Myosin II isoforms promote internalization of spatially distinct clathrin-independent endocytosis cargoes through modulation of cortical tension downstream of ROCK2

Jessica Wayt, Alexander Cartagena-Rivera, Dipannita Dutta, Julie Donaldson, and Clare Waterman

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E20-07-0480
TITLE: Myosin II isoforms promote internalization of spatially distinct clathrin-independent endocytosis cargoes through modulation of cortical tension downstream of ROCK2

Dear Dr. Waterman,

Two reviewers have evaluated your manuscript entitled "Myosin II isoforms promote internalization of spatially distinct clathrin-independent endocytosis cargoes through modulation of cortical tension downstream of ROCK2". As you will see, both reviewers thought that the work presented is technically sound and potentially very interesting. However, they also both expressed reservations regarding the lack of mechanistic insight into how myosin contractility regulates CIE. I am also worried about the second comment of Rev 1, about the integrity of epithelial morphology in the Caco2 depleted cells. You submitted the ms to be considered as a Brief Report, which could represent a good fit for the work. However, as it currently stands, it is exceeding the character count.
Here is what I suggest:
1. Attend to the comments of Rev1 and provide a more "informed discussion on how you envision that myosin II contractility plays a role in CIE"
2. Please be sure to clearly state the number of biological replicates and samples in the figure legends
3. Edit the text to fit within the limits of a Brief Report
I think that your work has the potential to simulate progress in the field and that MBoC is a good fit.

Sincerely,

Carole Parent
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Waterman,

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

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

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

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

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

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

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

Sincerely,

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

Reviewer #1 (Remarks to the Author):

Wayt et al show that depletion of rho-kinase 2 (ROCK2), but not ROCK1, inhibits internalization of two known clathrin independent endocytosis (CIE) cargos, MHCI and CD59 (a GPI-anchored protein), in HeLa cells. Surprisingly, the defect is due to a differential decrease in the activity of myosin IIA (MHCI) and myosin IIB (CD59) resulting from a reduction in regulatory light chain phosphorylation. The preferential internalization of MHCI by myosin IIA and CD59 by myosin IIB could be simply due to their different subcellular localizations. This was examined using a polarized epithelium of Caco2 cells where the two myosins exhibit differential localization either at the apical (myosin IIB) or basolateral (myosin IIA) region. Depletion of myosin IIB results in reduced uptake of CD59 from the apical region that is accompanied by a decrease in apical tension. Depletion of myosin IIA reduces MHCI uptake from that basolateral region. Since sites of internalized cargo are not specifically localized with either myosin II isoform, the authors conclude that general myosin II contractility, localized to specific regions of the cell, plays a significant role in CIE.

The experiments presented here are straightforward and the results provide strong evidence for the role of myosin II activity in clathrin-independent endocytosis. Demonstrating this in two different cell types, one of which has differential localization of the two different myosins supports a general contractile role for this motor and is consistent with the lack of a direct link between internalized cargo and myosin II. While the overall conclusion is quite interesting and perhaps even unexpected,

it is challenging to discern that actin is directly associated with the endocytic sites from the images provided (e.g. Fig 2H, 3D). If it is present, as would be consistent with other work in the field (recently discussed in Shafaq-Zadah et al, 2020, COCB), it is still somewhat difficult to envision how forces generated by myosin II are driving the internalization events. This is not an easy question to address but readers could benefit from the authors' informed discussion on how they consider myosin II contractility might play a role in efficient CIE.

Major comments -

The authors show that depletion of ROCK2 inhibits both the internalization of MHCI and CD59 (Fig 1B). In contrast, ROCK1 depletion has little impact on internalization of MHCI but actually increases CD59 uptake. Although it is stated (pg 5) that this is 'likely due to compensatory ROCK2' as they state that reduction of ROCK1 resulted in 'some compensatory upregulation of ROCK2'. However, the data presented in Fig 1A do not support this. While the bar graph shows a higher level of ROCK2, the increase is not statistically significant so it is difficult to attribute the enhanced uptake of CD59 to increased expression of ROCK2. It is also odd that the effect would then be so selective for CD59.

