Fibroblast to Myofibroblast Transition is Enhanced by Increased Cell Density

Mary Doolin, Ian Smith, and Kimberly Stroka

Corresponding author(s): Kimberly Stroka, University of Maryland

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RE: Manuscript #E20-08-0536
TITLE: Fibroblast to Myofibroblast Transition is Enhanced by Increased Cell Density

Dear Dr. Stroka:

Thank you very much for your submission to MBoC, the society journal of the ASCB. Two Reviewers with expertise in the mechanobiology of differentiation have provided comments on your submission, with many comments indicating a need for quantitation for the existing data.
Some additional methods to confirm the key trends would also seem desirable, and you can certainly be selective if you choose to
submit a revision. A responsive revision should summarize the key changes and key changes to the text should be made clear (e.g. red font, etc.).

Sincerely,

Dennis Discher
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Stroka,

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.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

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.

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):

Refer to the attached file.

Reviewer #2 (Remarks to the Author):

Doolin et al. present a study on the effect of mechanical confinement on fibroblast to myofibroblast transition (FMT) in response to TGF beta1. They found that human lung fibroblasts cultured at high density have increased expression of alpha SMA, a marker of myofibroblast but cell confinement imposed by micropillars has no effect on FMT. The hallmark of FMT transition is known to be associated with a decrease in N-cadherin and an increase in OB - cadherins, a transition that the authors also found in their dense culture studies.

I find this paper to be limited both in concepts and experimental design.

1. Fig 1 and Fig 2 are not very informative. For example, in Fig.1, in the case of 24 hours of treatment with the lowest TGF beta1 concentration F-actin is visually more than in the case of 72 hours with the highest concentration. It is not clear if this is because of the choice of field of view or because of the signal threshold of the image. Images need to be quantified.
2. Fig.3, the authors claim that at very low density HLFs did not express a significant amount of alpha SMA even for high dosages of TGF beta 1 but visually it looks like alpha SMA expression on an individual cell level is similar to that in Fig.2 and even in Fig.1. So it is possible that the total amount of alpha SMA in dense cultures is higher simply because there are a lot more cells. Here again a quantification needs to be done.
3. How can the authors separate the effect of the matrix from the effect of cell-cell mechanical interactions?
4. One of the main weaknesses of the paper is that it is not clear from the manuscript why the authors hypothesize that nuclear compression can contribute to FMT. The manuscript will improve if the authors put their investigation in context. Several studies have shown the link between YAP/TAZ signaling in fibrosis, among them Liu et al., Am J Physiol Lung Cell Mol Physiol, 2015, which shows that mechanosensors YAP and TAZ accumulate in the nuclei in fibroblasts on pathologically stiff matrices. Liang et al., J Am Soc Nephrol 2017, showed that inhibition of YAP/TAZ signaling blocked TGF beta 1 induced FMT. If the authors have in mind other pathways or nuclear mechanosensors that should also be specified.
5. In addition, there is no data showing how nuclear compression or deformation changes with increased cell density. How do nuclear deformations in 2D dense cultures compare to nuclear morphology on micropillar substrates? Analysis of nuclear morphology is needed to address this question.
6. In Fig 7, I am not sure why the alpha SMA expression on micropillars is compared to 2D TCPC when clearly the cell density on micropillar images is much lower compared to the density on TCPC. Similar densities and cell-cell contacts are needed here for fair comparison.

Minor:
Some images have higher background then the others.

Reviewer #1:

Reviewer comments: In this study by Doolin and Stroka, the authors reported the importance of cell density in fibroblast to myofibroblast transition (FMT). Densely cultured cells more readily undergo FMT in response to TGF-β1. The authors suggested elevated OB-cadherin expression or increased quiescence in the high-density cultures might contribute to this change. Using PDMS micropillars, the authors have also demonstrated that mechanical confinement does not affect FMT induction.

Overall, it is an interesting observation, but the study fails to establish a mechanism that can explain high fibroblast to myofibroblast transition rate in densely cultured cells. While the second part of the study established that mechanical confinement has no significant effect on FMT, the authors fail to address the rationale behind choosing confinement from all the different parameters. The Western blotting result shows OB-cadherin expression is increased in high-density culture and the authors suggested that this could be a possible mechanism for high FMT. More experiments to establish the role of OB-cadherin (for example, whether OB-cadherin knockdown in densely cultured cells causes low FMT) could shed some light on the mechanisms and will also strengthen the study.

