nature research

Peer Review Information

Journal: Nature Cell Biology
Manuscript Title: Rear traction forces drive adherent tissue migration in vivo
Corresponding author name(s): Holger Knaut

Reviewer Comments & Decisions:

Decision Letter, initial version:

Dear Holger,

Your manuscript, "A rear-engine drives adherent tissue migration in vivo", has now been seen by 2 referees, who are experts in cell migration, development (referee 1); cell migration, biophysics (referee 2), with our sincere apologies for the delayed review process. Despite our best efforts, we have not yet received comments from Reviewer #3, but we will pass them on to you if/when we do receive them. As you will see from their comments (attached below) Reviewers 1 and 2 find this work of potential interest, but have raised substantial concerns, which in our view would need to be addressed with considerable revisions before we can consider publication in Nature Cell Biology.

Nature Cell Biology editors discuss the referee reports in detail within the editorial team, including the chief editor, to identify key referee points that should be addressed with priority, and requests that are overruled as being beyond the scope of the current study. To guide the scope of the revisions, I have listed these points below. We are committed to providing a fair and constructive peer-review process, so please feel free to contact me if you would like to discuss any of the referee comments further.

I should stress that points #1 and #2 from reviewer 2 about the conceptual advance over Shellar et al. present a significant concern in our view, and reconsideration of the study for this journal and re-engagement of referees would depend on the strength of these revisions to yield data that raises the conceptual advance.

In particular, it would be essential to:

A) Carry out additional experiments to test whether cells in the side/rear form adhesions differently from the front, or whether cell density is the driver of the 'rear engine', and further probe the link between the molecular machinery to address how cells in the rear and sides produce greater force.

B) Conduct further studies to clarify the roles of the chemokine CcCl12a gradient and Cxcr4b receptor as a means for directional movement.

C) As mentioned above, address the comments of Reviewer 2 with regard to the the conceptual advance provided (in particular, Shellard et al, Science 2018; Olson and Nechiporuk Dev Biol 2021) with new experimentation.

D) All other referee concerns pertaining to strengthening existing data, providing controls, methodological details, clarifications and textual changes, should also be addressed.

E) Finally please pay close attention to our guidelines on statistical and methodological reporting (listed below) as failure to do so may delay the reconsideration of the revised manuscript. In particular please provide:

- a Supplementary Figure including unprocessed images of all gels/blots in the form of a multi-page pdf file. Please ensure that blots/gels are labeled and the sections presented in the figures are clearly indicated.

- a Supplementary Table including all numerical source data in Excel format, with data for different figures provided as different sheets within a single Excel file. The file should include source data giving rise to graphical representations and statistical descriptions in the paper and for all instances where the figures present representative experiments of multiple independent repeats, the source data of all repeats should be provided.

We would be happy to consider a revised manuscript that would satisfactorily address these points, unless a similar paper is published elsewhere, or is accepted for publication in Nature Cell Biology in the meantime.

When revising the manuscript please:

- ensure that it conforms to our format instructions and publication policies (see below and https://www.nature.com/nature/for-authors).

- provide a point-by-point rebuttal to the full referee reports verbatim, as provided at the end of this letter.

- provide the completed Reporting Summary (found here https://www.nature.com/documents/nr-reporting-summary.pdf). This is essential for reconsideration of the manuscript will be available to editors and referees in the event of peer review. For more information see http://www.nature.com/authors/policies/availability.html or contact me.

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.

-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

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This journal strongly supports public availability of data. Please place the data used in your paper into a public data repository, or alternatively, present the data as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories appears below.

Please submit the revised manuscript files and the point-by-point rebuttal to the referee comments using this link:

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*This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We would like to receive a revised submission within six months.

We hope that you will find our referees' comments, and editorial guidance helpful. Please do not hesitate to contact me if there is anything you would like to discuss.

