Strain induced mechanoresponse depends on cell contractility and BAG3-mediated autophagy

Lukas Loevenich, Georg Dreissen, Christina Hoffmann, Jens Konrad, Ronald Springer, Joerg Hoehfeld, Rudolf Merkel, and Bernd Hoffmann

Corresponding author(s): Bernd Hoffmann, Forschungszentrum Jülich GmbH

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(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 #E21-05-0254
TITLE: Strain induced mechanoresponse depends on cell contractility and BAG3-mediated autophagy

Dear Dr. Hoffmann:

Thank you very much for submitting to ASCB's scientific journal, MBoC.
Two society member reviewers with deep expertise in key aspects of your studies have provided comments that indicate enthusiasm for the work but also a need for revision. Please provide a detailed point-by-point response and make sure that each response is reflected in clearly indicated changes to the text or figures. We look forward to receiving a responsive revision from you and your colleagues.

Respectfully,

Dennis Discher
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Hoffmann,

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).

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To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

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):

Strain induced mechanoresponse depends on cell contractility and BAG3-mediated autophagy
Lovenich et al.

This is an interesting paper that raises important questions about the relationship between cytoskeletal and chaperone machineries, and between processes of force-mediated protein conformational change as means of mechanosensing and protein turnover. The manuscript is generally clearly written and succinct, and accompanying figures are of high quality. I think the data and concepts discussed in the paper will spark interest and discussion in the community, and I am therefore in favour of publication. I would however recommend address of the following points:

Introduction:
+ It's not clear why mechanosensitivity is stressed as being established 'at the single cell level'. Mechanical stimuli in most tissues (as described in this paragraph) propagate through cell-cell and cell-matrix interactions; cell-cell interactions are broadly studied, for example, in development.
+ The transition to the second paragraph is not smooth. Is cellular reorientation with respect to strain a universal phenomenon? Is it observed in all cell types? What about in monolayers of cells, or in three-dimensions? How/why does reorientation occur (e.g. to minimize the strain across individual cells)? Moreover, does it occur in tissues (e.g. heart and muscle, as described in the previous paragraph)?
+ A range of mechanosensing mechanisms have been identified (e.g. ion channels and protein conformational changes are mentioned here), but I don't think they are mutually exclusive or under dispute... I'm not sure where 'controversy' would arise.
+ 'Since mechanosensing primarily defines [...] high mechanical load.' - I don't follow the logic or evidence for this statement.
+ '[...] cell-matrix, i.e. focal adhesions [...]' - although there are force-responsive proteins in the extracellular matrix (ECM), should this statement refer to the 'cell-matrix interface'? A clear definition of the FA would be helpful, as it is discussed later. Are its components the same as the 'consensus integrin adhesome'?
+ Expand on the significance of 'dual-site phosphorylation'?
+ The last paragraph, summarising experimental results, doesn't clearly define what features of 'cell stress' or 'mechanoresponse' are being examined. Perhaps this could be linked to the discussion of cell orientation?

Results/Data:
+ More detail could be given on the cyclic stretch regime. Although the stiffness of the substrate, frequency and strain are given in the methods section, there are no references in support of these parameters representing a particular physiology.
+ Data should generally be described in the context of where significance can be demonstrated e.g. when referring to 'massively increased' numbers of LC3B spots in the first paragraph. Can quantitative analysis be performed on the images in Fig. 1? Fig. 2 contains plots of image quantification and statistical analysis, but this is not referred to in the text.
+ A little more explanation could be given on how the LC3B assay works. The marker is presumably not being expressed in response to autophagy, but rather is being localised into spots that can be counted. Is the total GFP signal, integrated across the entire cell, therefore constant throughout the duration of the experiment, or is the marker also turned over? And should this affect the interpretation of the longer strain-cycle experiments?
+ Although I am not keen to suggest additional experiments as a requirement for publication (particularly under the current circumstances), it would be interesting to see some data on the dynamics of the recovery process. As a control, do the unstrained cells behave the same at t = 0 as at t = 6 hours? And do the cells return to their original states after different durations of strain, or does remodelling persist? Maintained correlation between orientation and autophagy machinery as cells re-equilibrate to an absence of cyclic strain would strengthen the correlative arguments that the authors make.