In its current form, the manuscript cannot be accepted. This reviewer urges the authors to provide more mechanistic detail. In addition, the following comments must be addressed:

Authors' Response: We sincerely appreciate the reviewer's thorough and critical review of our manuscript. We have responded to each point below through additional experiments analysis, text in the manuscript, and discussion here.

Reviewer Comment 1. In figure-1 it is observed that α-SMA expression (integrated density) is highest in 5ng/mL of TGF-β1. Is there any reason why the authors have used 10ng/mL TGF-β1 for all subsequent experiments?

Authors' Response: After quantification, we determined that all concentrations worked similarly well. We chose 10 ng/mL because this condition demonstrated the least variability and the most consistent increase in alpha-SMA with time.

Reviewer Comment 2. To assess the α-SMA expression, more quantitative techniques like RT-PCR/ western blotting must be performed.

Authors' Response: We indeed included quantification of α-SMA expression via western blot. We refer the reviewer to Figures 5 (medium vs. high density cells) and 9 (cells in micropillar arrays).

Reviewer Comment 3. In Figure 5, there is no significant difference in the expression of OB-cadherin between high and medium density and +/- TGF-β1. This suggests that the shift from N-cadherin to OBcadherin is not prominent as the cell density increases.

Authors' Response: We appreciate this comment from the reviewer, as it prompted us to repeat this experiment several more times. We now find a robust increase in OB-cadherin expression going from medium to high cell density, with further increase in OB-cadherin expression for cells treated with TGF-β1. These data are presented in the new Figure 6.

Reviewer Comment 4. In Figure 6 also, the data is not significant. Authors could have used RT-PCR to probe the transition.

Authors' Response: Indeed, after repeating this experiment several more times, we now find statistical significance from the western blot quantitative analysis. Please see the new Figure 6.

Reviewer Comment 5. Since IPF causes increased ECM deposition, in high-density/medium density fibroblast culture condition, what is the status of ECM deposition? The authors should perform collagen staining to address this question.

Authors' Response: We thank the reviewer for this excellent suggestion. We have performed this experiment and found that HLFs at high density, and treated with TGF-β1, deposit significantly more collagen 1 (COL1A1, specifically) matrix in comparison with low and medium cell density and control treatment. These data are included in the new Figure 7 and discussed in the manuscript on p. 7, para 3.

Reviewer Comment 6. For studying the effect of confinement, the authors can seed cells in 3D collagen with increased density to mimic high ECM deposition in vivo. Alternatively, the authors may adopt a patterning approach in 2D.

Authors' Response: This is a very interesting suggestion worthy of exploration in future work. However, we believe it is outside of the scope of this current work.

Reviewer Comment 7. FMT is also dependent on stiffness. The seeding density (low, medium, and high) will likely change the cell layer stiffness via modulation of ECM deposition. To address this issue, it would be good if the authors can provide some measurements of cell layer stiffness and its influence on FMT.

Authors' Response: This is a very interesting and important point. Dr. Dennis Discher's lab has shown that cells plated atop gels respond to the stiffness of the underlying plastic when gels are less than 5 μm thick (Buxboim et al., J Phys Condens Matter 22(19): 194116, 2010). Cells still sense the underlying rigidity with decreasing intensity up to a critical thickness of 20 μm, whereafter they fully sense the stiffness of the gel directly under the cell. While ECM deposition may modulate the microenvironment, we do not anticipate enough ECM accumulated to create an environment thick enough to shield the cells from the stiffness of the underlying PDMS.

Reviewer Comment 8. The manuscript heavily relies on conclusions drawn from microscopy images (fig. 1, 2, 3, 4, 7), while these images are not quantified. This is a very big concern. Assuming the figures showcase only representative images, I would strongly recommend the authors to acquire images from multiple fields and quantify the images using image

quantification software like ImageJ and show population statistics (for example, integrated density of individual cells or ROI for multiple cells/positions).

Authors' Response: We have taken this recommendation and completed additional quantification of the immunofluorescence images. This quantification is now shown in the new Figures 3 and 4.

