Benoit's work, this requirment is no longer needed.

Response: Many thanks for this insightful comment. In our system, even αE-catenin KO cells can undergo collective migration but at lower speeds, indicating that AJ-independent mechanisms to support the collective migration of epithelial cells also exist. The idea proposed by the reviewer is quite intriguing, but we would not include it in the manuscript to avoid too much speculation.

4.In Figure, 1 using DLD1 and Caco-2, the authors provide convincing evidence that removal of alphaE-catenin impairs wound closure, mainly by disrupting directional motility. This phenotype is shown to be an emergent property of cell collective as individual cell migration is marginally affected by alphaEcatenin removal. It would be critical to determine the status and the dynamics of lamellipodia at the leading edge under these conditions. It is critical to determine whether the removal of alphaE-catenin impact on the epithelial vs mesenchymal state of these cells, the morphology of which appear significantly more elongated with respect to control cells. The experiment showing that a full EMT as induced by SNAIL expression in MDCK or TGFbeta stimulation in A549 is not sufficient to rule out the possibility that a partial or plastic EMT is induced by the removal of alphaECatenin.

Response: In Video 1, αE-catenin KO DLD1 cells indeed look a bit elongated, but this is not a general morphology of αE-catenin KO epithelial cells. For example, singly isolated αE-catenin KO Caco2 cells show a disc-like shape, which do not resemble mesenchymal cells. Also, they keep the expression of E-cadherin, desmosomal proteins and tight junction

proteins, which are generally not expressed by mesenchymal cells. All these data strongly suggest that αE-catenin KO epithelial cells maintain the epithelial phenotypes. To confirm these points, we have prepared a Western blot which demonstrates that αE-catenin KO DLD1 cells do not express mesenchymal markers, as shown below:

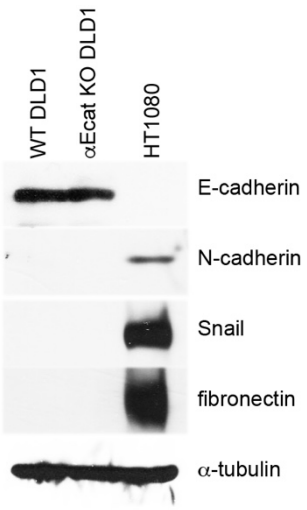

Figure legend. Western blot for E-cadherin (an epithelial marker; and N-cadherin, Snail and fibronectin (mesenchymal markers). HT1080 cells were used as a representative of mesenchymal cells.

It would also be important to determine the direction of motion not only of the leader cells, but also of the followers. Is the motion and directionality of cells within the wounded epithelia affected by the removal of alphaE-catenin. Cell tracking using nuclear markers or PIV analysis would seem necessary to describe the collective behavior of these cells.

Response: As requested, we have analyzed the direction of motion not only for leader cells but also for followers, and added the results of this analysis to the revised Figure 1C. Thanks to the suggestion by the reviewer, these new data can illustrate the collective behavior of cells in more details than before.

5.In Figure 2, the analysis of AJ in control vs alphaECatenin null cells is provided. These experiments are neat and clear. It would be important to restore the expression of alphaECatenin and show that the phenotype is indeed formally dependent on it. The process of generating KO requires cloning, which might display spurious effects due to genetic drifts or unwanted genetic alterations.

Response: We have been extremely careful to avoid observing potential cloning effects. Therefore, we always prepared multiple KO clones, and confirmed whether we are observing the phenotypes common to all the isolated clones. We also actually examined the effect of re-introduction of the αE-catenin gene into αE-catenin KO clones. These points are now mentioned at the section 'Isolation of cells lines using CRISPR/Cas9 plasmids-mediated gene knockout' in the Materials & Methods'. One example of such experiments is presented below.

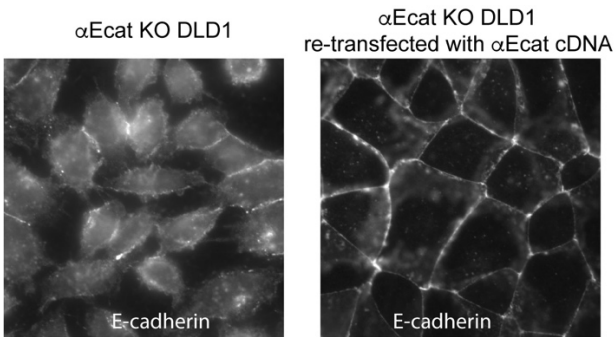

Figure legend. The αE-catenin KO DLD1 line used in the present experiments (left) was re-transfected with αE-catenin cDNA (right). The KO cells restore wild-type junctions.

6.In Video 6 it is shown the dynamics of Life-Act expressing single and dual cells. From the analysis, of these movies, the authors conclude that:" Thus, the AJ begins to control cell motility at the two-cell stage, but it requires more cells to organize a polarized cell sheet in order to conduct directed migration." This is a neat observation but would require to be supported by the analysis of cell protrusion and their orientation as a function of the number of cells.

