

# Three-Dimensional Mechanical Cooperativity Optimises Epithelial Wound Healing

Shu En Lim, Pablo Vicente-Munuera, Robert Tetley, Martin Huang-Zhang, Samantha Warrington, Besaiz Sánchez-Sánchez, Brian Stramer, Jose Muñoz, and Yanlan Mao

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## Review Timeline:

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*Editor: Ieva Gailite*

## 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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Yanlan,

Thank you for submitting your manuscript for consideration by the EMBO Journal. I apologise for the unusually extended review process in this case. We have now received comments from a full set of reviewers, which are included below for your information.

As you can see, all reviewers express interest in the study and appreciate its contribution to the research field. At the same time, they indicate a number of partially overlapping and substantive concerns that will need to be addressed before they can support publication here. In particular, these concerns include:

- Providing further support to the relevance of cell intercalations during wound healing (reviewers #1 and #2),
- Further clarification of the contribution of the apical vs lateral cables for establishment of cortical tension and wound closure (all reviewers),
- Improvement of the imaging of the lateral cables and laser ablation experiments (reviewers #2 and #3),
- Clarification of the contribution of basement membrane deformation and cell volume preservation for wound closure in the model vs the in vivo situation (all reviewers).

Based on the general interest expressed in the reports, I would like to invite you to submit a revised manuscript in response to these comments. I would also be happy to discuss the revision in more detail via email or phone/videoconferencing - please let me know what you prefer.

We generally allow three months as standard revision time, which can be extended to six months in the case of major revisions. Should you foresee a problem in meeting this deadline, please let us know in advance to discuss an extension.

As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, please contact me as soon as possible upon publication of any related work to discuss the appropriate course of action.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File and will therefore be available online to the community. For more details on our Transparent Editorial Process, please review our Editorial Policies: <https://link.springer.com/partners/embo-press/editorial-policies>. Please also see the attached instructions for further guidelines on preparation of the revised manuscript.

Please feel free to contact me if you have any further questions regarding the revision. Thank you for the opportunity to consider your work for publication. I look forward to discussing your revision!

With best wishes,

Ieva

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Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

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- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist
- Expanded View files (replacing Supplementary Information)
- a Reagents and Tools Table as part of the Methods section

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (7th May 2026). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

<https://emboj.msubmit.net/cgi-bin/main.plex>

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Referee #1:

Lim and colleagues investigate the three-dimensional aspect of wound healing in a columnar epithelium. Using a powerful combination of novel 3D *in silico* modelling, and the *Drosophila* wing imaginal disc, they show that, in addition to a myosin cable around the perimeter of the wound, myosin cables also form from the apex to the base of the cells around the wound. These lateral cables pull down on the apical cell surface and up on the basement membrane, causing a reduction in cell height that displaces the cell volume in the XY plane, contributing to wound closure. In addition, the authors propose that the cables contribute to cell remodelling in 3D to facilitate the expansion of cells into the wound.

This is a nice study that takes advantage of mathematical modelling to make predictions on how lateral cables contribute to wound repair. To this reviewer's knowledge, lateral cables had not been previously described in wound healing, and thus this work is likely to inspire further studies in other 3D tissues. The work is limited in that experimental manipulations to disrupt apical or lateral myosin cables in discs are somewhat indirect. While the shibire approach is elegant and clean, the Mbs RNAi experiments are hard to interpret, as Mbs presumably controls myosin activity both in apical and lateral cables. The study would greatly benefit from the use of optogenetic approaches to specifically disrupt myosin in apical or lateral pools, but I appreciate that those experiments can be technically very challenging and could easily become another paper. Instead, I think the authors should address the following points, which are more manageable both technically and in terms of their scope.

#### MAJOR

1. The authors repeatedly mention that supporting cell intercalations in 3D is a key innovation of their mathematical model. I agree with this, but does it matter? What are the predictions of the model regarding number and distribution of 3D intercalations in controls and shi[TS] or Mbs RNAi scenarios? Do 3D intercalations happen during wound healing in wing discs? If that has been demonstrated before, they should cite the work. If not, they should test if there are 3D intercalations during wound healing *ex vivo*, and if model predictions on the effect of shi[TS] or Mbs RNAi with respect to 3D intercalations hold when looking *ex vivo*. Otherwise, what is the value of the 3D model (could one just use a simpler 2D model of a cross-section of the epithelium)?
2. Lateral cables seem to be unstable when the apical cable is disrupted (Figure 4F). How does this happen? *In vitro* and *in vivo* work showed that myosin can be stabilized by tension/strain. Does loss of the apical cable reduce tension in the lateral cables? This should be tested both *in silico* and *ex vivo*.
3. Related to the previous point, in page 10 (end of second paragraph) the authors argue that shi[TS] disrupts wound healing by stabilizing cortical tension. But don't their results suggest that the disruption of wound closure is due to a de-stabilization of cortical tension? They should conduct FRAP experiments to measure the turnover of myosin in the lateral cables, both in control and shi[TS] discs and determine the effect of losing the apical cable on lateral myosin turnover.
4. Figure 5G, 6K-L: how do the authors explain that removing basal adhesion in the model increases basal indentation? Wouldn't one expect that cells need to adhere to the substrate to be able to pull on it? I appreciate that this is difficult to assess in discs,

but given that both talin and integrin loss of function both lower myosin levels in lateral cables, I have a hard time seeing how the prediction of the model of increased basal indentation with reduced adhesion could hold ex vivo. Can the authors try a partial loss of function of talin or integrin (e.g. using a weaker Gal4 driver) to assess whether basal indentation increases or decreases ex vivo?

#### MINOR

5. Fig. S2A-B: I found it very interesting that the "continuity" between the apical and lateral actomyosin cables seems to happen at points in which adherens junctions are excluded. The authors should discuss how they think that continuity arises to enable the lateral cables to pull on the apical cell surface (e.g. crosslinking?).

6. Fig 2D.: the position of the wound should be indicated (e.g. with a square bracket at the top of the image). There are many lateral cables, and I was wondering if all of them are associated with cells adjacent to the wound, or if one can also find lateral cables away from the wound edge or in an unwounded epithelium? The authors should show similar images in an intact disc to demonstrate the specificity (or not) of lateral cable formation to wounded epithelia.

7. Fig. 2J-K: please, add images that demonstrate the quantitative results. From the images shown in 2I it is hard to see what is happening, as most of the differences in J and K only become significant after 30-60 sec.

8. Pages 2, 14: I would suggest citing the original papers showing an actomyosin purse string around wounds in *Drosophila* embryos (Kiehart, 2000 and Wood, 2002). Reference 19 came much later, and although beautiful work, credit should be given to the original papers.

9. Fig. 2H: report the statistical tests used in the figure legend.

#### TYPOS/GRAMMAR

10. Supplement, page 14: please revise "Once the environment is set and compiled the cython files as explained in the README.md"

#### Referee #2:

The manuscript by Lim et al. investigates the mechanisms of wound healing in *Drosophila* and presents a 3D vertex model to analyze the underlying processes. The authors show that wound closure is not driven solely by the classical purse-string mechanism, but also involves an important contribution from lateral actomyosin cables. They combine ex vivo experiments with computational modeling to interpret the relative roles of purse-string contraction and lateral cable formation. While several results are interesting, the manuscript would benefit from substantial rewriting to improve clarity, coherence, and precision, and to avoid potential overstatements or misinterpretations. In particular, the rationale behind several experiments should be clarified to help readers better understand the authors' intentions and interpretations.

##### 1. Cell volume (Fig. 1).

The authors state that cell volume remains constant during wound healing; however, the projected cell area also appears largely unchanged (e.g., Fig. S1B). This raises questions about how cell volume is measured and how sensitive the method is to subtle changes. Additional methodological details are needed to clarify the volume quantification. Moreover, at the wound edge, one might expect local volume perturbations (e.g., due to membrane damage or leakage), which are not discussed.

##### 2. Origin and localization of lateral cables.

It is unclear whether the lateral actomyosin cables emerge specifically in response to wounding or whether they are also present under control conditions. Are these structures localized only around the wound margin, or do they also form elsewhere in the tissue? In Fig. 2C, the difference between the pre-wound condition and the 7:30 min post-wounding time point is difficult to appreciate. Clearer visualization or quantitative analysis would help to support the authors' claims.

##### 3. Increase of the apical purse string (Fig. 2).

The authors claim that the apical purse-string signal increases over time (p. 4, Fig. 2E), but this trend is not readily apparent in the images shown (Fig. 2C). How was this increase quantified? Providing representative examples and a clearer description of the analysis pipeline would strengthen this conclusion.

##### 4. Continuity of the apical cable.

The authors claim that the apical cable is continuous, yet this is not clearly evident in the figures. Additional clarification and/or higher-resolution images would help to support this statement.