Reviewer Comment 9. Please mention the details of Western blot quantification method. As per my understanding, the Y-axis in the graphs shows a ratio between the protein of interest with GAPDH (OBcadherin/ GAPDH, α-SMA/GAPDH, etc) where some values are 0. How is this possible? Are the values normalized such that the lowest ratio is considered 0? If yes, mention that in the materials-methods section or the figure legend.
Authors' Response: The reviewer is correct; the Y-axis in the graphs shows a ratio between the protein of interest with GAPDH. If the value is zero, it means the protein is not expressed highly enough to be visualized by the western blot. We have added this point to the "Data analysis" subsection of the Methods section (p. 14).

Reviewer Comment 10. In quantification of OB-cadherin western blot (Figure 5C), the graph makes it seem like OBcadherin expression is non-significant across all the conditions (that includes high density vs medium density group), but from the figure 5B and also from the result seems that is not the case (i.e. the high density group has higher OB-cadherin than medium density group). Please use separate lines to indicate pairwise statistical comparison to make it clearer.

Authors' Response: After repeating these western blot experiments, we now find statistical significance between multiple conditions. We have clearly indicated the difference using separate lines to indicate the pairwise statistical comparisons. These data are now included in the new Figure 6.

Reviewer Comment 11. In Figure 5C, western blots show very faint OB-cadherin signal. To make it more clearly visible the author might consider repeating the experiment with (i) increase the exposure during the blot detection or (ii) Use a more sensitive detection reagent or (iii) load more amount of protein per well. Similar to α-SMA blots, the OB-cadherin western blotting experiments can also be accompanied by immunostaining.

Authors' Response: We have taken this excellent suggestion of the reviewer, and also repeated our western blot experiments. These updated data are shown in the new Figure 6.

Reviewer's Minor comments:

Reviewer Minor Comment 1. Since the culture plastic wares are very stiff and enhance FMT, the authors might consider using a soft substrate (Polyacrylamide gels or soft PDMS substrate coated with collagen)

Authors' Response: This is a very interesting suggestion, and indeed, it has been shown that stiff substrates promote more FMT than soft substrates. While this was not the scope of our study, it should be an avenue for future work.

Reviewer Minor Comment 2. "…HLFs seeded at high density expressed more OB-cadherin than the medium density group, and TGF-β1 treatment increased OB-cadherin…" should be: "…HLFs seeded at high density expressed more OB-cadherin than the medium density group, and TGF-β1 treatment increased OB-cadherin…"

Authors' Response: We thank the reviewer for catching this typo. We have made the correction.

Reviewer Minor Comment 3. Incomplete Referencing, Missing journal name: "Swift, J et al. (2013). Nuclear Lamin-A Scales with Tissue Stiffness and Enhances Matrix Directed Differentiation. 341"

Authors' Response: We thank the reviewer for catching this omission. We have made this correction.

Reviewer #2:

Reviewer Comments: Doolin et al. present a study on the effect of mechanical confinement on fibroblast to myofibroblast transition (FMT) in response to TGF beta1. They found that human lung fibroblasts cultured at high density have increased expression of alpha SMA, a marker of myofibroblast but cell confinement imposed by micropillars has no effect on FMT. The hallmark of FMT transition is known to be associated with a decrease in N-cadherin and an increase in OB - cadherins, a transition that the authors also found in their dense culture studies.

I find this paper to be limited both in concepts and experimental design.

Authors' Response: We sincerely appreciate the reviewer's thorough and critical review of our manuscript. We have responded to each point below through additional experiments analysis, text in the manuscript, and discussion here.

Reviewer Comment 1. Fig 1 and Fig 2 are not very informative. For example, in Fig.1, in the case of 24 hours of treatment with the lowest TGF beta1 concentration F-actin is visually more than in the case of 72 hours with the highest concentration. It is not clear if this is because of the choice of field of view or because of the signal threshold of the image. Images need to be quantified.
Authors' Response: We thank the reviewer for this comment. We have taken this recommendation and completed additional quantification of the fluorescence images. This quantification is now shown in Figures 3 and 4.

Reviewer Comment 2. Fig.3, the authors claim that at very low density HLFs did not express a significant amount of alpha SMA even for high dosages of TGF beta 1 but visually it looks like

alpha SMA expression on an individual cell level is similar to that in Fig.2 and even in Fig.1. So it is possible that the total amount of alpha SMA in dense cultures is higher simply because there are a lot more cells. Here again a quantification needs to be done.