With best wishes,

Christina

Christina Kary, PhD
Chief Editor
Nature Cell Biology
1 New York Plaza
Tel: +44 (0) 207 843 4924

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
The authors identify a rear based engine that drives the migration of primordial cells along the basement membrane. They use two approaches. First, imaging by TEM and fluorescent laminin (an exciting new tool). The imaging is clear and is of high quality. Second is Embryogram to measure the deformation of BM by primordium. Embryogram works by mapping fixed points on the basement membrane and then measuring how they change over time. This is used to construct a map of basement membrane deformation under the primordium as it moves. To derive traction force measurments, they measure the physical property of the substrate. By knowing how stiff the substrate is and how much it moves due to presence of the primordium, they can calculate traction force generated by the primordium.
Using Embryogram they identify a mode of migration that is characterized by increased traction force focused on the sides and back of the primordium. The front provides minimal traction stress on BM. There is compelling and reproducible (Embryogram measurements coupled with more conventional line scan showing LamC1 crumpling) to show the physical dynamics of primordium BM interaction.
The next sections describes the molecular details of how these forces are generated. The authors identify integrin and talin as the primary drivers of migration through their interaction with actin and the matrix. Using genetic approaches the authors show that Itgb1b and Tln1 form relatively transient adhesions with the basement membrane in vivo, and are required for tissue migration.
This study provides novel insight into the movement of cells/tissue in vivo. They identify that transient, rather than stable, adhesions are utilized. Further, they identify a "rear-engine" that drives motility. This observation, that traction forces are highest toward the rear and sides, is interesting, but more could be done to link this feature of tissue migration to the molecular components (integrin/talin) that produce the required force.

Questions and Concerns

1. The authors note that cells of the primordium contribute equally to directed migration (Discussion) and hypothesize that the increased density of cells in the rear accounts for increased force production there rather than the front of the tissue. Can this be measured? This is a crux of the paper, but they don't do experiments designed to ask whether the cells in the side/rear form adhesions differently than the front. It is conceivable that they do, which would open interesting questions about how this is done, or it is possible that adhesions are transient throughout and cell density is the driver of the "rear-engine". The authors could assess stability of integrin/talin/actin using the existing approaches in the paper (FRAP, actin flow measurement) and the chimeric primordium to look at cells in different regions that correspond to their traction force map.

2. They initially introduce the chemokine CxCl12a gradient and Cxcr4b receptor as the means for directional movement. How does this work with the rear-engine (i.e. cells in the front sense directionality, but those in the rear produce majority of traction force)?

Specific concerns:
1. Related to figure 1e-g: Do they have data that compares WT primordial migration (LamC1 +/+ and Cxcl12a +/+) with LamC1 +/+, Cxcl12a null larvae injected with acta1:cxcl12a?

The comparisons shown adequately show that whatever level of Cxcl12a expression is achieved in muscle is capable of promoting migration in the presence of BM, but we do not know if this is equivalent to the WT context. Comparing migration distance between the genotypes above would provide a convincing control that the comparisons shown in Fig. 1g are replicating to some degree the normal chemokine environment.
Currently, the evidence that there is sufficient Cxcl12a to be "functional" is the receptor internalization data (Fig. 1h-j). But it still seems possible that they are undershooting the WT Cxcl12a expression. While not probable, it may be possible that more Cxcl12a could overcome the lack of BM in lamC1 -/-. Thus they should show the Cxcl12a expression they achieve ectopically is close to WT level. Doing so by comparing migration distance with the genotypes above would be sufficient.