Depletion of either myosin IIA or myosin IIB from Caco2 cells is said to not perturb the morphology of the epithelium (pg 9). This seems a bit unexpected given the role of myosin II in junctional adhesion and also because the authors find a reduction in apical tension in the myosin IIB depleted cells (Fig 4I). CIE is implicated in apicobasal polarity and one might anticipate that this could be perturbed if this pathway is inhibited. Have the authors examined markers of epithelial morphology other than actin that might reveal disruption of the integrity of the apical versus basolateral compartments? There are at least three different modes of CIE (fast endophilin-mediated endocytosis, via clathrin-independent carriers and glycolipid-lectin) and actin has been implicated in each. Which pathway plays a role in MHCI internalization? Do all three rely on myosin contractility in HeLa cells?

The steady state localization of MHCI or CD59 is not perturbed but there is a significant decrease in uptake of CD59 when myosin IIA is depleted and MHCI when myosin IIB is depleted (Fig 4E H). Is the overall steady-state level of each endocytic markers different in the siRNA-treated cells at the terminal time point of incubation?

Minor comments -

While the field has unfortunately lapsed into using the designation MLC (myosin light chain) to refer to the target of ROCK activity (either via inhibition of myosin phosphatase activity or direct phosphorylation of the phosphorylation by ROCK and other kinases), the designation is incomplete and ambiguous as there are two distinct MLCs, regulatory and essential. The authors are strongly encouraged to correctly refer to the target of ROCK as the myosin II regulatory light chain (RLC) both in the text and figures.

The number of biological replicates and number of samples should be indicated in the figure legend.

Reviewer #2 (Remarks to the Author):

Wayt et al

In this manuscript, the authors begin to explore the mechanisms of clathrin-independent endocytosis (CIE). This form of endocytosis has been known for a number of years, but not very well understood. They identify a role for Rock2 through an siRNA screen and go on to show that the downstream targets of Rock, myosin and cofilin, are somehow involved in CIE. Although the work presented is technically sound, the story is really too preliminary and poorly developed to be all that interesting at this stage. They come to the relatively unsatisfying conclusion that somehow myosin contractility is involved in CIE, but they do not explore any possible mechanisms for this effect. Thus, my enthusiasm for this work is limited.

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Carole Parent
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Parent,

Enclosed please find our revised version of the manuscript "**Myosin II isoforms promote internalization of spatially distinct clathrin-independent endocytosis cargoes through modulation of cortical tension downstream of ROCK2.**" (Manuscript Number: E20-07-0480) that we are resubmitting for consideration for publication as a Brief Report in *Molecular Biology of the Cell*.

We were encouraged by the positive response of the reviewers to our initial submission, and have now added new figure panels and made changes to the manuscript text that speak to the important concerns raised by the reviewers.

One of the major concerns raised by both reviewers was the lack of mechanistic insight regarding how myosin II contractility regulates CIE. To speak to this concern, we have added additional text to the discussion where we speculate how this phenomenon might occur. Briefly, we think that myosin II plays a major role in maintaining membrane tension through the interaction between the actin cytoskeleton and the plasma membrane. Maintaining membrane tension homeostasis is essential for efficient CIE to progress, perhaps by providing a conducive environment for pit formation to occur. Too little membrane tension might favor fewer smaller pits (hence a decrease in CIE) because there isn't enough membrane tension to anchor and counterbalance the newly formed pit. An increase in membrane tension might favor increased pit formation (which we see in cells with increased myosin II activation, as in the case of cofilin silenced cells), possibly through larger pit formation.

A second major concern was whether silencing of myosin II in polarized epithelial cells resulted in altered epithelial morphology, especially in regards to maintaining apicobasal polarity. To address this issue, we have created 3 new figure panels. In Supplemental Figure 3A, we label the endogenous steady state localization of MHCI and CD59 in polarized caco-2 cells depleted of myosin IIA or IIB by siRNA, and show that the steady state localization of these CIE cargoes is unchanged by myosin II isoform knockdown. To further demonstrate that apicobasal polarity is unchanged, in Supplemental Figure 3B we look at the localization of the apical microvillar protein ezrin. The localization of this apical protein is also unchanged in myosin II isoform knockdown cells, which we feel demonstrates that apicobasal polarity is maintained following the loss of either myosin II protein. In spite of these evidences of maintenance of polarity, we do document that myosin II knockdown does in fact have some effect on epithelial morphology. As shown in Supplemental Figure 3C, we measured the epithelial height in myosin II isoform-silenced cells and show that loss of myosin IIA induces a decrease in overall height of the monolayer.