Authors' Response: We appreciate this suggestion and have included quantification of the fluorescence images. Although there is some basal expression of alpha-SMA expression in low density cells, our main point is that we did not observe a substantial increase in alpha-SMA expression with TGFβ1 treatment compared to control conditions, as we do in high-density cultures. We have modified the sentence describing this result in the Results section (p. 4, para 3) to be, "At an even lower density, HLFs treated with 20 ng/mL TGF-β1 did not display the pronounced increase in α-SMA (Figure 4) that was observed in higher density HLFs."

We also note that the images in Figure 4 are from a separate experiment and therefore their fluorescence intensity magnitude should not be compared directly with the images in Figures 1 and 2. We have made this note by modifying the last sentence in the "Immunofluorescence" Methods section: "The settings for each fluorescent channel were maintained across all images acquired within a given experiment. Image intensity can be compared within figures (and between Figures 1 and 2) but should not be directly compared across other figures since they are from different experiments."

Reviewer Comment 3. How can the authors separate the effect of the matrix from the effect of cell-cell mechanical interactions?

Authors' Response: We thank the reviewer for this comment. We point out that the matrix is the same (i.e., plastic or collagen I coated PDMS) for all groups within a particular experiment. However, we chose not to directly compare experiments on plastic to those on PDMS, due to possible differences in how the collagen I attaches to the substrate for these two materials.

Reviewer Comment 4. One of the main weaknesses of the paper is that it is not clear from the manuscript why the authors hypothesize that nuclear compression can contribute to FMT. The manuscript will improve if the authors put their investigation in context. Several studies have shown the link between YAP/TAZ signaling in fibrosis, among them Liu et al., Am J Physiol Lung Cell Mol Physiol, 2015, which shows that mechanosensors YAP and TAZ accumulate in the nuclei in fibroblasts on pathologically stiff matrices. Liang et al., J Am Soc Nephrol 2017, showed that inhibition of YAP/TAZ signaling blocked TGF beta 1 induced FMT. If the authors have in mind other pathways or nuclear mechanosensors that should also be specified.

Authors' Response: We appreciate the suggestion and have incorporated these suggested references into our Results/Discussion section (p. 8, para 2). In this same paragraph, we have also added a few more sentences discussing that stiff substrates promote FMT and that stiff substrates promote nuclear compression, in order to better motivate our hypothesis.

Reviewer Comment 5. In addition, there is no data showing how nuclear compression or

deformation changes with increased cell density. How do nuclear deformations in 2D dense cultures compare to nuclear morphology on micropillar substrates? Analysis of nuclear morphology is needed to address this question.

Authors' Response: This is an excellent suggestion made by the reviewer. To address this comment, we have quantified nuclear area for control and TGF-β-treated cells at low, medium, and high density. We have included these data in the new Figure 8.

Reviewer Comment 6. In Fig 7, I am not sure why the alpha SMA expression on micropillars is compared to 2D TCPC when clearly the cell density on micropillar images is much lower compared to the density on TCPC. Similar densities and cell-cell contacts are needed here for fair comparison.

Authors' Response: Based on previous data in this manuscript showing an increasing trend in alpha-SMA on 2D TCPS, a 2D TCPS group was selected as a positive control for the micropillar experiments. While the density on 2D TCPS appears to be higher, the western blot data is normalized such that the same amount of protein is loaded across all groups. Also of note, it was shown in a previous publication (M.T. Doolin & K.M. Stroka, Tiss Eng C, 2019), that the cell density between micropillar groups is consistent at short time points. However, differences in cell-cell contacts may certainly play a role in the results and could be an area to further explore in future work.

Reviewer's Minor comments:

Reviewer Minor Comment 1. Some images have higher background then the others.

Authors' Response: We appreciate this comment from the reviewer. Indeed, this is true. As we took an array of images across each well, we believe this is due to different locations within the well causing varying degrees of refraction of the light.

RE: Manuscript #E20-08-0536R
TITLE: Fibroblast to Myofibroblast Transition is Enhanced by Increased Cell Density

Dear Dr. Stroka:

Thank you very much for your revision, but please add some additional analyses of existing data per below. I believe these will strengthen your story and better satisfy some concerns.
(1) Given your comments on 'quiescence' in the abstract, please add % mitotic cells to Fig.8 as possible direct evidence of quiescence. You should also consider making histograms of total DNA intensity per nucleus to quantify %cells not replicating.
(2) Clarify if 'ns' in Fig.9B applies also to Tissue Culture Plastic; if it does, then please explain consistency with earlier data. (3) Add to Fig.10 for all conditions: a plot of average nuclei per field for all conditions, and a plot of nuclear area per Fig.8A.
(4) Also in Fig.10, the 2D PDMS + TGFb condition appears to have heterogeneous cell density and also some cells that strongly express SMA compared to the rest. Please discuss this issue and perhaps add some analysis or more images where you consider local cell density in relation to SMA.