##### 5. Laser ablation and recoil measurements.

The authors use nano-ablation to measure recoil following the disruption of either the lateral cables or the apical purse string (p. 4). However, the data shown for recoil (Fig. 2F) appear to correspond to the apical cable only. Is this measured at sites where the purse string colocalizes with lateral cables? It remains unclear to me. It would be helpful to clarify this point and, if possible, to also present recoil measurements from appropriate control regions (as referenced in Fig. S2C).

##### 6. Vertex model and cell intercalation.

The authors justify the use of a 3D vertex model in part by stating that cell intercalation plays an important role during wound

healing. However, it is not clear from the experimental data where, when, or to what extent such intercalation occurs. This point is not convincingly demonstrated in the manuscript. Because cell intercalation appears to be a key assumption underlying the modeling framework, it should be explicitly shown, quantified, and clearly justified using the experimental data. Also, that would help to understand what the model brings. For instance, the model predicts that removing lateral cables will change cell intercalation. Is it true in the experiments?

7. Definition and quantification of "apical junction remaining."

The term apical junction remaining is used throughout the manuscript but is never clearly defined. How is this quantity measured and normalized? Based on the description in the Materials and Methods, it is difficult to reconcile the reported ~50% change in Fig. 6D with the example shown in Fig. 6. Please clarify the definition, provide a precise quantification procedure, and include additional representative examples.

8. Interpretation of adhesion perturbations (integrin and talin).

The statement, "Due to weakened BM attachment, the basal surface can deform more easily, even with weaker lateral cables," is difficult to interpret. Along similar lines, it is unclear how apical wound-healing dynamics can remain largely unaffected by Talin knockdown if the lateral cables are connected to the basal membrane. How are forces transmitted in this case? Overall, the section on adhesion perturbations (integrin and talin) is not clearly explained. This part would benefit from substantial rewriting to clarify the mechanical interpretation and the authors' main message.

9. Definition and quantification of suprabasal myosin.

Although a minor point, this reflects a broader issue throughout the manuscript. The term suprabasal myosin is used only 3 times, never clearly defined, despite appearing to be important for the authors' interpretation. How is suprabasal myosin identified and quantified?

Referee #3:

The manuscript by Y. Mao and colleagues proposes a 3D description of wound healing in the *Drosophila* wing disk, combining high resolution live imaging and physical modeling with a 3D vertex model. With high resolution confocal microscopy, the authors identified that wound closure is associated with apical basal indentations and 3D cell shape changes. They hypothesized that these cell shape changes could contribute to wound closure and developed a thorough analysis of the process. They identified the presence of lateral actomyosin cables potentially acting as force generators. With perturbation of endocytosis and myosin activity, they could correlate changes in tissue geometrical shape, closure kinetics and myosin enrichment in the different perturbed cases. These data support a model in which the lateral myosin cables generate force promoting the indentation and contributing, because of constant cellular volume, to the wound closure. The authors eventually investigate the connection with the basement membrane and identify a delay in the indentation suggesting a more complex mechanical picture.

Overall the study is very interesting and innovative. The claims are well supported by experimental observations and modeling approaches. Some experimental data are not very clear and needs to be improved, particularly the imaging of the lateral cables. Some aspect of the modeling also would need to be clarified, particularly on some differences observed between the output of the model and the experiments in the apical and basal indentation. With some modification, I believe this work has the potential to be published in EMBO Journal.

Major comments:

-A central element of the manuscript is the presence of lateral myosin cables potentially generating out of plane active forces. While the data shows the presence of these cables relatively clearly, it is very difficult to evaluate with the actual figures when do they really appear, where they are located and how many cables are present. On the Fig 2A and C, we can observe the presence of cables before wounding is performed. It seems that these cable shows an enrichment of myosin during closure. But it is difficult to evaluate with the quality of the images. I would encourage the authors to improve the quality of the images and perhaps to provide quantifications showing the arguments defining lateral cables.

-Also, the exact location of the cables within the tissue and within the cells is difficult to assess with the shown data. For example, in fig 2D, we see several myosin dot-like enrichments in the XY plane. Are all of these enrichments associated to lateral cables? I would recommend the authors to complement the data with new images showing the location of the lateral cables in cell (is it at junctional vertices?) and in the tissue. It is also very difficult to evaluate the density of cells with lateral cables (particularly density of leading edge cells that display a lateral cable. Such a quantification would be useful for the comparison with the vertex model which includes 1 lateral cable/cell (from what I understood).

- The laser ablation of the lateral cable shows a recoil of the wound edge, indicating a reduction in the forces promoting wound closure. Consistent with it the authors show also a reduction in myosin intensity. What happen at the level of the apical indentation, do the indentation also shows a reduction consistent with the in-plane recoil?

- About the model: The 3D model appears very nicely done and seems to recapitulate well the kinetics of wound closure. The different supplemental movies are very helpful to visualize the kinetics of closure in the simulation. However, it is not clear to me what the volume labels are indicating as apical and basal volumes appear different. Is it cell volume? Also what is the scale (It's difficult to evaluate whether we look at small volume differences or not)?

From these movies, The volume seems to be not conserved during healing in any of the conditions and therefore it is different from the ex vivo case. It is however difficult to estimate volumes only based on the color coding. The authors should control this parameter and include quantifications in the manuscript (as a supp. Figure for example).

-Related to my previous point, In the Fig 3D in absence of lateral cables, the quantification shows an absence of apicobasal indentation. I find this result surprising as I would expect that purse string force generation + constant volume would generate indentation anyway. What could be the cause of this observation, is it due to an increase in cell volume? The authors should explain this result.

-From the Figure 3 onward, the authors introduce the quantification of apical junction loss. It is however not very well introduced with a lack of definition of which apical junction loss is quantified. In the remaining of the manuscript, it is not very clear how these quantifications bring a mechanistic understanding of the process. I would invite the authors to introduce and explain more extensively the relationship between this junctional loss potential cell intercalations and the lateral myosin cables.

-Finally, the imaging of the basement membrane is particularly interesting, the fig 5 A-B shows a delay in the basal indentation compared to the apical indentation, suggesting a change in the force balance at mid-closure and two potential different phases of closure. Is it correct? As it is presented, the model seems to not recapitulate this sequential deformation. The authors could discuss the limitations of the model to recapitulate this sequential deformation and probably the need to include changes in parameters at mid closure. Also, the different perturbations already used (shi, MbsRNAi, Talin RNAi and integrin DN) may have an impact on this delay. It would be interesting to see the changes in basement membrane indentation in these different cases. I believe this is an important observation of the manuscript that could be highlighted or discussed if there are really two sequential phases of apico basal deformations.

#### Minor comments:

-In the Fig 1B, the cadherin signal is depleted in the region that undergo apico basal deformation, is it correct? Or is it a coincidence in this particular example? This may have some mechanical consequences if it's the case.

-In the Fig 1E, the cadherin signal is not visible. I would suggest to change the image

-In the Fig 2A, the contrast is very weak, it is very difficult to identify structures (like wound edge or cell edges). I would suggest to change the image for one with better contrast.

-In the figure 2E the axis seems to be not correct (inverted).

-The Fig S3A needs a legend in the figure to clearly distinguish the simulations from the experiment. Also an explanation should be added in the main text to explain that the motivation is to approach experimental geometry prior ablation.

-Fig 3C apical indentation curves are slightly different between simulation and ex vivo, with a faster increase followed by a decrease in speed in the simulation compared to ex vivo that steadily increases. Is it due to progressive changes in purse string or lateral cable tension that are not included in the model? Or perhaps to different cell volume contribution in ex vivo and in the simulation? This could be discussed.

-In the model the volume contribution  $W_v$  is kept for the debris, I don't see clear reason for that as I believe that ablated cells will have a strong change in volume. Is there a physical reason to keep the energy term in the simulations?

-Introduction of the previous 3D models could be a bit extended particularly in the context to understand why inclusion of scutoids forms is novel and interesting and what it actually brings in the context of this study.

Dear Reviewers,

Thank you for your interest in our manuscript and for considering a revised version.

Please find below our detailed point-by-point response to the reviewers' comments. For your ease, reviewers' comments are in black, our responses are in blue. Within the manuscript content, all newly added text is in blue.

Referee #1:

Lim and colleagues investigate the three-dimensional aspect of wound healing in a columnar epithelium. Using a powerful combination of novel 3D in silico modelling, and the *Drosophila* wing imaginal disc, they show that, in addition to a myosin cable around the perimeter of the wound, myosin cables also form from the apex to the base of the cells around the wound. These lateral cables pull down on the apical cell surface and up on the basement membrane, causing a reduction in cell height that displaces the cell volume in the XY plane, contributing to wound closure. In addition, the authors propose that the cables contribute to cell remodelling in 3D to facilitate the expansion of cells into the wound.