Response: Thanks for this comment. The sentence "the AJ begins to control cell motility at the two-cell stage" was overstatement. Therefore, we rephrased it. On the other hand, the number of cells and the position/orientation of protrusions in a cell colony, which are associated with the onset of their migration, were very variable from colony to colony. It was therefore difficult to describe these phenomena in a quantitative manner.

7.The experiments depicted in figure 3 to 5 are largely confirmatory of previous data (Das et al., 2015; Malinverno et al., 2017; Palamidessi et al., 2019) and fully consistent with a RAC1/WAVE2 complex/ARP2/3 axis operative in cryptic lamellipodia formation. This should be openly acknowledged.

Response: We thank the reviewer for this comment and also for providing a useful list of references. We have now cited more literatures relevant to the present study, including those listed by the reviewer.

8.Fig. 6 and 7 depict some interesting set of finding, supporting the notion that disruption of junctional strength may lead to altered of RHOA and RHOA mediated actomyosin contractility, which in turn correlate with cryptic lamellipodia formation. This is fine, but not particularly novel albeit the images and experiments in support of this contention are convincing.

Response: The RhoA-myosin II system has broadly been studied as a regulator of junctional integrity. However, to our knowledges, no other studies have convincingly demonstrated that RhoA is specifically activated at cell junctions in correlation with their disruption, leading to the subsequent events that affect the migration of cells.

What is missing from all these experiments is the mechanisms through which the RAC1/WAVE2/ARP2/3 axis becomes aberrantly elevated following junctional disruption and actomyosin contractility perturbation. In the absence of new insights, the manuscript is solid

but advances marginally our knowledge of collective motion and cryptic lamellipodia formation.

Response: We admit that we have not presented novel 'biochemical' pathways that regulate cryptic lamellipodia formation. However, we have revealed detailed cellular processes of how cryptic lamellipodia form and how cell junctions influence on their formation, which have not been reported before. We believe that deeper documentation of cell-level processes is equally important to the discovery of novel biochemical pathways in cell biology fields.

July 15, 2020

RE: JCB Manuscript #202006196T

Dr. Masatoshi Takeichi
RIKEN
Minatojima-Minamimachi, Chuo-ku
Kobe, Hyogo 650-0047
Japan

Dear Dr. Takeichi:

Thank you for submitting your revised manuscript entitled "Adherens junction regulates cryptic lamellipodia formation for epithelial cell migration". We have assessed your revised paper and response to the reviewer comments and we would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

In your response to reviewer #3, you noted that you "...did not obtain any evidence that Rac1 activity differs between normal and disrupted junctions, when we assessed it by using active Rac1 sensors (data not shown). These suggest that Rac1 is unlikely the target of junction weakening." We feel it is important that this information be made clear to the reader and so we would like you to add this Rac1 sensor data to the paper (as a supplementary figure) and to mention and discuss it in the text. Please note that final acceptance of the paper is contingent on the addition of this data and discussion.

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

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2) Figures limits: Articles and Tools may have up to 10 main text figures.

3) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Molecular weight or nucleic acid size markers must be included on all gel electrophoresis, including cropped gels. Thus, you must add weight markers to the gel in figure 6c.

4) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph

must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please also be sure to indicate the statistical tests used in each of your experiments (both in the figure legend itself and in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, since you used parametric tests in your study (e.g. t-tests, ANOVA, etc.), you should have first determined whether the data was normally distributed before selecting that test. In the stats section of the methods, please indicate how you tested for normality. If you did not test for normality, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

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b. Type, magnification, and numerical aperture of the objective lenses
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g. Acquisition software
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8) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed.

9) Supplemental materials: There are normally strict limits on the allowable amount of supplemental data. Articles/Tools may usually have up to 5 supplemental figures. At the moment, you currently have 6 such figures (and may need to add another to accommodate the Rac1 sensor data). Given the circumstances, though, we will be able to allow you the extra space so 6 (or 7) supplementary figures will be fine.
Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section.

10) eTOC summary: A ~40-50 word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person. It should begin with "First author name(s) et al..." to match our preferred style.

11) Conflict of interest statement: JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the following statement: "The authors declare no competing financial interests." If competing interests are declared, please follow your statement of these competing interests with the following statement: "The authors declare no further competing financial interests."

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Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Kenneth Yamada, MD, PhD
Senior Editor
The Journal of Cell Biology

Tim Spencer, PhD
Executive Editor
Journal of Cell Biology

Responses to the editorial requests

Responding to your request that Rac1 sensor data should be added to the paper as a supplementary figure, we have added a set of these data to Fig. S5C and S5D, describing them at the first paragraph of the section ‘AJ disruption induces myosin II activation’ in Results (on pages 12 to 13), and adding some comments to Discussion. We also have made a number of editorial revisions, referring to the guidelines for authors. The number of supplemental figures is kept 7, as this was permitted in the editorial decision e-mail dated July 15.