We look fwd to receiving your revision addressing the above.

Sincerely,

Dennis Discher
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Stroka,

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.

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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.

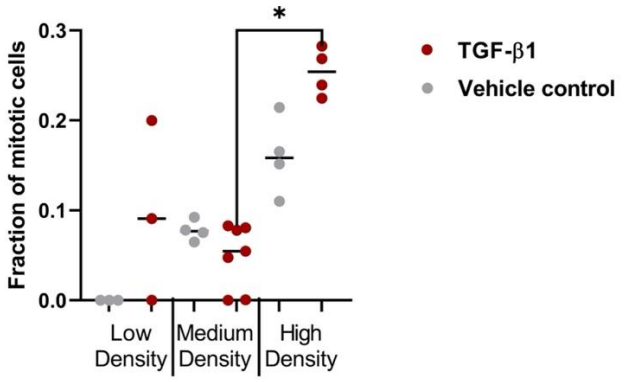
Sincerely,

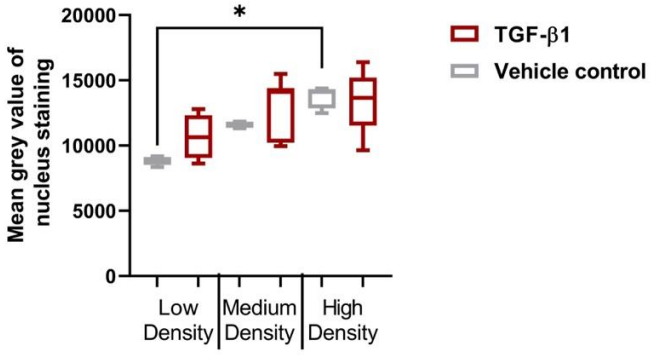
Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Response to Editor's comments

Editor's comment (1): Given your comments on 'quiescence' in the abstract, please add % mitotic cells to Fig.8 as possible direct evidence of quiescence. You should also consider making histograms of total DNA intensity per nucleus to quantify %cells not replicating.

Authors' response: We thank the Editor for this suggestion. As suggested, we analyzed each image for low, medium, and high density by counting the number of cells whose nuclei visually appeared mitotic and divided by the total number of nuclei, to obtain the fraction of mitotic cells. Interestingly, with TGF-β1 treatment, the high-density cells were more mitotic than the medium-density cells (see first figure below), while the intensity of nuclear Hoechst staining (see second figure below) increased for vehicle control cells going from low to high density conditions. Hence, we have decided to remove mention of the possible role of quiescence from both the abstract and the last paragraph of the results/discussion section. We agree with the Editor, that without solid evidence for quiescence in our experiments, we should not speculate on its role in the density-dependent results.





Editor's comment (2): Clarify if 'ns' in Fig.9B applies also to Tissue Culture Plastic; if it does, then please explain consistency with earlier data.

Authors' response: Yes, "ns" in Figure 9B also applies to tissue culture plastic. Figure 9B is consistent with Figure 5C. In both sets of data, there is a qualitative increase in

αSMA with TGF-β1 treatment on TCPS in the high-density condition, but this increase is not statistically significant in either set of experiments. Note that we have moved Figure 8 into Figure 5 and renumbered the subsequent figures, so that Figure 9 is now Figure 8.

Editor's comment (3): Add to Fig.10 for all conditions: a plot of average nuclei per field for all conditions, and a plot of nuclear area per Fig.8A.

Authors' response: As suggested, we have added plots of average nuclei per field (new Fig. 9C) and average nuclear area (new Fig. 9D) for all conditions. We also added the following text to the second paragraph in the Results and Discussion subsection titled "Cell confinement may not affect FMT: "Furthermore, our analysis of number of nuclei per image (Fig. 9C; statistics in Supplemental Table S2) and mean nuclear area (Fig. 9D; statistics in Supplemental Table S3) for cells in the micropillar devices suggested that there were no systematic differences in these values between micropillar spacings that may have created confounding effects between cell density and confinement, especially when considering the seeding densities of our low, medium, and high density conditions were 10-fold different from each other."