2. Related to figure 1h-j: As noted, the purpose of this is to show that their muscle-specific Cxcl12a expression is capable of stimulating the primordium. This method of quantification seems very reliant on the plane of imaging (i.e. the Kate signal is still present but if you imaged lower into the cells you could achieve a higher RFP/GFP ratio even after Cxcl12a expression). The important part to this is not the intensity, but the localization of signal. Can authors show 3D representation of Z-stack to show internalization of Cxcr4b-Kate (and this movement away from memGFP? Line-scan of memGFP illustrating strong co-localization without Cxcl12a, but loss of co-localization with Cxcl12a would also work.
3. Do the authors ever link the molecular force production machinery (Itgb1a and Tln1) to the traction stress measurements from the first half of the paper? For example, they show in great detail that higher traction stress is produced at the sides and back of the primordium as it moves forward. Does this coincide with more stable complexes in these locations? They describe the transient nature of integrin/talin interaction with actin in the primordium (mostly Extended data 8), but would these still be predicted to be more stable (i.e. less FRAP recovery) in areas where increased force generation occurs. Can the authors measure Itgb1b/Tln1/Actin interaction across the primordium (front/side/rear)?
Question: Where did they measure the integrin/talin/actin interactions? They note it is in the basal portion of the primordium cell membrane, but where in relation to the front/side/rear?
Again this goes back to the general question I have related to how the cells in the rear and sides are producing greater force. Is it simply that there are more of them, or is it that they are capable of forming more mature adhesions to produce greater force?

4. Related to Extended data 6k: They mention forming the chimeric primordium in which Talin depletion was concentrated in cells toward the front of the primordium. It is difficult to assess whether that was the case, but would they expect this to actually affect migration more-so than talin depletion in the rear of the primordium given that additional force is required in the rear?
It is believable that talin depletion generally would be expected to reduce migration, but if the authors are capable of finding chimeric primordia with enriched depletion in the front vs. back, it would be interesting to see if one of these affected migration more than the other. The same could be said for Itgb1b expression.
This is a related experiment to the thought above about linking their traction force measurements to the molecular components that produce such force.
5. Related to figure 4i-k: The authors assess actin flow as an indirect measure of the stability of the adhesions primordium cells are making as they migrate. Increased actin retrograde flow and polymerization rate in Itgb1b -/- indicates that integrins (and talin) are needed to produce the traction force for migration. However, it would be more impactful if the authors could assess cells located within different regions of the primordium as described above and link this to the traction forces they

have measured.
This would indicate whether cells at different regions of the primordium produce fundamentally different types of adhesions, thus accounting for the increased traction force (and providing some cool additional biological questions) or whether the increased traction force is somehow a product of cell density or regional differences in BM stiffness, etc.
This raises an overall question, that should be at least discussed more in the text- related to the question above is: How are cells sensing a chemokine gradient in the front, but responding primarily with force generation toward the back? It is most intuitive to think of the leader cell reading the chemical environment and forming physical adhesions to pull the tissue forward. The interesting aspect of this paper is the "rear-engine". The authors identify well the molecular machinery that produces the migratory force, but they don't address how this machinery seems to preferentially produce force in the rear vs. the front.

Minor concerns:
1. The organization of figures and extended data makes the paper very hard to follow. There is a lot of mixing data across figures and going backward to reference data from previous figures that had not been previously discussed. Requiring the reader to bounce back and forth between figures unnecessarily affects comprehension.
2. Text in the figures, specifically labels on data plots, is often very small and difficult to read. Text within image panels is often much larger and therefore easy to see. There is plenty of unused space within figures that should allow for making all the text larger and more easily readable.
3. I don't see a reason for listing "data not shown". This seems to be in reference to the ability of Itgb1b and Tln1 constructs to restore viability in mutant backgrounds. The authors should show any relevant data or remove the text that it refers to.

Reviewer #2:
Remarks to the Author:
This clearly presented manuscript establishes a novel method for quantifying stress forces in vivo. The authors examine in detail the mechanical forces involved in zebrafish posterior lateral line primordium migration. They establish that the collective migration of this population of cells occurs on a basement membrane substrate using transient rather than the more prolonged adhesion to substrates reported for cells in tissue culture. They then establish that these cells deform the underlying basement membrane during cell migration with a "maximal stress close to 1 kPa." This adhesive force is similar to those described for other integrin-based adhesions. This integrin-dependent adhesion is transient and is coupled to the stalling of retrograde actin flow that is suggested to result in forward movement. The nature of the cell-substrate traction or stresses is downward, sideways, and backwards, thereby transmitting stresses along the sides of this tissue at the rear of the mass of migrating cells. The authors conclude that this mechanism can be summarized as a novel tissue migration system in which a "rear-engine drives adherent tissue migration in vivo."