These major revisions, along with clarifications and corrections to figures and the text have substantially improved the manuscript and strengthened our previous conclusions. We are grateful to the reviewers for this feedback, and we hope that our responses to their concerns, which are detailed point-by-point in the following pages, alleviate their concerns and make the manuscript acceptable for publication in Molecular Biology of the Cell.

Sincere Regards,

Clare M. Waterman, PhD

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Reviewer #1 (Remarks to the Author):

Wayt et al show that depletion of rho-kinase 2 (ROCK2), but not ROCK1, inhibits internalization of two known clathrin independent endocytosis (CIE) cargos, MHCI and CD59 (a GPI-anchored protein), in HeLa cells. Surprisingly, the defect is due to a differential decrease in the activity of myosin IIA (MHCI) and myosin IIB (CD59) resulting from a reduction in regulatory light chain phosphorylation. The preferential internalization of MHCI by myosin IIA and CD59 by myosin IIB could be simply due to their different subcellular localizations. This was examined using a polarized epithelium of Caco2 cells where the two myosins exhibit differential localization either at the apical (myosin IIB) or basolateral (myosin IIA) region. Depletion of myosin IIB results in reduced uptake of CD59 from the apical region that is accompanied by a decrease in apical tension. Depletion of myosin IIA reduces MHCI uptake from that basolateral region. Since sites of internalized cargo are not specifically localized with either myosin II isoform, the authors conclude that general myosin II contractility, localized to specific regions of the cell, plays a significant role in CIE.

We respectfully offer to correct this kind reviewer's summary of our work. The reviewer notes that:

> *"…polarized epithelium of Caco2 cells where the two myosins exhibit differential localization either at the apical (myosin IIB) or basolateral (myosin IIA) region. Depletion of myosin IIB results in reduced uptake of CD59 from the apical region that is accompanied by a decrease in apical tension. Depletion of myosin IIA reduces MHCI uptake from that basolateral region."*

Our results are unfortunately not so straightforward. We found that in polarized Caco2 cells, myosin IIA is localized apically and at the basal cortex, and is required for the basolateral internalization of MHCI, and myosin IIB is localized to apical cell junctions and the basal cortex and is required for the apical internalization of CD59. We have included the following summary statement in the manuscript to hopefully make this more clear to the reader:

> *"Thus, in polarized intestinal epithelial cells, myosin IIA localizes at the basal cortex and apical brush border and mediates basolateral internalization of MHCI, while myosin IIB localizes to the basal cortex and apical cell-cell junctions and promotes CD59 uptake from the apical membrane."*

The experiments presented here are straightforward and the results provide strong evidence for the role of myosin II activity in clathrin-independent endocytosis. Demonstrating this in two different cell types, one of which has differential localization of the two different myosins supports a general contractile role for this motor and is consistent with the lack of a direct link between internalized cargo and myosin II. While the overall conclusion is quite interesting and perhaps even unexpected, it is challenging to discern that actin is directly associated with the endocytic sites from the images provided (e.g. Fig 2H, 3D). **If it is present, as would be consistent with other work in the field (recently discussed in Shafaq-Zadah et al, 2020, COCB), it is still somewhat difficult to envision how forces generated by myosin II are driving the internalization events. This is not an easy question to address but readers could benefit from the authors' informed discussion on how they consider myosin II contractility might play a role in efficient CIE.**

We have now included a more in-depth discussion regarding how we believe myosin II contractility might play a role in efficient CIE in the last section of the manuscript. The text changes are bolded in the manuscript for ease of reading and quoted here:

> *"Our results support the contention that myosin II-mediated regulation of cortical tension, through interaction between the actin cytoskeleton and the plasma membrane, may be critical for efficient CIE. Decreased membrane tension has been shown to increase endocytic events in cases where actin polymerization (macropinocytosis, (Loh et al., 2019)) or coat proteins (CME, (Saleem et al., 2015)) are recruited to endocytic sites. As CIE has no clear requirement for either, it is not unreasonable to assume that lateral tension generated from myosin II contractility planar to the forming endocytic pit plays a large role in efficient CIE pit formation. When membrane tension is decreased, perhaps there is insufficient tension surrounding the pit to anchor the invaginating plasma membrane, and thus an endocytic pit cannot be efficiently formed. Further, the observation that endocytosis of CIE cargoes is stimulated in the case of excess NMII activation (in cofilin-silenced cells) would also suggest that balanced tension generated from NMII contractility is required for normal CIE. In support of this, the influence of NMII contractility on the endocytosis of CME cargoes has been observed by others (Chandrasekar et al., 2014). Although we cannot rule out other possible roles for myosin II in cortical cytoskeleton organization, which may be required for nano-clustering of plasma membrane proteins (Goswami et al., 2008), or vesicle fission through the*

recruitment of membrane bending proteins (Galic et al., 2012), these possibilities remain to be tested in the future."

Major comments -

(1) The authors show that depletion of ROCK2 inhibits both the internalization of MHCI and CD59 (Fig 1B). In contrast, ROCK1 depletion has little impact on internalization of MHCI but actually increases CD59 uptake. Although it is stated (pg 5) that this is 'likely due to compensatory ROCK2' as they state that reduction of ROCK1 resulted in 'some compensatory upregulation of ROCK2'. However, the data presented in Fig 1A do not support this. While the bar graph shows a higher level of ROCK2, the increase is not statistically significant so it is difficult to attribute the enhanced uptake of CD59 to increased expression of ROCK2. It is also odd that the effect would then be so selective for CD59.

We agree that the increase in ROCK2 expression upon ROCK1 knockdown is not statistically significant and have altered the language of the text to reflect this. We have instead changed the text to emphasize that there is a significant increase in activated myosin II, as measured by an increase in phosphorylated myosin light chain (pRLC) in ROCK1 knockdown cells (Fig2A) and it is this increase in myosin II activation that is promoting increased uptake of both MHCI and CD59. This explanation is also more in line with what we observe in cofilin silenced cells (Fig 2J), in which increased internalization of cargo is actually promoted by increased myosin II activation. The relevant text is as follows:

"…In contrast, silencing ROCK1 did not inhibit uptake, and in fact stimulated internalization of both cargoes (Fig 1B)."

And

"…Western blot analysis showed that ROCK2 silencing or pharmacological ROCK inhibition decreased the levels of phosphorylated RLC (pRLC) and cofilin (pCFL) phosphorylation. In contrast, silencing ROCK1 had no significant effect on pCFL, and mildly increased pRLC, ***which might explain the increase in cargo uptake caused by silencing ROCK1*** *(Fig 2A, B)."*

(2) Depletion of either myosin IIA or myosin IIB from Caco2 cells is said to not perturb the morphology of the epithelium (pg 9). This seems a bit unexpected given the role of myosin II in junctional adhesion and also because the authors find a reduction in apical tension in the myosin IIB depleted cells (Fig 4I). CIE is implicated in apicobasal polarity and one might anticipate that this could be perturbed if this pathway is inhibited. Have the authors examined markers of epithelial morphology other than actin that might reveal disruption of the integrity of the apical versus basolateral compartments?

While we have demonstrated that tight and adhesion junctions are intact following depletion of myosin IIA and IIB (Supp Fig 2G), to address changes to overall epithelial morphology we have created a new supplemental figure (Supp 3). To ensure that apico-basal polarity is unchanged we visualized the steady state localization of our CIE cargo proteins (Supp Fig 3A) in myosin II isoform-silenced polarized caco-2 cells and show that their steady state distributions are unchanged in either knockdown condition. As GPI anchor proteins, like

CD59, have been demonstrated to be exclusively apically localized, we feel that this is a suitable marker for apicobasal polarity. To bolster this contention, we have also looked at the steady state localization of the apically localized microvillar protein ezrin, which is also unchanged in either knockdown condition (Supp Fig 3B). We feel that these two panels address the justified concern regarding apicobasal polarity in myosin II knockdown cells.

The concern about morphology led us to look closer at other phenotypes produced by myosin isoform knockdown in our polarized epithelial cells. In spite of our evidence described above of maintenance of polarity, we do find that myosin II knockdown has some effect on epithelial morphology which is shown in Supplemental Figure 3C, where we measure the epithelial height in myosin II isoform-silenced cells and show that loss of myosin IIA induces a decrease in overall epithelial height.