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MAJOR

1. The authors repeatedly mention that supporting cell intercalations in 3D is a key innovation of their mathematical model. I agree with this, but does it matter? What are the predictions of the model regarding number and distribution of 3D intercalations in controls and shi[TS] or Mbs RNAi scenarios? Do 3D intercalations happen during wound healing in wing discs? If that has been demonstrated before, they should cite the work. If not, they should test if there are 3D intercalations during wound healing ex vivo, and if model predictions on the effect of shi[TS] or Mbs RNAi with respect to 3D intercalations hold when looking ex vivo. Otherwise, what is the value of the 3D model (could one just use a simpler 2D model of a cross-section of the epithelium)?

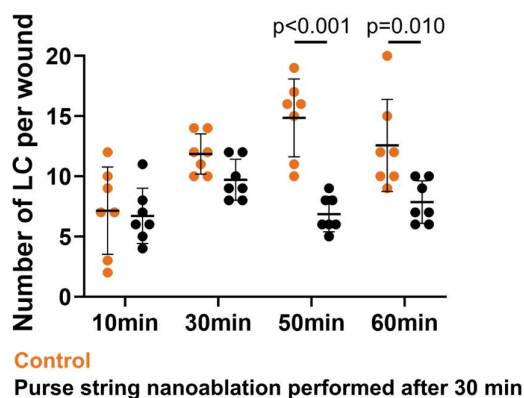
To our knowledge, 3D intercalations during wound healing have not been quantified experimentally in any model system. To quantify this, full 3D cell segmentation would be required and in the wing disc this is extremely challenging even in unwounded discs as we have described in our lab's latest protocols paper (Paci et al, 2025). However, our lab has found that during morphogenesis, the apical 30  $\mu\text{m}$  of the wing disc undergoes dynamic 3D intercalations (Paci et al, 2026), supporting the biological relevance of including this behaviour in the model. Therefore, in our biophysical model, cells intercalate away from the wound edge apically, whereas basal topology is kept during the first 60 minutes due to the extracellular matrix additional 'viscosity' (Kumar et al, 2024; Santillán et al, 2024). Thus, apical and basal cells intercalate differently, and we can use apical intercalations as a proxy for 3D intercalations. In this context, using a simpler 2D model of a cross-section of the epithelium, won't be able to show the importance of cells moving away from the edge (cell-cell intercalation) while

preserving the cell volumes, whereas in our 3D vertex model, we can assess the role of 3D intercalations together with cellular volumes, indentation, and purse string on wound healing efficiency (Fig. 3D, D', D'', No intercalations). In addition, initially in the computational model, there are around 50% of scutoids on each of the 4 different topologies used. Allowing different topologies between apical and basal is important for different mechanical properties between the three different cellular domains (apical, basal, and lateral) consistent with Yanin Guerra Santilla et al., 2024. We added a clarification on the importance of including 3D intercalations in our computational model in the introduction to vertex models (Introduction, 3rd paragraph).

Regarding 3D intercalations predictions of the model on shi[TS] or Mbs RNAi, there are less predicted intercalations in shi[TS] and Mbs RNAi, as shown in Fig. 4P. This prediction holds true for Mbs RNAi ex vivo (Fig. 4K) but not for shi[TS] ex vivo (Fig. 4D), suggesting other behaviours may contribute to intercalations, as discussed in the manuscript, Results section: 'Lateral Cable Assembly And Apical Indentation Require The Apical Purse String For Stabilisation But Not Formation' subsection, last paragraph.

2. Lateral cables seem to be unstable when the apical cable is disrupted (Figure 4F). How does this happen? In vitro and in vivo work showed that myosin can be stabilized by tension/strain. Does loss of the apical cable reduce tension in the lateral cables? This should be tested both in silico and ex vivo.

To directly test whether the loss of apical cable tension affects the lateral cables, we performed nano-ablations of the apical purse string 30 min after wounding, to release tension in the apical cable, and quantified the number of lateral cables before and after the nano-ablation. There is a significant decrease in the number of lateral cables after purse string nano-ablation compared to control wounds (new Figure EV2F and below). This finding demonstrates that maintenance of the lateral cables depends on the integrity of the apical purse string. Testing this hypothesis in silico with the current model is not possible given that we use two different mechanical parameters for purse string strength and lateral cable strength. However, we think the ex vivo results are convincing enough to show that the lateral cables are indeed stabilised by apical cables.



3. Related to the previous point, in page 10 (end of second paragraph) the authors argue that shi[TS] disrupts wound healing by stabilizing cortical tension. But don't their results suggest that the disruption of wound closure is due to a de-stabilization of cortical tension? They should conduct FRAP experiments to measure the turnover of myosin in the lateral cables, both in control and shi[TS] discs and determine the effect of losing the apical cable on lateral myosin turnover.

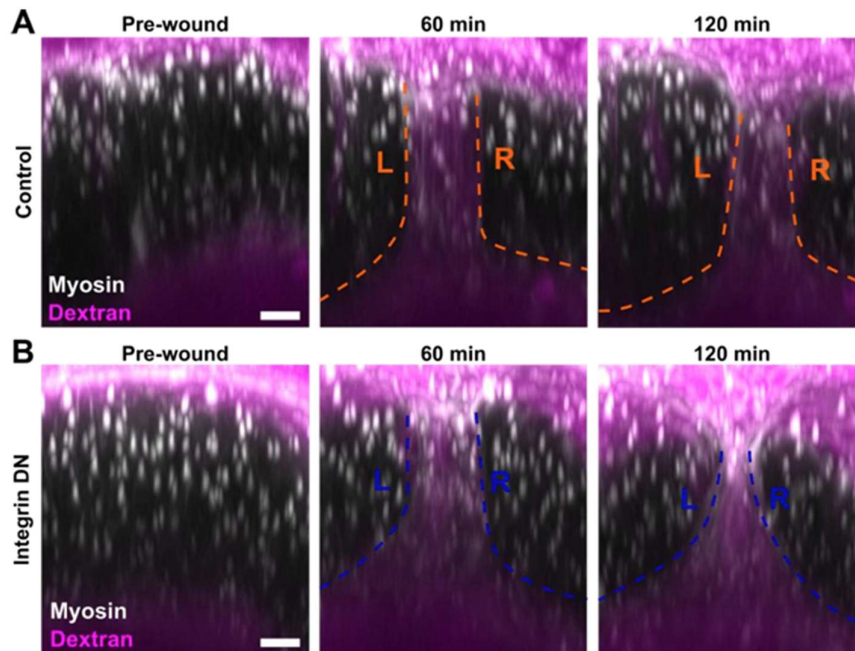
We thank the reviewer for this feedback, and we apologise that our concluding comments for Figure 4 were misleading. By “stabilised cortical tension”, we refer to the overall global increase of tissue tension (because of Mbs-RNAi) rather than elevated tension in the lateral cable itself. Our model proposes that successful wound closure is a dynamic competition between the tension generated by wound healing structures and the overall tissue-wide tension levels. As the reviewer pointed out, in shi[TS], impaired purse string prevents sufficient tension to overcome the resting cortical tension. We have amended the text to remove the term “stabilised cortical tension”.

Nevertheless, we believe that our purse string nano-ablation experiments in the figure above directly test the effect of losing the apical cable on lateral myosin stability. Acute laser nano-ablation of the apical purse string resulted in a reduced number of lateral cables. This finding supports the conclusion that lateral cables are stabilised by tension generated by the purse string.

4. Figure 5G, 6K-L: how do the authors explain that removing basal adhesion in the model increases basal indentation? Wouldn't one expect that cells need to adhere to the substrate to be able to pull on it? I appreciate that this is difficult to assess in discs, but given that both talin and integrin loss of function both lower myosin levels in lateral cables, I have a hard time seeing how the prediction of the model of increased basal indentation with reduced adhesion could hold *ex vivo*. Can the authors try a partial loss of function of talin or integrin (e.g. using a weaker Gal4 driver) to assess whether basal indentation increases or decreases *ex vivo*?

Our working hypothesis is that cells adhere to a stiff basement membrane basally, and this adherence prevents the lateral cables from pulling the stiff basal surface upwards. If we remove this adhesion to the stiff basement membrane, then the basal surface can deform more easily. We would like to clarify that the lateral cables are linked to the basal cell surface rather than directly to the substrate (basement membrane). Our *ex vivo* experiments indicate that loss of basal adhesion does not prevent lateral cable formation, suggesting that these structures do not require substrate attachment. Therefore, in the model, removal of basal adhesion to a stiff substrate would reduce resistance to the pull of the lateral cables, allowing the basal surface of the cells to indent more. We added additional text to clarify this conclusion.