Reviewer #2 (Remarks to the Author):

This manuscript reports that the process of strain-induced reorientation of the actin cytoskeleton, typically observed in cells exposed to uniaxial strain, is sensitive to the formation of autophagosomes and the co-chaperone BAG3. To test the role of autophagosome formation for the reorientation response of cells, the authors expressed a GFP-tagged version of the autophagy-marker LC3B and exposed smooth muscle cells (A7r5) and murine embryonic fibroblasts (MEFs) to mechanical straining using elastomeric silicone rubber substrates. The experiments show that A7r5 cells and MEFs increase autophagosome formation upon stretch. Blocking the autophagy pathway with chloroquine impairs actin reorientation in A7r5 cells, whereas MEFs appear rather insensitive to chloroquine treatment. However, inducing RhoA-activity by LPA treatment leads to a more pronounced reorientation response in MEFs, which is then sensitive to autophagy inhibition by chloroquine. Finally, the authors show that inhibition of the previously described CASA-pathway by overexpression of a dominant negative BAG3 (BAG3-WAWA) impairs cellular reorientation in A7r5 cells.
Overall, this study describes an interesting phenomenon, namely a critical role of autophagy during the reorientation response upon mechanical stretch. The major limitation is the rather descriptive nature of the experiments, which do not provide details on the underlying molecular mechanisms. Still, the study strikes me as a worthwhile addition to the small but growing list of studies demonstrating a functional link between cell mechanics and autophagy. I therefore wish to recommend publication with revisions. Please find more detailed comments below.

Data presentation:

a) The authors start by comparing autophagosome formation in A7r5 cells, MEFs, and murine keratinocytes. The experiments show that A7r5 cells and MEFs display autophagosomes (upon GFP-LC3B transfection), whereas keratinocytes appear to lack these structures under steady-state conditions. I find this to be an interesting observation but, unfortunately, keratinocytes are not mentioned any further in the manuscript. I think the authors should either perform additional experiments with keratinocytes to determine whether they induce autophagy upon straining (biologically interesting because those cells have a prominent keratin network and should handle external strain differently). Alternatively, the authors should delete the keratinocyte finding from Fig. 1A. What's the point in showing the steady-state condition only.
b) The authors observe a strong nuclear GFP-LC3B staining in A7r5 cells, but not in MEFs (Fig. 1A). Is this a representative image? Furthermore, the scale bars for A7r5 cells and MEFs are very different in Fig. 1A; MEFs seem to be much smaller compared to A7r5. However, in Fig. 1B the cell size seems to be about the same. Can the authors check whether they got the scales bars right, or find more representative images of the here used cell lines?
c) The provided data seem to indicate that autophagosomes often form in close proximity (e.g. Fig. 2C, 10min), which will be challenging to quantify. Independent reproduction of the data will require a detailed understanding of how this particular analysis was conducted. Thus, the authors, in addition to their section in the 'materials and methods' (LC3 spot quantification), should provide a workflow describing this particular analysis. This workflow should indicate how complex signals (e.g. from cells as shown in Fig. 2C, 10min) can be isolated and whether there are important exclusion criteria? In addition, it should be described in detail how Z-stacks were acquired (e.g. how many optical sections were used per cell). Finally, I recommend to present the data (Fig. 2B, Fig. 2D, Fig. 6B) in some other form than a bar graph, so individual data points and the distribution of the data are discernible.
d) Even though the authors have published much of the here used methodology, it would be helpful if experimental conditions and analyses were described in more detail in the main text. For instance, please indicate which stretch amplitude and frequency were used for straining directly in the main text. Please also describe shortly in the main text how the actin fiber reorientation was analyzed and quantified.

Biological interpretation:
e) The authors state in the abstract that "strain-induced cell reorientation is massively delayed upon inhibition of autophagy". It seems that this statement is inconsistent with the observation that MEFs have a rather quick autophagy response (Fig. 2C, D) but still undergo reorientation in the presence of chloroquine (Fig. 4). It would be helpful if the authors could discuss this aspect in more detail, because cells (MEFs) can obviously reorient without a functional autophagy pathway, albeit not quite as efficiently as A7r5 cells.
f) The authors show that elevating cellular contractility increases the sensitivity for autophagy during cell reorientation. However, 2D culture and high substrate stiffness often correlate with enhanced myosin activation and the authors here use comparably stiff (50 kPa) 2D matrices. Would it be possible to use substrates that are softer (e.g. 1-10 kPa) to demonstrate that the effect does also occur on physiologically relevant stiffnesses? If this is technically not possible, I suggest that the authors discuss this issue. The observed effects may be still relevant under conditions of pathologically increased matrix stiffness (fibrosis, cancer, etc).