Editor's comment (4): Also in Fig.10, the 2D PDMS + TGFb condition appears to have heterogeneous cell density and also some cells that strongly express SMA compared to the rest. Please discuss this issue and perhaps add some analysis or more images where you consider local cell density in relation to SMA.

Authors' response: The addition of TGF-β1 can cause human lung fibroblasts to form gaps in their monolayer, as seen in the PDMS condition of Figure 10 (now Figure 10), likely due to enhanced cell contractility (Epa et al., 2015; Liu et al., 2001). This effect is also visible in Figure 5A (medium density). In order to reduce this artifact, we could consider treating the TGF-β1 groups with an inhibitor of myosin activity, such as blebbistatin, to reduce contractility. However, this would also prevent FMT (Southern et al., 2016; Sun et al., 2021) and create a confounding variable. While we agree modulating local density would be interesting, it is beyond the scope of this work. To address this comment, we have added new text, along with the references below, into the end of the second paragraph in the Results/Discussion section on "Cell confinement may not affect FMT."

References cited above; also added to manuscript:

Epa, A. P., Thatcher, T. H., Pollock, S. J., Wahl, L. A., Lyda, E., Kottmann, R. M., Phipps, R. P., & Sime, P. J. (2015). Normal human lung epithelial cells inhibit transforming growth factor-β induced myofibroblast differentiation via prostaglandin E2. *PLoS ONE*, *10*(8), 1–19. https://doi.org/10.1371/journal.pone.0135266

Liu, X. D., Umino, T., Ertl, R., Veys, T., Skold, C. M., Takigawa, K., Romberger, D. J.,

Spurzem, J. R., Zhu, Y. K., Kohyama, T., Wang, H., & Rennard, S. I. (2001). Persistence of TGF-beta1 induction of increased fibroblast contractility. *In Vitro Cellular & Developmental Biology. Animal*, *37*(3), 193–201. https://doi.org/10.1290/1071-2690(2001)037<0193:POTIOI>2.0.CO;2

Southern, B. D., Grove, L. M., Rahaman, S. O., Abraham, S., Scheraga, R. G., Niese, K. A., Sun, H., Herzog, E. L., Liu, F., Tschumperlin, D. J., Egelhoff, T. T., Rosenfeld, S. S., & Olman, M. A. (2016). Matrix-driven myosin II mediates the pro-fibrotic fibroblast phenotype. *Journal of Biological Chemistry*, *291*(12), 6083–6095. https://doi.org/10.1074/jbc.M115.712380

Sun, X., Zhu, M., Chen, X., & Jiang, X. (2021). MYH9 Inhibition Suppresses TGF-β1-Stimulated Lung Fibroblast-to-Myofibroblast Differentiation. *Frontiers in Pharmacology*, *11*(January), 1–9. https://doi.org/10.3389/fphar.2020.573524

RE: Manuscript #E20-08-0536RR
TITLE: "Fibroblast to Myofibroblast Transition is Enhanced by Increased Cell Density"

Dear Dr. Stroka:

One last and quick revision needed. Unless i somehow missed it, please add as Supplement figures your plots of mitotic counts and DNA intensity that you show in "Response to Editor's comments / Editor's comment (1)". Please also add a brief discussion of this data in relation to your studies and perhaps those of others.

Sincerely,
Dennis Discher
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Stroka,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) 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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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.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL):
Link Not Available

Authors of Articles and Brief Communications whose manuscripts have returned for minor revision ("revise only") are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

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

Response to Editor’s comments

Editor’s comment (1): Please add as Supplement figures your plots of mitotic counts and DNA intensity that you show in "Response to Editor's comments / Editor's comment (1)". Please also add a brief discussion of this data in relation to your studies and perhaps those of others.

Authors’ response: We have added the plots of mitotic counts and DNA intensity into the supplemental material file. We also added a brief discussion of this data in the main text Results and Discussion section, indicated by the yellow highlighted text.

RE: Manuscript #E20-08-0536RRR
TITLE: "Fibroblast to Myofibroblast Transition is Enhanced by Increased Cell Density"

Dear Dr. Stroka:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell, the scientific journal of the ASCB. We hope that you found the review process fair and helpful, and we encourage further submissions from your lab as well as your broader participation in support of the society.

Sincerely,
Dennis Discher
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Stroka:

Congratulations on the acceptance of your manuscript.

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