We considered including basal surface images from dextran experiments (below), although these data are qualitative as we could not extract the exact basal surface. We attach an example image below for the reviewer's reference. This data suggests that reducing integrin adhesion to the basement membrane allows the basal surface to indent more, particular at 120min.



Orthogonal max projection of A) control and B) Integrin DN wounds with myosin II in grey and 30kDa Dextran AF647 in magenta. The dextran floods the space without intact cells showing the wound volume and tissue surfaces. Scale bar, 5µm.

#### MINOR

5. Fig. S2A-B: I found it very interesting that the "continuity" between the apical and lateral actomyosin cables seems to happen at points in which adherens junctions are excluded. The authors should discuss how they think that continuity arises to enable the lateral cables to pull on the apical cell surface (e.g. crosslinking?).

We added the following line to our discussion on page 15: "Whilst the mechanism behind lateral cable formation remains unclear, we speculate that actin regulators, such as Arp2/3, may contribute to their branching from the apical purse string and will be explored in future work."

6. Fig 2D.: the position of the wound should be indicated (e.g. with a square bracket at the top of the image). There are many lateral cables, and I was wondering if all of them are associated with cells adjacent to the wound, or if one can also find lateral cables away from the wound edge or in an unwounded epithelium? The authors should show similar images in an intact disc to demonstrate the specificity (or not) of lateral cable formation to wounded epithelia.

We do observe lateral enrichments of myosin in unwounded wing discs due to cell rounding during cell division. However, in comparison to lateral cables, these are only located in regions of dividing cells and have a different structure, rather than the significant accumulation at the wound edge shown in previous Figure 2A (now Figure 2B). We have added additional representative images of cross sections away from wound in our updated Figure 2 and we have indicated the wound positions as suggested.

7. Fig. 2J-K: please, add images that demonstrate the quantitative results. From the images shown in 2I it is hard to see what is happening, as most of the differences in J and K only become significant after 30-60 sec.

We have added additional images on control and lateral ablations as updated Fig. 2G and J.

8. Pages 2, 14: I would suggest citing the original papers showing an actomyosin purse string around wounds in *Drosophila* embryos (Kiehart, 2000 and Wood, 2002). Reference 19 came much later, and although beautiful work, credit should be given to the original papers.

We thank the reviewer for pointing this out and have added these references.

9. Fig. 2H: report the statistical tests used in the figure legend.

We have added the statistical test used in the figure legend of Figure 2: "For I, the plot shows individual data points with the mean (horizontal line), SD (bars) and p-values from two-tailed Mann-Whitney tests."

TYPOS/GRAMMAR

10. Supplement, page 14: please revise "Once the environment is set and compiled the cython files as explained in the README.md"

We thank the reviewer for this comment. We have improved the GitHub repository instructions to reproduce our work. We have also made it available in pypi: <https://pypi.org/project/pyVertexModel/>

Referee #2:

The manuscript by Lim et al. investigates the mechanisms of wound healing in *Drosophila* and presents a 3D vertex model to analyze the underlying processes. The authors show that wound closure is not driven solely by the classical purse-string mechanism, but also involves an important contribution from lateral actomyosin cables. They combine ex vivo experiments with computational modeling to interpret the relative roles of purse-string contraction and lateral cable formation. While several results are interesting, the manuscript would benefit from substantial rewriting to improve clarity, coherence, and precision, and to avoid potential overstatements or misinterpretations. In particular, the rationale behind several experiments should be clarified to help readers better understand the authors' intentions and interpretations.

1. Cell volume (Fig. 1).

The authors state that cell volume remains constant during wound healing; however, the projected cell area also appears largely unchanged (e.g., Fig. S1B). This raises questions about how cell volume is measured and how sensitive the method is to subtle changes. Additional methodological details are needed to clarify the volume quantification. Moreover, at the wound edge, one might expect local volume perturbations (e.g., due to membrane damage or leakage), which are not discussed.

We acknowledge the Fig S. 1B may be misleading as the cells are very elongated, so the projected area does not accurately represent their true size. Figure 1E gives a more accurate depiction of the clones projected in the cross-sectional view. It is also unclear where in the wound and the quantified clone, so we have added clarifying outline annotations in our updated Fig EV 1B. As the cells twist and curve, the cell volume is not calculated by simply area  $\times$  h to get volume. We have added additional methodological details describing how cell volume was quantified: "Finally, the segmented cell volume was extracted from the 3D masks by summing the number of voxels. The cell height was extracted from the number of z slices each mask spanned."

2. Origin and localization of lateral cables.

It is unclear whether the lateral actomyosin cables emerge specifically in response to wounding or whether they are also present under control conditions. Are these structures localized only around the wound margin, or do they also form elsewhere in the tissue? In Fig. 2C, the difference between the pre-wound condition and the 7:30 min post-wounding time point is difficult to appreciate. Clearer visualization or quantitative analysis would help to support the authors' claims.

We have added additional images of unwounded cross sections to help clarify the appearance and timing of the lateral cables as well as updated image intensities. We would like to clarify with the reviewer that the lateral myosin enrichment seen in pre-wounding cross sections is due to myosin from a dividing cell that is coincidental in the image (Figure 2A-B, magenta asterisk). We have observed this in our lab's previous work (Kirkland et al, 2020). We would like to highlight that this myosin is much more diffuse and not in the thin cable-like structure as seen in later wounded timepoints. Updated Fig 2A shows the first 8 min of wound healing where the lateral cables are still emerging, but by 60min as shown in updated Fig 2F and in our quantifications in Fig 4A and 4H, they can be clearly distinguished.

3. Increase of the apical purse string (Fig. 2).

The authors claim that the apical purse-string signal increases over time (p. 4, Fig. 2E), but this trend is not readily apparent in the images shown (Fig. 2C). How was this increase quantified? Providing representative examples and a clearer description of the analysis pipeline would strengthen this conclusion.

We apologise, the timescales for the quantifications and images were not the same. We have added text to clarify the relative timescales of each figure. To show the increase in apical purse string myosin intensity, we have included a montage from a representative movie used for quantifications in Fig. 2E that reviewer referred to (now new Fig. 2D) in our new Fig. EV2B, which clearly shows the increase in apical purse string myosin over the first 20min. Also, we reference our previous work, Tetley et al 2019, for further details about purse string during wound healing in *Drosophila* wing disc. The whole Figure 2 is updated to clarify the lateral cable event timings.

#### 4. Continuity of the apical cable.

The authors claim that the apical cable is continuous, yet this is not clearly evident in the figures. Additional clarification and/or higher-resolution images would help to support this statement.

We apologise for the confusion. Through our imaging we show that there are lateral myosin enrichments, or lateral cables that appear to form "a continuous structure with the apical purse string (Fig. EV2D)", now correct on Page 4. However, we do not claim that the apical purse string is continuous - in fact, it is not (Fig. EV2D, XY view).

#### 5. Laser ablation and recoil measurements.

The authors use nano-ablation to measure recoil following the disruption of either the lateral cables or the apical purse string (p. 4). However, the data shown for recoil (Fig. 2F) appear to correspond to the apical cable only. Is this measured at sites where the purse string colocalizes with lateral cables? It remains unclear to me. It would be helpful to clarify this point and, if possible, to also present recoil measurements from appropriate control regions (as referenced in Fig. S2C).

We have clarified that the apical purse string serves as a marker for the apical surface. The terms "Apical recoil" and "Initial velocity" have been updated to "Lateral recoil" and "Lateral velocity," respectively. We also clarified that the orange and light blue datapoints correspond to separate control conditions and added image of control to Fig. 2G and updated the Fig 2 legend. Section 'Contractile Lateral Actomyosin Cables Form at the Wound Edge and Deform the Tissue', 4<sup>th</sup> paragraph, emphasised lateral and apicobasal axis.

#### 6. Vertex model and cell intercalation.

The authors justify the use of a 3D vertex model in part by stating that cell intercalation plays an important role during wound healing. However, it is not clear from the experimental data where, when, or to what extent such intercalation occurs. This point is not convincingly demonstrated in the manuscript. Because cell intercalation appears to be a key assumption underlying the modeling framework, it should be explicitly shown, quantified, and clearly justified using the experimental data. Also, that would help to understand what the model brings. For instance, the model predicts that removing lateral cables will change cell intercalation. Is it true in the experiments?