Manuscript Lövenich et al. rebuttal letter

Dear editor, dear reviewers,
We thank for the very helpful comments and experimental suggestions that further improved our manuscript. In a step-by-step commentary, we have addressed all aspects in this rebuttal letter and also changed the manuscript accordingly. For better visibility, all manuscript adjustments are also indicated in tracking mode in the uploaded PDF version.

Reviewer 1:

This is an interesting paper that raises important questions about the relationship between cytoskeletal and chaperone machineries, and between processes of force-mediated protein conformational change as means of mechanosensing and protein turnover. The manuscript is generally clearly written and succinct, and accompanying figures are of high quality. I think the data and concepts discussed in the paper will spark interest and discussion in the community, and I am therefore in favour of publication. I would however recommend address of the following points:

Introduction

+ It's not clear why mechanosensitivity is stressed as being established 'at the single cell level'. Mechanical stimuli in most tissues (as described in this paragraph) propagate through cell-cell and cell-matrix interactions; cell-cell interactions are broadly studied, for example, in development.

The reviewer is absolutely right. Our original intention simply was to refer to early publications that specifically characterized focal adhesions and actin stress fibers as mechanosensitive structures. Nevertheless, this restriction is misleading and we have changed it in the introduction.

+ The transition to the second paragraph is not smooth. Is cellular reorientation with respect to strain a universal phenomenon? Is it observed in all cell types? What about in monolayers of cells, or in three-dimensions? How/why does reorientation occur (e.g. to minimize the strain across individual cells)? Moreover, does it occur in tissues (e.g. heart and muscle, as described in the previous paragraph)?

Thanks a lot for this comment. We have now smoothened the transition from the first to the second paragraph. Furthermore we have included additional aspects regarding the reorientation process as requested by the reviewer in order to make the introduction more informative.

+ A range of mechanosensing mechanisms have been identified (e.g. ion channels and protein conformational changes are mentioned here), but I don't think they are mutually exclusive or under dispute... I'm not sure where 'controversy' would arise.

Very true! We have adapted the text accordingly.

+ 'Since mechanosensing primarily defines [...] high mechanical load.' - I don't follow the logic or evidence for this statement.

We may have expressed this aspect in an unclear manner in the manuscript. However, the basic statement behind it is very important for the principle of mechanosensitivity as well as for the understanding of our work. Crucial for a response to mechanical stimuli is their efficient detection. Underlying proteins must therefore be integrated in such a way that the corresponding stimulus also

reaches these proteins. In the case of stretch, structures that actually experience the mechanical stress are particularly suitable for its detection, while cytoplasmic proteins or those with only a single binding site would not well function as mechanosensors for strain.

However, the reviewer is certainly correct that such structures do not necessarily have to experience a "high" mechanical load upon elongation, as they must primarily exhibit sensitivity in the case of a protective mechanism. We have therefore adapted the corresponding text passage and hope to have clarified this misunderstanding.

+ '[...] cell-matrix, i.e. focal adhesions [...]' - although there are force-responsive proteins in the extracellular matrix (ECM), should this statement refer to the 'cell-matrix interface'? A clear definition of the FA would be helpful, as it is discussed later. Are its components the same as the 'consensus integrin adhesome'?

We fully agree with the reviewer that also ECM molecules as e.g. fibronectin can function as mechnosensors or at least are force-responsive elements and we are not trying to exclude them from the still unknown molecular architecture of the identified interaction between autophagy and mechanoresponse. However, since autophagosomes are formed inside the cell we try to keep it simple at this stage and do not mention ECM molecules. We have clarified this aspect in the text.

For the same simplicity reasons we had also mentioned the consensus integrin adhesome which is certainly not identical to and just a small fraction of the complete integrin adhesome characterized by Benny Geiger and coworkers in various publications with more than 200 reported components. Further experiments by e.g. mass spectrometry will help to identify the underlying mechanism connecting mechanosensitivity and autophagy. However, to avoid confusion, we have now clarified the text and talk only about the complete “integrin adhesome”.

+ Expand on the significance of 'dual-site phosphorylation'?

Since there is no added value for understanding our data on the fact that filamin-C degradation is regulated by dual-site phosphorylation, we decided not to expand this aspect in the introduction. Instead we just say “by phosphorylation”.

+ The last paragraph, summarising experimental results, doesn't clearly define what features of 'cell stress' or 'mechanoresponse' are being examined. Perhaps this could be linked to the discussion of cell orientation?

We are sorry for this unclear definition. We modified the text accordingly.