. We have addressed these new findings in the text as follows:

> *"Myosin IIA or IIB silencing substantially diminished protein levels in confluent polarized monolayers by 92 h post-transfection (Fig 4G, Supp Fig 2D), with minimal perturbation of epithelial morphology (Supp Fig 2F,G),* ***steady state localization of cargo (Supp Fig 3A) or apico-basal polarity (Supp Fig 3B). However, inhibition of myosin IIA did result in a slight decrease in epithelial height (Supp Fig 3C)****."*

(3)There are at least three different modes of CIE (fast endophilin-mediated endocytosis, via clathrin-independent carriers and glycolipid-lectin) and actin has been implicated in each. Which pathway plays a role in MHCI internalization? Do all three rely on myosin contractility in HeLa cells?

MHCI and CD59 are classically defined as Arf6-associated CIE cargo proteins, although GPI-anchored proteins like CD59 have also been described to participate in other CIE pathways in a cell-type dependent manner (Kalia *et al.*, 2006)*. To the best of our knowledge, myosin II contractility is not required for these other modes of CIE. We would like to emphasize that we concentrate on cargo that simply enter the cell via a pathway that does not require clathrin. We are focused on describing specific requirements for entry of distinct* ***cargo*** *and not specific* ***pathway*** *requirements. We thus feel that the question of the specific mode of CIE that is mediated by myosin II contractility is outside the scope of the current Brief Report.*

(4) The steady state localization of MHCI or CD59 is not perturbed but there is a significant decrease in uptake of CD59 when myosin IIA is depleted and MHCI when myosin IIB is depleted (Fig 4E H). Is the overall steady-state level of each endocytic markers different in the siRNA-treated cells at the terminal time point of incubation?

We interpret this question as either a question about the surface levels of cargo after internalization or as a question regarding potential defects in recycling of cargo back to the plasma membrane in Caco2 cells. In regards to the surface levels following antibody internalization, experimental limitations make it so that we are unable to look at surface protein

following antibody internalization. The antibodies we use for internalization are mouse monoclonal antibodies and we don't have a suitable second antibody raised to cargo in a different species which would allow visualization of the protein remaining at the surface following our antibody internalization assay.

Attempting to dissect a recycling defect in polarized epithelial monolayers grown on transwell filters presents another technically difficult challenge, as the assay is already challenging in dishes (acid washing, resting, and visualizing antibody return to the surface), the added layer of using filters (which are already difficult to efficiently acid strip) makes this technically exceptionally difficult if not impossible. Because of this, we feel that while this question is very interesting, it is outside the scope of the current study.

Minor comments -

(1)While the field has unfortunately lapsed into using the designation MLC (myosin light chain) to refer to the target of ROCK activity (either via inhibition of myosin phosphatase activity or direct phosphorylation of the phosphorylation by ROCK and other kinases), the designation is incomplete and ambiguous as there are two distinct MLCs, regulatory and essential. The authors are strongly encouraged to correctly refer to the target of ROCK as the myosin II regulatory light chain (RLC) both in the text and figures.

We appreciate this suggestion and have changed all references of "MLC" to "RLC" in both the text and the figures.

(2)The number of biological replicates and number of samples should be indicated in the figure legend.

We have now included the number of biological replicates and number of samples in the figures and figure legends.

Reviewer #2 (Remarks to the Author):

Wayt et al

In this manuscript, the authors begin to explore the mechanisms of clathrin-independent endocytosis (CIE). This form of endocytosis has been known for a number of years, but not very well understood. They identify a role for Rock2 through an siRNA screen and go on to show that the downstream targets of Rock, myosin and cofilin, are somehow involved in CIE. Although the work presented is technically sound, the story is really too preliminary and poorly developed to be all that interesting at this stage. They come to the relatively unsatisfying conclusion that somehow myosin contractility is involved in CIE, but they do not explore any possible mechanisms for this effect. Thus, my enthusiasm for this work is limited.