To our knowledge, 3D intercalations during wound healing have not been quantified experimentally in any model system. To quantify this, full 3D cell segmentation would be required and in the wing disc this is technically challenging even in unwounded discs as we have described in our lab's latest protocols paper (Paci et al, 2025). However, our lab has found that during morphogenesis, the apical 30um of the wing disc undergoes dynamic 3D intercalations (Paci et al, 2026) supporting the biological relevance of including this behaviour in the model. Therefore, in our biophysical model, cells intercalate away from the wound edge apically, whereas basal topology is kept during the first 60 minutes due to the extracellular matrix additional 'viscosity' (Kumar et al, 2024; Santillán et al, 2024). Thus, apical intercalations are a proxy for 3D intercalations. Using our 3D vertex model, we can then assess the role of 3D intercalations on wound healing efficiency (Fig. 3D, D', D'', No intercalations). In addition, initially in the computational model, there are around 50% of scutoids on each of the 4

different topologies used. Allowing to have different topologies between apical and basal is important to have different mechanical properties between the three different cellular domains (apical, basal, and lateral) consistent with Santillán et al., 2024. We added a clarification on the importance of including 3D intercalations in our computational model in introduction to vertex models (3rd paragraph).

Regarding model predictions on removal of lateral cables and its effect on 3D intercalations ex vivo: for now, it is impossible to genetically separate the formation of lateral cables vs apical purse string, hence we cannot experimentally prevent just the lateral cables from forming. Therefore, we cannot clearly assess the importance of lateral cables in changing intercalations experimentally, hence the need for the modelling approach.

#### 7. Definition and quantification of "apical junction remaining."

The term apical junction remaining is used throughout the manuscript but is never clearly defined. How is this quantity measured and normalized? Based on the description in the Materials and Methods, it is difficult to reconcile the reported ~50% change in Fig. 6D with the example shown in Fig. 6. Please clarify the definition, provide a precise quantification procedure, and include additional representative examples.

We agree with the reviewer and have replaced the term "apical junctions remaining" with "apical wound edge cells". We have also clarified the definition in the text under "A 3D Vertex Model Predicts Cooperative Forces Accelerate Wound Closure", 1<sup>st</sup> paragraph: and the Methods: this metric represents the proportion of apical cells at the wound edge relative to the number of wound edge cells at the first timepoint after wounding. Before wounding, this value is defined as 100%, corresponding to the number of cells surrounding the future wound. At each subsequent time point, the number of cells at the wound edge is counted and normalized to this initial number.

#### 8. Interpretation of adhesion perturbations (integrin and talin).

The statement, "Due to weakened BM attachment, the basal surface can deform more easily, even with weaker lateral cables," is difficult to interpret. Along similar lines, it is unclear how apical wound-healing dynamics can remain largely unaffected by Talin knockdown if the lateral cables are connected to the basal membrane. How are forces transmitted in this case? Overall, the section on adhesion perturbations (integrin and talin) is not clearly explained. This part would benefit from substantial rewriting to clarify the mechanical interpretation and the authors' main message.

We thank the reviewer for this. We have rewritten it and added certain explanations to further illustrate our point.

In short, our working hypothesis is that cells adhere to a stiff basement membrane basally, and this adherence prevents the lateral cables from pulling the stiff basal surface upwards. If we remove this adhesion to the stiff basement membrane, then the basal surface can deform more easily. We would like to clarify that the lateral cables are linked to the basal cell surface rather than directly to the substrate (basement membrane). Our ex vivo experiments indicate that loss of basal adhesion does not prevent lateral cable formation, suggesting that these structures do not require substrate attachment. Therefore, in the model, removal of basal adhesion to a stiff substrate would reduce resistance to the pull of the lateral cables, allowing the basal surface of the cells to indent more.

#### 9. Definition and quantification of suprabasal myosin.

Although a minor point, this reflects a broader issue throughout the manuscript. The term suprabasal myosin is used only 3 times, never clearly defined, despite appearing to be important for

the authors' interpretation. How is suprabasal myosin identified and quantified?

We apologise for this. We have removed the term 'suprabasal' and instead use the terms 'basal myosin' and 'basolateral myosin'. Basal myosin refers to myosin structure present at cell-ECM adhesion puncta whereas basolateral myosin refers to myosin above these cell-ECM puncta.

Referee #3:

The manuscript by Y. Mao and colleagues proposes a 3D description of wound healing in the *Drosophila* wing disk, combining high resolution live imaging and physical modeling with a 3D vertex model. With high resolution confocal microscopy, the authors identified that wound closure is associated with apico basal indentations and 3D cell shape changes. They hypothesized that these cell shape changes could contribute to wound closure and developed a thorough analysis of the process. They identified the presence of lateral actomyosin cables potentially acting as force generators. With perturbation of endocytosis and myosin activity, they could correlate changes in tissue geometrical shape, closure kinetics and myosin enrichment in the different perturbed cases. These data support a model in which the lateral myosin cables generate force promoting the indentation and contributing, because of constant cellular volume, to the wound closure. The authors eventually investigate the connection with the basement membrane and identify a delay in the indentation suggesting a more complex mechanical picture.

Overall the study is very interesting and innovative. The claims are well supported by experimental observations and modeling approaches. Some experimental data are not very clear and needs to be improved, particularly the imaging of the lateral cables. Some aspect of the modeling also would need to be clarified, particularly on some differences observed between the output of the model and the experiments in the apical and basal indentation. With some modification, I believe this work has the potential to be published in EMBO Journal.

Major comments:

-A central element of the manuscript is the presence of lateral myosin cables potentially generating out of plane active forces. While the data shows the presence of these cables relatively clearly, it is very difficult to evaluate with the actual figures when do they really appear, where they are located and how many cables are present. On the Fig 2A and C, we can observe the presence of cables before wounding is performed. It seems that these cable shows an enrichment of myosin during closure. But it is difficult to evaluate with the quality of the images. I would encourage the authors to improve the quality of the images and perhaps to provide quantifications showing the arguments defining lateral cables.

We have now revised Figure 2 to provide better images. We have added additional images of unwounded cross sections to help clarify the appearance and timing of the lateral cables as well as updated image intensities. We would like to clarify with the reviewer that the lateral myosin enrichment seen in pre-wounding cross sections is due to myosin from a dividing cell that is coincidental in the image (Figure 2A-B, magenta asterisk). We have observed this in our lab's previous work (Kirkland et al, 2020). We would like to highlight that this myosin is much more diffuse and not in the thin cable-like structure as seen in later wounded timepoints. Updated Fig 2A shows the first 8 min of wound healing where the lateral cables are still emerging, but by 60min as shown in updated Fig 2F and in our quantifications in Fig 4A and 4H, they can be clearly distinguished.

-Also, the exact location of the cables within the tissue and within the cells is difficult to assess with the shown data. For example, in fig 2D, we see several myosin dot-like enrichments in the XY plane. Are all of these enrichments associated to lateral cables? I would recommend the authors to complement the data with new images showing the location of the lateral cables in cell (is it at junctional vertices?) and in the tissue. It is also very difficult to evaluate the density of cells with lateral cables (particularly density of leading edge cells that display a lateral cable. Such a quantification would be useful for the comparison with the vertex model which includes 1 lateral cable/cell (from what I understood).

We have clarified in the text the importance of evaluating orthogonal views when assessing cable-like structures, as these views allow the presence or absence of cables to be determined more clearly. There may be dots in XY but the only way to determine if these spots correspond to cables is from the XZ views (Fig EV 2C, D). To aid interpretation, we have added additional movies showing the apical purse string and lateral cable structures in 3D, including sequential cross-sections through the same wound and movie showing 3D views of the wound (Figure 2A, B, F). We did show the location of the lateral cables in the cell in previous Fig. S2B (now Fig EV 2D) and have clarified this in the text.

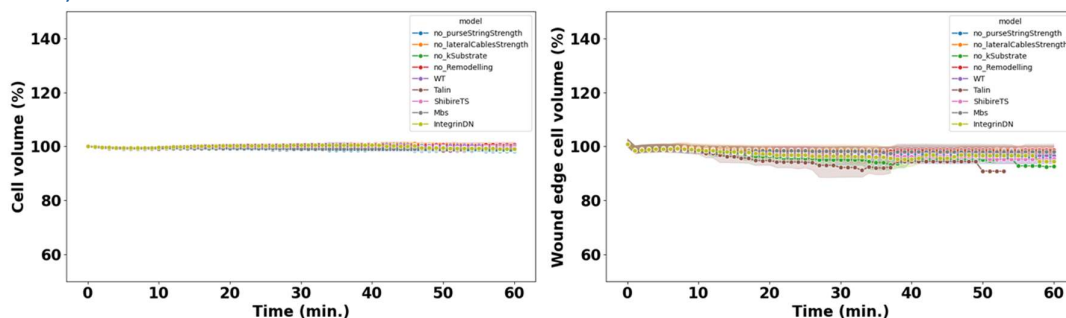
- The laser ablation of the lateral cable shows a recoil of the wound edge, indicating a reduction in the forces promoting wound closure. Consistent with it the authors show also a reduction in myosin intensity. What happen at the level of the apical indentation, do the indentation also shows a reduction consistent with the in-plane recoil?