Results/Data:
+ More detail could be given on the cyclic stretch regime. Although the stiffness of the substrate, frequency and strain are given in the methods section, there are no references in support of these parameters representing a particular physiology.

Mimicking physiological conditions is certainly of great importance. For smooth muscle cells amplitudes of 20% strain with every heart beat are described. We have therefore chosen this amplitude and included the respective references in the material and methods section. As frequency we decided to stay slightly below the human heart rate at rest to prevent medium flow in the stretching chambers which would have caused inhomogeneous cell reorientation within the same

sample due to strain- and flow-induced cell orientation. At our chosen frequency of 300 mHz all cells within a chamber respond the same and are therefore exclusively responding to strain. This information is now also included in the same paragraph of the manuscript.

+ Data should generally be described in the context of where significance can be demonstrated e.g. when referring to 'massively increased' numbers of LC3B spots in the first paragraph. Can quantitative analysis be performed on the images in Fig. 1? Fig. 2 contains plots of image quantification and statistical analysis, but this is not referred to in the text.

Figure 1 is intended solely to provide an overview of the induction of autophagosome formation under stretch and to show that autophagosomes are subject to high turnover (blocking by CQ). The quantitative evaluation of such images, including additional time points, has then been performed and is shown in Figure 2. Therefore we refrained from showing the same evaluations again in Figure 1. The only result that was not quantitatively analyzed was the CQ block experiment. This has now been made up and is incorporated in the results section.

Incidentally, we of course fully agree with the reviewer that only effects with real significance can be shown as such. In order to illustrate the significance levels not only in the figures, we have now also modified the text to show this more clearly.

+ A little more explanation could be given on how the LC3B assay works. The marker is presumably not being expressed in response to autophagy, but rather is being localised into spots that can be counted. Is the total GFP signal, integrated across the entire cell, therefore constant throughout the duration of the experiment, or is the marker also turned over? And should this affect the interpretation of the longer strain-cycle experiments?

We thank the reviewer for this question since we obviously did not clarify well enough how we performed the LC3B assay. As assumed by the reviewer, using GFP as marker for autophagosomes in principle results identical results compared to immunocytochemical staining of LC3B. This is also indicated in Figure 1A (LC3B-GFP) and Figure 1B (IF staining of LC3B). Since use of GFP as marker over time was difficult, all quantifications have been performed by IF stainings at indicated time points. Possible GFP bleaching artifacts or turnover of the marker did therefore not occur even for longer stretching times. Since we obviously did not made ourselves clear enough we have now included this information in the first paragraph of the results section as well as in the figure legend of Fig. 1.

+ Although I am not keen to suggest additional experiments as a requirement for publication (particularly under the current circumstances), it would be interesting to see some data on the dynamics of the recovery process. As a control, do the unstrained cells behave the same at t = 0 as at t = 6 hours? And do the cells return to their original states after different durations of strain, or does remodelling persist? Maintained correlation between orientation and autophagy machinery as cells re-equilibrate to an absence of cyclic strain would strengthen the correlative arguments that the authors make. #1

The reviewer suggested two very interesting experiments and we were able to perform both of them. Results clearly show that cells are not “frozen” in the reoriented state. Instead, cells keep their dynamic abilities and return to a more unordered orientation distribution already within 4 hours after stretch. In a second experiment we tested cellular dynamics of reoriented cells after straining by rotating the chamber by 90° and induction of cyclic straining for different times of up to 4 h. This experiment therefore started with cells almost homogeneously directed in strain direction due to a

reorientation process that had just ended. Interestingly, cells fully kept their ability to respond to these changed mechanical conditions and reoriented again almost perpendicular to the new strain direction within 4 h. These data are now included in the results section. However, since the data sets are based on a low number of independent experiments and imply further very interesting experiments that would be sufficient of an additional manuscript, we are showing these data as new supplementary figure 2.

Reviewer 2

This manuscript reports that the process of strain-induced reorientation of the actin cytoskeleton, typically observed in cells exposed to uniaxial strain, is sensitive to the formation of autophagosomes and the co-chaperone BAG3. To test the role of autophagosome formation for the reorientation response of cells, the authors expressed a GFP-tagged version of the autophagy-marker LC3B and exposed smooth muscle cells (A7r5) and murine embryonic fibroblasts (MEFs) to mechanical straining using elastomeric silicone rubber substrates. The experiments show that A7r5 cells and MEFs increase autophagosome formation upon stretch. Blocking the autophagy pathway with chloroquine impairs actin reorientation in A7r5 cells, whereas MEFs appear rather insensitive to chloroquine treatment. However, inducing RhoA-activity by LPA treatment leads to a more pronounced reorientation response in MEFs, which is then sensitive to autophagy inhibition by chloroquine. Finally, the authors show that inhibition of the previously described CASA-pathway by overexpression of a dominant negative BAG3 (BAG3-WAWA) impairs cellular reorientation in A7r5 cells.