As noted above, we have now included in the manuscript a more in-depth discussion regarding how we envision the mechanism by which myosin II contractility might play a role in efficient CIE. The text changes are bolded in the manuscript for ease of reading and quoted here:

> *"Our results support the contention that myosin II-mediated regulation of cortical tension, through interaction between the actin cytoskeleton and the plasma membrane, may be critical for efficient CIE. Decreased membrane tension has been shown to increase endocytic events in cases where actin polymerization (macropinocytosis, (Loh et al., 2019)) or coat proteins (CME, (Saleem et al., 2015)) are recruited to endocytic sites. As CIE has no clear requirement for either, it is not unreasonable to assume that lateral tension generated from myosin II contractility planar to the forming endocytic pit plays a large role in efficient CIE pit formation. When membrane tension is decreased, perhaps there is insufficient tension surrounding the pit to anchor the invaginating plasma membrane, and thus an endocytic pit cannot be efficiently formed. Further, the observation that endocytosis of CIE cargoes is stimulated in the case of excess NMII activation (in cofilin-silenced cells) would also suggest that balanced tension generated from NMII contractility is required for normal CIE. In support of this, the influence of NMII contractility on the endocytosis of CME cargoes has been observed by others (Chandrasekar et al., 2014). Although we cannot rule out other possible roles for myosin II in cortical cytoskeleton organization, which may be required for nano-clustering of plasma membrane proteins (Goswami et al., 2008), or vesicle fission through the recruitment of membrane bending proteins (Galic et al., 2012), these possibilities remain to be tested in the future."*

References

Chandrasekar, I, Goeckeler, ZM, Turney, SG, Wang, P, Wysolmerski, RB, Adelstein, RS, and Bridgman, PC (2014). Nonmuscle myosin II is a critical regulator of clathrin-mediated endocytosis. Traffic 15, 418–432.

Galic, M, Jeong, S, Tsai, FC, Joubert, LM, Wu, YI, Hahn, KM, Cui, Y, and Meyer, T (2012). External push and internal pull forces recruit curvature-sensing N-BAR domain proteins to the plasma membrane. Nat Cell Biol 14, 874–881.

Goswami, D, Gowrishankar, K, Bilgrami, S, Ghosh, S, Raghupathy, R, Chadda, R, Vishwakarma, R, Rao, M, and Mayor, S (2008). Nanoclusters of GPI-Anchored Proteins Are Formed by Cortical Actin-Driven Activity. Cell 135, 1085–1097.

Kalia, M, Kumari, S, Chadda, R, Hill, MM, Parton, RG, and Mayor, S (2006). Arf6-independent GPI-anchored protein-enriched early endosomal compartments fuse with sorting endosomes via a Rab5/phosphatidylinositol-3′-kinase- dependent machinery. Mol Biol Cell 17, 3689–3704.

Loh, J, Chuang, MC, Lin, SS, Joseph, J, Su, YA, Hsieh, TL, Chang, YC, Liu, AP, and Liu, YW (2019). An acute decrease in plasma membrane tension induces macropinocytosis via PLD2 activation. J Cell Sci 132.

Saleem, M, Morlot, S, Hohendahl, A, Manzi, J, Lenz, M, and Roux, A (2015). A balance between membrane elasticity and polymerization energy sets the shape of spherical clathrin coats. Nat Commun 6, 1–10.

RE: Manuscript #E20-07-0480R
TITLE: "Myosin II isoforms promote internalization of spatially distinct clathrin-independent endocytosis cargoes through modulation of cortical tension downstream of ROCK2"

Dear Dr. Waterman:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Congratulations!

Sincerely,
Carole Parent
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Waterman:

Congratulations on the acceptance of your manuscript.

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

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

Sincerely,

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

Reviewer #1 (Remarks to the Author):

The authors have addressed the specific points raised in the previous review. Most importantly they show that general epithelial integrity is not substantially altered upon depletion of either myosin IIA or myosin IIB. They also now suggest in the Discussion that inhibition of CIE of a specific cargo could be attributed to a reduction of membrane tension in different regions of the epithelial cell (i.e. apical vs basolateral) generated by a particular myosin.

The authors make the point that MHC I and CD59 are regarded simply as 'cargo' for the purposes of this study so the differential reduction in CIE of either cargo would seem to make general sense in the context of myosin IIA and B localization in epithelial cells. However, it remains difficult to reconcile the observed specificity of uptake inhibition in Hela cells with the observed overlapping localization of myosin IIA and myosin IIB.

Comment: A number of studies implicate myosin IIA in membrane tension in mammalian cells, is there any evidence that myosin IIB contributes as well? If so, it might be worth referring to it in support of a role for myosin IIB in CIE in Hela cells.