Yes, that is correct. We previously quantified the reduction of this apical indentation in Figure 2G–H using the terms “Apical recoil” and “Initial velocity.” We recognize that this terminology may be confusing, as “apical recoil” suggests motion parallel to the epithelial plane, whereas in this panel we intended to describe the movement apically. To clarify this, we have changed the axis label to “lateral recoil” and “lateral velocity” in the new Figure panel 2H and 2I, and edited the text (P4).

- About the model: The 3D model appears very nicely done and seems to recapitulate well the kinetics of wound closure. The different supplemental movies are very helpful to visualize the kinetics of closure in the simulation. However, it is not clear to me what the volume labels are indicating as apical and basal volumes appear different. Is it cell volume? Also what is the scale (It's difficult to evaluate whether we look at small volume differences or not)?

From these movies, The volume seems to be not conserved during healing in any of the conditions and therefore it is different from the ex vivo case. It is however difficult to estimate volumes only based on the color coding. The authors should control this parameter and include quantifications in the manuscript (as a supp. Figure for example).

To clarify this, we have provided additional panels plotting how cells’ volume changes over time (Fig. EV 3F). We have also plotted how wound edge cells’ volume evolves over time. All conditions are tissue average volume: 100%  $\pm$  3%; whereas wound edge average volume: 100%  $\pm$  10% (1 outlier with +15%).



-Related to my previous point, In the Fig 3D in absence of lateral cables, the quantification shows an absence of apicobasal indentation. I find this result surprising as I would expect that purse string force generation + constant volume would generate indentation anyway. What could be the cause of this observation, is it due to an increase in cell volume? The authors should explain this result.

We agree that this is indeed an interesting result. There is a slight increase in tissue average volume (0.1%), with the same change at the wound edge (0.2%) with respect WT in silico simulations. We

believe that, given the mechanical parameters used in the model, the consequence of no indentation is reflected in a delayed healing (apical wound closure Fig. 3D', green dots).

-From the Figure 3 onward, the authors introduce the quantification of apical junction loss. It is however not very well introduced with a lack of definition of which apical junction loss is quantified. In the remaining of the manuscript, it is not very clear how these quantifications bring a mechanistic understanding of the process. I would invite the authors to introduce and explain more extensively the relationship between this junctional loss potential cell intercalations and the lateral myosin cables.

We agree with the reviewer, we have changed the term, “apical junctions remaining” to “apical wound edge cells” as also pointed out by reviewer 2.

-Finally, the imaging of the basement membrane is particularly interesting, the fig 5 A-B shows a delay in the basal indentation compared to the apical indentation, suggesting a change in the force balance at mid-closure and two potential different phases of closure. Is it correct? As it is presented, the model seems to not recapitulate this sequential deformation. The authors could discuss the limitations of the model to recapitulate this sequential deformation and probably the need to include changes in parameters at mid closure. Also, the different perturbations already used (shi, MbsRNAi, Talin RNAi and integrin DN) may have an impact on this delay. It would be interesting to see the changes in basement membrane indentation in these different cases. I believe this is an important observation of the manuscript that could be highlighted or discussed if there are really two sequential phases of apico basal deformations.

We agree with the reviewer. There should be indeed a change of force balance around 60 minutes when the basement membrane starts indenting upwards. However, we acknowledge this is beyond the scope of the model and would require more experimental data to properly model it with the computational model. We expanded our discussion on this matter in Discussion 4<sup>th</sup> paragraph and in Results 'Basement Membrane Deformation Correlates with Lateral Cable Myosin II Intensity' subsection, 2<sup>nd</sup> paragraph.

Minor comments:

-In the Fig 1B, the cadherin signal is depleted in the region that undergo apico basal deformation, is it correct? Or is it a coincidence in this particular example? This may have some mechanical consequences if it's the case.

Similar depletion of E-cadherin in this region was previously reported in Fig. 1 of (Tetley et al, 2019). While the signal appears reduced in the area undergoing apicobasal deformation, we have not directly investigated the functional consequences of this in the current study. In the model, we restrict the analysis to the first four rows of cells surrounding the wound edge. We therefore believe that the current modelling computational framework remains appropriate despite this observation.

-In the Fig 1E, the cadherin signal is not visible. I would suggest to change the image

We have removed the E-cadherin channel from these images as they are not used for any quantification in the subsequent subfigures. The E cadherin signal was used to mark the wound and is shown clearly in Fig. EV 1B.

-In the Fig 2A, the contrast is very weak, it is very difficult to identify structures (like wound edge or cell edges). I would suggest to change the image for one with better contrast.

We agree with the reviewer. We have added additional XZ slice images and annotations as Figure 2A and B and updated the image intensities to clearly show the lateral cables (yellow arrows).

-In the figure 2E the axis seems to be not correct (inverted).

We thank the reviewer for spotting this error and have corrected this.

-The Fig S3A needs a legend in the figure to clearly distinguish the simulations from the experiment. Also an explanation should be added in the main text to explain that the motivation is to approach experimental geometry prior ablation.

We have added an explanation in the main text and added the legend to Fig. EV3A to differentiate between ex vivo average (orange) and simulations (blue).

-Fig 3C apical indentation curves are slightly different between simulation and ex vivo, with a faster increase followed by a decrease in speed in the simulation compared to ex vivo that steadily increases. Is it due to progressive changes in purse string or lateral cable tension that are not included in the model? Or perhaps to different cell volume contribution in ex vivo and in the simulation? This could be discussed.

We thank the reviewer for this comment and appreciate the differences between computational and ex vivo model. For simplicity, in the model there is a cable per cell throughout the simulation that follows the same trajectory as updated Fig 2E (previously Fig. 2E') lateral cables myosin intensities. However, the number of cables change dynamically over time in all conditions (Fig. 4F,M and Fig. 6F) with varying myosin intensities (Fig. 4G,N and Fig. 6G). We think that is a plausible reason for that change.

-In the model the volume contribution  $W_v$  is kept for the debris, I don't see clear reason for that as I believe that ablated cells will have a strong change in volume. Is there a physical reason to keep the energy term in the simulations?

We apologise for the confusion. Debris cells do only contribute to the  $W_v$ , but their contribution is much lower ( $\lambda_{v_{debris}} = 1e-8$ ) compared to regular cells ( $\lambda_v = 1$ ), as stated in Expanded View.

-Introduction of the previous 3D models could be a bit extended particularly in the context to understand why inclusion of scutoids forms is novel and interesting and what it actually brings in the context of this study.

We agree with the reviewer and have extended our introduction of previous 3D models.

## Summary of new text changes

### Throughout

Changed “apical junctions” to “apical wound edge cells”.

Changed “junction loss” to “apical wound edge cell loss”

Changed “suprabasal” to “basolateral”.

Specified that replicates were biological replicates in figure legends.

### Abstract

Added previously to emphasise that tissue fluidisation is paper Tetley et al., 2019.

### Introduction

3<sup>rd</sup> paragraph expanded why scutoids are important for computational models.

### Results

‘Contractile Lateral Actomyosin Cables Form at the Wound Edge and Deform the Tissue’ section.

1<sup>st</sup> paragraph – Added details about figure 2A and Figure S2C, D.

2<sup>nd</sup> paragraph – Added times of experiments related to Fig. 2C, D. Emphasised explanation of Figure 2D. Added new information about Figure 2B.

3<sup>rd</sup> paragraph – Emphasised ‘lateral recoil velocity in the apicobasal axis’ Figure 2I.

4<sup>th</sup> paragraph – Updated new panel order.

Figure Legend 2 – Emphasised time explicitly. Statistical tests detailed. Added information about new panels A, B, G, H.

‘A 3D Vertex Model Predicts Cooperative Forces Accelerate Wound Closure’ section.

1<sup>st</sup> paragraph – Quantifying metrics rather than mechanisms to be precise and added a sentence to define apical wound edge cells.

‘Lateral Cable Assembly And Apical Indentation Require The Apical Purse String For Stabilisation But Not Formation’ section.

2<sup>nd</sup> paragraph – New results from nanoablations of the apical purse string detailed.

7<sup>th</sup> paragraph – Corrected comment about ‘stabilising cortical tension’.

‘Basement Membrane Deformation Correlates with Lateral Cable Myosin II Intensity’ section.

2<sup>nd</sup> paragraph – Improve explanation of results and hypothesis of in silico simulations.

### Discussion

3<sup>rd</sup> paragraph – extended to discuss mechanism on lateral cable formation.