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Data presentation:

a) The authors start by comparing autophagosome formation in A7r5 cells, MEFs, and murine keratinocytes. The experiments show that A7r5 cells and MEFs display autophagosomes (upon GFP-LC3B transfection), whereas keratinocytes appear to lack these structures under steady-state conditions. I find this to be an interesting observation but, unfortunately, keratinocytes are not mentioned any further in the manuscript. I think the authors should either perform additional experiments with keratinocytes to determine whether they induce autophagy upon straining (biologically interesting because those cells have a prominent keratin network and should handle external strain differently). Alternatively, the authors should delete the keratinocyte finding from Fig. 1A. What's the point in showing the steady-state condition only.

We thank the reviewer for this helpful comment. Based on the hypothesis that stably adhered stress fibers are important for autophagosome formation under strain we have analyzed keratinocytes as highly dynamic cell type and could see, indeed, that this cell type was not visible forming autophagosomes. However, the reviewer is absolutely right that due to the fact that we did not analyze keratinocytes further in all subsequent experiments, this information is kind of lost and not well supported. We have therefore removed the keratinocyte experiment completely from this manuscript.

b) The authors observe a strong nuclear GFP-LC3B staining in A7r5 cells, but not in MEFs (Fig. 1A). Is

this a representative image? Furthermore, the scale bars for A7r5 cells and MEFs are very different in Fig. 1A; MEFs seem to be much smaller compared to A7r5. However, in Fig. 1B the cell size seems to be about the same. Can the authors check whether they got the scales bars right, or find more representative images of the here used cell lines?

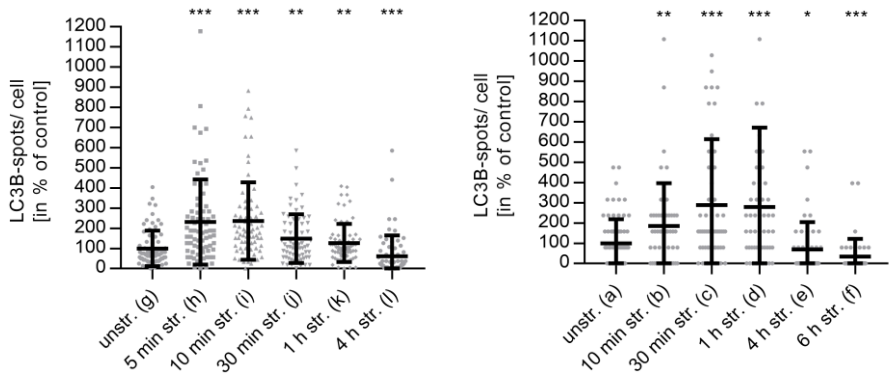
What you see in Figure 1A is a signal of GFP-LC3B transiently transfected cells. Surprisingly, in all A7r5 cells transfection caused high nuclear background staining while this was not visible in MEF cells. Tests for specificity with antibodies against LC3B proved nuclear staining as artifact in smooth muscle cells since no such signal was detectable in immune stainings. Since all cytoplasmic LC3B signals remained present at an even better signal to noise ratio we decided to perform all subsequent experiments and evaluations via immunocytochemical approaches.

Furthermore, we carefully checked once more all scale bars and they are correct. A7r5 cells are simply highly variable in size which causes the different scale bars. This broad size distribution of A7r5 cells is also well visible in the overview images of figure 3. However, all cells behave the same independent on size and given cells are representative for the overall results we received.

c) The provided data seem to indicate that autophagosomes often form in close proximity (e.g. Fig. 2C, 10min), which will be challenging to quantify. Independent reproduction of the data will require a detailed understanding of how this particular analysis was conducted. Thus, the authors, in addition to their section in the 'materials and methods' (LC3 spot quantification), should provide a workflow describing this particular analysis. This workflow should indicate how complex signals (e.g. from cells as shown in Fig. 2C, 10min) can be isolated and whether there are important exclusion criteria? In addition, it should be described in detail how Z-stacks were acquired (e.g. how many optical sections were used per cell). Finally, I recommend to present the data (Fig. 2B, Fig. 2D, Fig. 6B) in some other form than a bar graph, so individual data points and the distribution of the data are discernible.