4<sup>th</sup> paragraph – extended discussion on basement deformation and why we only model the first 60 minutes in silico.

### Methods

Added to table of Experimental models two new rows that were missing.

Added in ‘Laser ablation’ explanations of the new nanoablations performed on the purse string.

Updated how clone height and volume was calculated based on reviewer’s comments.

Rewritten ‘Lateral recoil and velocity’ subsection for clarity.

Updated explanation for the apical wound edge cells metric.

Added justification for statistical tests chosen.

### Expanded View Methods

Updated section ‘Running the code’.

Updated repository instructions for installation.

#### New figures

Fig 1B: Images replaced to show E-cadherin signal more clearly.

Fig 1E: We have removed the e-cadherin channel.

Fig EV1B: Wound outlined (white dotted line), and quantified clone annotated (white asterisk).

Fig 2: New panels A and B with images highlighting the wound (yellow dotted line), lateral cables (yellow arrow), and other structures that may look like lateral cables (red arrow, and magenta asterisk), but they are not. Also, new panel G, H with new images of purse string and lateral cables after nano-ablations.

Fig EV2: New panels A, B, E and F. A shows more timepoints from the previous Figure 2C to clarify the timings of purse string and lateral cable formation. B shows an examples of the complete timelapse of the apical wound area used in quantifications for 2D-F. E shows the corresponding nano-ablation ROIs for the wounds shown in 2G and J as an example. F is the new purse string nano-ablation data acquired in revisions.

Fig EV3F: New panel with simulation volumes.

Fig 5: Changed the order of subfigures to match the order appearance in the text.

Dear Yanlan,

Thank you for submitting a revised version of your manuscript to The EMBO Journal. I apologise for the delay in the assessment process due to summer holidays. I have now received input from all original reviewers, who are generally satisfied with the performed revisions and now suggest only minor suggestions for the final polishing of the article.

There are also a few formatting aspects that need to be implemented before I can extend the official acceptance of the manuscript:

1. Please note that it is The EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letters) to be published as an online supplement to each paper. If you should prefer removal of any referee-only figures included in the point-by-point response(s), e.g. because they may still be used for future publication or because they have been reproduced from published work by others, please do let us know immediately via response email.

More information is available here: [https://www.embopress.org/transparent-process#Review\\_Process](https://www.embopress.org/transparent-process#Review_Process)

2. Please check that the funding information is correct and identical both in the manuscript and our online system. Currently, ID2020-116141GB-I00 funded by MCIN/AEI, SGR 01049 from the local government Generalitat de Catalunya, and EMBO Young Investigator Program are mentioned in the Acknowledgments but not in the funders list in our system.

3. Email to Robert J Tetley (r.tetley@ucl.ac.uk) was returned to the sender. Please update his contact details.

4. Please update the order and the headings of the manuscript sections to: Abstract / Introduction / Results / Discussion / Methods / Data Availability / Acknowledgements / Disclosure and Competing Interests Statement / References / Figure Legends / Expanded View Figure Legends.

5. CRediT has replaced the traditional author contributions section because it offers a systematic, machine-readable author contributions format that allows for more effective research assessment. Please remove the Authors Contributions from the manuscript and use the free text boxes beneath each contributing author's name in our online submission system to add specific details on the author's contribution. More information is available in our guide to authors.

6. Please add the supplementary methods to the Methods section in the manuscript text to enhance their accessibility.

7. For the Expanded View items, please correct the nomenclature to Figure EV1-EV4 and Table EV1 (including in the figure legends and citations).

8. Please remove the legend for Table EV1 from the expanded view content file and from the manuscript text and add it directly to the excel file Table EV1.

9. Please remove movie legends from the expanded view file and manuscript text and zip each legend as a simple text file with the corresponding movie file.

10. In our standard image check, we noted that some of the immunofluorescence images are reused between:

- Figure 4H and Figure 6H.

- Figure 4H and Figure EV2B.

Please check - if this is intentional, please state this explicitly in the figure legends.

11. Our data editors have flagged the following issues in figure legends that need correcting:

- Please indicate the statistical test used for data analysis in the legends of figures 6J,K.

- Please define the scale bar in the legend of figure EV1B.

12. Papers published in The EMBO Journal are accompanied online by a 'Synopsis' to enhance discoverability of the manuscript. It consists of A) a short (1-2 sentences) summary of the findings and their significance, B) 3-4 bullet points highlighting key results (the highlights currently included in the manuscript can be used here) and C) a synopsis image that is 550x300 pixels large (width x height, jpeg or png format). You can either show a model or key data in the synopsis image. Please note that the image size is rather small and that text needs to be readable at the final size.

Please let me know if you have any questions regarding any of these points. You can use the link below to upload the revised files.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal. I look forward to receiving the final version!

With best wishes,

Ieva

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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (10th Dec 2026). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

<https://emboj.msubmit.net/cgi-bin/main.plex>

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Referee #1:

The authors have addressed most of my concerns. I would encourage them to include grayscale versions of the two separate images that make up each of the panels in Figure 2J: as it stands, it is very hard to see the proposed reduction in myosin II intensity. Showing the before and after images in grayscale, next to the colour merge, would facilitate visualization of changes in intensity (the merge is useful to visualize changes in wound area).

Also, I have an additional (optional) suggestion that would directly demonstrate the power of using the 3D model. I understand the argument provided by the authors about the importance of using a 3D model to show the role of cell intercalation with volume preservation. The authors could drive that point home by showing the predictions of a 2D version of their model vs. their 3D model, thus demonstrating the superior predictive ability of the 3D version.

Referee #2:

The authors have convincingly addressed my concerns, and I recommend the paper for publication.

Referee #3:

The authors have performed a substantial revision of the manuscript and the quality of the manuscript significantly improved. My initial main concerns on the quality of the images showing the basolateral actomyosin cables have been very well addressed. My additional concerns on the details and interpretation of the model also have been addressed correctly. Therefore, I believe now that this work is close to be suitable for publication. I have only a couple of minor points that the authors could address before.

-The author mention the inclusion of supplementary movie highlighting the localization of the cables in the response to reviewer. These movies are not present. Having them may help the reader for assessment of the cables.

-related to my point on the contribution of cables. In the absence of cable, if I understand well, the reduced wound edge cell deformation is compensated by a global tissue volume increase. This should be clearly stated in the text.

17 September 2026

Dear Reviewers,

Thank you for reading our revised manuscript and for suggesting some further minor edits before acceptance.

Please find below our detailed point-by-point response to the reviewers' comments. For your ease, reviewers' comments are in black, our responses are in blue. Within the manuscript content, all newly added text is in blue.

Referee #1:

The authors have addressed most of my concerns. I would encourage them to include grayscale versions of the two separate images that make up each of the panels in Figure 2J: as it stands, it is very hard to see the proposed reduction in myosin II intensity. Showing the before and after images in grayscale, next to the colour merge, would facilitate visualization of changes in intensity (the merge is useful to visualize changes in wound area).

We have added the proposed greyscale images as Fig. EV2F.

Also, I have an additional (optional) suggestion that would directly demonstrate the power of using the 3D model. I understand the argument provided by the authors about the importance of using a 3D model to show the role of cell intercalation with volume preservation. The authors could drive that point home by showing the predictions of a 2D version of their model vs. their 3D model, thus demonstrating the superior predictive ability of the 3D version.

We thank the reviewer for their helpful comments. Unfortunately, comparing the results of a 2D model and a 3D one seems out of the scope of this work given that to faithfully comparing them, equations, geometry, and mechanical parameters should be coupled between 2D and 3D. However, the reader can compare results from previous works such as Tetley et al., 2019 (2D model) and ours (3D model).

Referee #2:

The authors have convincingly addressed my concerns, and I recommend the paper for publication.

We thank the reviewer for their positive comment.

Referee #3:

The authors have performed a substantial revision of the manuscript and the quality of the manuscript significantly improved. My initial main concerns on the quality of the images showing the basolateral actomyosin cables have been very well addressed. My additional concerns on the details and interpretation of the model also have been addressed correctly. Therefore, I believe now that this work is close to be suitable for publication. I have only a couple of minor points that the authors could address before.

-The author mention the inclusion of supplementary movie highlighting the localization of the cables in the response to reviewer. These movies are not present. Having them may help the reader for

assessment of the cables.

All the movies and additional information have been uploaded to zenodo and it is publicly available:  
<https://www.ebi.ac.uk/biostudies/studies/S-BSST3135>

-related to my point on the contribution of cables. In the absence of cable, if I understand well, the reduced wound edge cell deformation is compensated by a global tissue volume increase. This should be clearly stated in the text.

We have added a sentence related to this in the MS: “Interestingly, in our simulations with no lateral cables (Fig. 3D), the reduced wound edge cell deformation is compensated with a slight increase of tissue volume of approximately 0.1% (Fig. EV3F).”

Dear Yanlan,

Thank you for incorporating the final editorial and formatting requests in the manuscript. I am now happy to inform you that your manuscript has been accepted for publication. Congratulations on a great study!

Before we forward your manuscript to our publishers, I would like to propose some minor edits in the manuscript title, abstract and synopsis. We have also selected a short blurb that will accompany the title of your manuscript in our online table of contents. Please take a look at the proposed textual changes in the attached file - any corrections from your side are very welcome.

Your manuscript will be processed for publication by EMBO Press. It will be copy edited and you will receive page proofs prior to publication. Please note that you will be contacted by Springer Nature Author Services to complete licensing and payment information.

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Once I have received your input on the final textual changes, I will be happy to forward your manuscript to our publishers for typesetting.

Thank you for this interesting contribution to The EMBO Journal!

Best wishes,

Ieva

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EMBO Press Author Checklist

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|----------------------------------------|
| Corresponding Author Name: Yanlan Mao  |
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Reporting Checklist for Life Science Articles (updated January

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: [10.31222/osf.io/9sm4x](#)). Please follow the journal's guidelines in preparing your

**Please note that a copy of this checklist will be published alongside your article.**

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple  $\chi^2$  tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.

Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

| Newly Created Materials                                                                                                                                                                                                     | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| New materials and reagents need to be available; do any restrictions apply?                                                                                                                                                 | Not Applicable                          |                                                                                                                                         |
| Antibodies                                                                                                                                                                                                                  | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| For <b>antibodies</b> provide the following information:<br>- Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number<br>- Non-commercial: RRID or citation                        | Not Applicable                          |                                                                                                                                         |
| DNA and RNA sequences                                                                                                                                                                                                       | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| <b>Short novel DNA or RNA including primers, probes:</b> provide the sequences.                                                                                                                                             | Yes                                     | Reagents and Tools Table                                                                                                                |
| Cell materials                                                                                                                                                                                                              | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| <b>Cell lines:</b> Provide species information, strain. Provide accession number in repository <b>OR</b> supplier name, catalog number, clone number, and <b>OR</b> RRID.                                                   | Not Applicable                          |                                                                                                                                         |
| <b>Primary cultures:</b> Provide species, strain, sex of origin, genetic modification status.                                                                                                                               | Not Applicable                          |                                                                                                                                         |
| Report if the cell lines were recently <b>authenticated</b> (e.g., by STR profiling) and tested for mycoplasma contamination.                                                                                               | Not Applicable                          |                                                                                                                                         |
| Experimental animals                                                                                                                                                                                                        | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| <b>Laboratory animals or Model organisms:</b> Provide species, strain, sex, age, genetic modification status. Provide accession number in repository <b>OR</b> supplier name, catalog number, clone number, <b>OR</b> RRID. | Yes                                     | Reagents and Tools Table                                                                                                                |
| <b>Animal observed in or captured from the field:</b> Provide species, sex, and age where possible.                                                                                                                         | Not Applicable                          |                                                                                                                                         |
| Please detail <b>housing and husbandry conditions</b> .                                                                                                                                                                     | Yes                                     | Methods                                                                                                                                 |
| Plants and microbes                                                                                                                                                                                                         | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| <b>Plants:</b> provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).                                         | Not Applicable                          |                                                                                                                                         |
| <b>Microbes:</b> provide species and strain, unique accession number if available, and source.                                                                                                                              | Not Applicable                          |                                                                                                                                         |
| Human research participants                                                                                                                                                                                                 | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.                                                                                            | Not Applicable                          |                                                                                                                                         |
| Core facilities                                                                                                                                                                                                             | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| If your work benefited from core facilities, was their service mentioned in the acknowledgments section?                                                                                                                    | Yes                                     | Acknowledgements                                                                                                                        |

Design

| Study protocol                                                                                                                                                           | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| If study protocol has been <b>pre-registered</b> , provide DOI in the <b>manuscript</b> . For clinical trials, provide the trial registration number <b>OR</b> cite DOI. | Not Applicable                          |                                                                                                                                         |
| Report the <b>clinical trial registration number</b> (at ClinicalTrials.gov or equivalent), where applicable.                                                            | Not Applicable                          |                                                                                                                                         |

| Laboratory protocol                                                                                     | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Provide DOI OR other citation details if <b>external detailed step-by-step protocols</b> are available. | Not Applicable                          |                                                                                                                                         |

| Experimental study design and statistics                                                                                                                                                                                                                                                                                                   | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Include a statement about <b>sample size</b> estimate even if no statistical methods were used.                                                                                                                                                                                                                                            | Yes                                     | Figures                                                                                                                                 |
| Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. <b>randomization procedure</b> )? If yes, have they been described?                                                                                                                                                     | Yes                                     | Methods                                                                                                                                 |
| Include a statement about <b>blinding</b> even if no blinding was done.                                                                                                                                                                                                                                                                    | Yes                                     | methods                                                                                                                                 |
| Describe <b>inclusion/exclusion criteria</b> if samples or animals were excluded from the analysis. Were the criteria pre-established?                                                                                                                                                                                                     | Yes                                     | Methods                                                                                                                                 |
| If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.                                                                                                                                                                                               |                                         |                                                                                                                                         |
| For every figure, are <b>statistical tests</b> justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared? | Yes                                     | Methods                                                                                                                                 |

| Sample definition and in-laboratory replication                                                  | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|--------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| In the figure legends: state number of times the experiment was <b>replicated</b> in laboratory. | Yes                                     | Figures                                                                                                                                 |
| In the figure legends: define whether data describe <b>technical or biological replicates</b> .  | Yes                                     | Figures                                                                                                                                 |

#### Ethics

| Ethics                                                                                                                                                                                                                                                                                                   | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Studies involving <b>human participants</b> : State details of <b>authority granting ethics approval</b> (IRB or equivalent committee(s), provide reference number for approval.                                                                                                                         | Not Applicable                          |                                                                                                                                         |
| Studies involving <b>human participants</b> : Include a statement confirming that <b>informed consent</b> was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report. | Not Applicable                          |                                                                                                                                         |
| Studies involving <b>human participants</b> : For publication of <b>patient photos</b> , include a statement confirming that consent to publish was obtained.                                                                                                                                            | Not Applicable                          |                                                                                                                                         |
| Studies involving experimental <b>animals</b> : State details of <b>authority granting ethics approval</b> (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.                                                           | Not Applicable                          |                                                                                                                                         |
| Studies involving <b>specimen and field samples</b> : State if relevant <b>permits</b> obtained, provide details of authority approving study; if none were required, explain why.                                                                                                                       | Not Applicable                          |                                                                                                                                         |

| Dual Use Research of Concern (DURC)                                                                                                                                                                                                                   | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Could your study fall under dual use research restrictions? Please check biosecurity documents and list of <b>select agents and toxins</b> (CDC): <a href="https://www.selectagents.gov/sat/list.htm">https://www.selectagents.gov/sat/list.htm</a> . | Not Applicable                          |                                                                                                                                         |
| If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?                                                                                                                                              | Not Applicable                          |                                                                                                                                         |
| If a study is subject to dual use research of concern regulations, is the name of the <b>authority granting approval and reference number</b> for the regulatory approval provided in the manuscript?                                                 | Not Applicable                          |                                                                                                                                         |

#### Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

| Adherence to community standards                                                                                                                                                                                                                                                                                                     | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| State if relevant guidelines or checklists (e.g., <b>ICMJE, MIBBI, ARRIVE, PRISMA</b> ) have been followed or provided.                                                                                                                                                                                                              | Not Applicable                          |                                                                                                                                         |
| For <b>tumor marker prognostic studies</b> , we recommend that you follow the <b>REMARK</b> reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.                                                                               | Not Applicable                          |                                                                                                                                         |
| For <b>phase II and III randomized controlled trials</b> , please refer to the <b>CONSORT</b> flow diagram (see link list at top right) and submit the <b>CONSORT</b> checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list. | Not Applicable                          |                                                                                                                                         |

#### Data Availability

| Data availability                                                                                                                                                                                 | Information included in the manuscript? | In which section is the information available?<br>(Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Have <b>primary datasets</b> been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section? | Yes                                     | Data Availability                                                                                                                       |
| Were <b>human clinical and genomic datasets</b> deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?  | Not Applicable                          |                                                                                                                                         |
| Are <b>computational models</b> that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?        | Yes                                     | Data Availability                                                                                                                       |
| If publicly available data were reused, provide the respective <b>data citations in the reference list</b> .                                                                                      | Not Applicable                          |                                                                                                                                         |