We fully agree with the reviewer that all evaluations and experiments must be accurately described. Since the procedure of LC3 spot quantification was obviously not sufficiently well explained we have now included an additional supplementary figure S4 explaining everything in a step by step manner. Furthermore, we included the information how Z-stacks were acquired in the material and methods / microscopy section.

Regarding data presentation in Figure 2 and 6 we are thankful for the recommendation and plotted the results also as dot plots as indicated below. Unfortunately, due to single outlier values the y-axis legend would nearly double. Although the differences over time are still visible, most data points are very much compressed. We therefore decided to stick to the original data presentation. If requested, we could also show dot plots as additional supplementary figure.



d) Even though the authors have published much of the here used methodology, it would be helpful if experimental conditions and analyses were described in more detail in the main text. For instance, please indicate which stretch amplitude and frequency were used for straining directly in the main text. Please also describe shortly in the main text how the actin fiber reorientation was analyzed and quantified.

Requested information are now included in the main text. For actin fiber reorientation analysis we kept this short since more detailed information are already published and additionally indicated in the material and methods section / Analysis of actin fiber reorientation.

Biological interpretation:
e) The authors state in the abstract that "strain-induced cell reorientation is massively delayed upon inhibition of autophagy". It seems that this statement is inconsistent with the observation that MEFs have a rather quick autophagy response (Fig. 2C, D) but still undergo reorientation in the presence of chloroquine (Fig. 4). It would be helpful if the authors could discuss this aspect in more detail, because cells (MEFs) can obviously reorient without a functional autophagy pathway, albeit not quite as efficiently as A7r5 cells.

The reviewer mentions one of the most relevant aspects of our manuscript. Our data argue that the interplay between autophagosome formation and mechanoresponse becomes more prominent and important the more cytoskeletal structures, i.e. stress fibers are present in the cell. With just a reduced number of filaments or a more unordered actin cytoskeleton, cells reorient with basically no impairment upon CQ addition. Only in the presence of a naturally well-structured stress fiber system (smooth muscle cells, A7r5) or cytoskeletal reinforcement (RhoA activation in MEF cells) autophagy becomes essential for a fully functional and fast mechanoresponse. We have discussed this aspect now in more detail in the discussion section and also relate our data to results in skeletal muscles in which autophagy seems to be even more important for function and development upon mechanical stimulation.

Furthermore, we have removed “massively” from the abstract, since there is no need for this kind of extreme wording.
f) The authors show that elevating cellular contractility increases the sensitivity for autophagy during cell reorientation. However, 2D culture and high substrate stiffness often correlate with enhanced myosin activation and the authors here use comparably stiff (50 kPa) 2D matrices. Would it be possible to use substrates that are softer (e.g. 1-10 kPa) to demonstrate that the effect does also occur on physiologically relevant stiffnesses? If this is technically not possible, I suggest that the authors discuss this issue. The observed effects may be still relevant under conditions of pathologically increased matrix stiffness (fibrosis, cancer, etc).

The reviewer suggested a very interesting and important experiment. For this reason we have overlayed our 50 kPa stretching chambers with a layer of soft PDMS elastomer (5 kPa) based on standardized indentation calibrations. Using such chambers, we repeated stretch experiments and characterized actin cytoskeletal structure, actin filament orientation and also autophagosome formation. The achieved data proved former work of an overall slowed down reorientation on soft substrates. At the same time, they confirm the identified correlation between cell strain and autophagy with a significant increase of autophagosomes within 1 h of stretch on soft substrates. Due to the importance of the data we have included them in the results section and as supplementary figure S3.

Once more, I would like to thank all reviewers for their very valuable input and hope to have addressed all aspects in a clear and sufficient way. In case you have additional questions, I am happy to help at any time.

With kind regards

Bernd Hoffmann

RE: Manuscript #E21-05-0254R
TITLE: "Strain induced mechanoresponse depends on cell contractility and BAG3-mediated autophagy"

Dear Dr. Hoffmann:

We are pleased to accept your manuscript for publication in Molecular Biology of the Cell. MBoC is the scientific publication of the ASCB and reflects its interests in technically sound, good science. We encourage you to again consider submitting such work to MBoC.

Sincerely,
Dennis Discher
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Hoffmann:

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.

Within approximately four weeks you will receive a PDF page proof of your article.

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Sincerely,

Eric Baker
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