This manuscript is carefully documented and is of generally quite high scientific quality, with excellent applications of zebrafish genetic approaches and large sample sizes. It describes a novel, ingenious, and useful new approach for evaluating tissue stresses in vivo without needing to incorporate foreign objects into the animal. It involves the clever approach of photobleaching a hexagonal pattern of dots in the basement membrane labeled with a chimeric fluorescent protein. The movement of cells in a tissue over this pre-marked substrate allows the authors to estimate stresses on the basement

membrane. This approach appears to be a valuable advance for a relatively straightforward procedure for evaluating forces in vivo, and as such deserves publication. Nevertheless, there are several substantive concerns that would need to be resolved before this otherwise outstanding manuscript can be considered further for publication.

1. A question about novelty will need to be resolved. A 2018 paper in Science 2018 (PMID: 30337409) titled: "Supracellular contraction at the rear of neural crest cell groups drives collective chemotaxis" established the concept of a rear-wheel drive for tissue migration, which seems to be at least superficially analogous to the authors' term "rear-engine." It will be important to establish how the current paper differs from the following abstract conclusion besides just a different cell type: "Studying Xenopus and zebrafish, we have shown that the neural crest exhibits a tensile actomyosin ring at the edge of the migratory cell group that contracts in a supracellular fashion. This contractility is polarized during collective cell chemotaxis: It is inhibited at the front but persists at the rear of the cell cluster. The differential contractility drives directed collective cell migration ex vivo and in vivo through the intercalation of rear cells. Thus, in neural crest cells, collective chemotaxis works by rear-wheel drive."

2. Because the paper in point #1 goes further than the current paper in providing a cellular mechanism for how this rear propulsion system supposedly works, the authors of the current paper need to stain for the actomyosin cytoskeleton to establish whether there is a similar or a different mechanism. In addition, it would be very helpful to know whether lateral line cell migration involves the intercalation process established by the Mayor lab.

3. A recent paper in Developmental Biology studying what appears to be the same zebrafish lateral line developmental system comes to rather different conclusions, including claims of the existence of focal adhesions and importance of interactions with the overlying skin: PMID: 33096063
"Lamellipodia-like protrusions and focal adhesions contribute to collective cell migration in zebrafish"
"…our results suggest a model where the coordinated dynamics of lamellipodia-like protrusions, making contact with either the overlying skin or the extracellular matrix through focal adhesions, promotes migration of pLLP cells."
It appears important that the authors determine whether the LLP cells make functionally significant adhesions to the skin, rather than having their migration driven solely by interactions with the basement membrane. A theoretical alternative explanation is that the tension forces that they describe are needed to break adhesions with the skin, rather than being the primary motive force For LLP cell migration, which ideally needs to be rolled out. A possible approach might be to characterize stress patterns during cell migration after removal of the skin if the process can still continue under those conditions. Until this point is resolved, it does not appear to be safe to state "This suggests that the PM serves as the primordium's substrate for migration."
A second point of comparison needs to involve whether the current study reporting what appear to be nascent adhesions somehow missed what the authors of the Developmental Biology paper claim to be focal adhesions. In particular, the authors need to clarify what types of cell adhesions exist between the LLP and the overlying skin. That is, are there any apical cell adhesions?

4. There is confusion about the interpretation of the photo bleaching data to determine dynamics. When evaluating the stability of laminin, the authors indicate that its half-life of 12.3 minutes with mobile fraction = 20.1% indicates a stable system for optical landmarks, yet the authors state that "the mobility of Itgb1b-sfGFP is high" with parameters of a half-life of 11.4 seconds and mobile fraction = 33.6%, which to this reviewer does not seem high. It is also not at all clear that these

differences are particularly significant, and the authors should be careful in their claims. Instead, the only safe comparison appears to be after experimental treatments that indicate a change in mobility, not absolute conclusions about mobility.

5. Although the authors convincingly establish an intriguing pattern of substrate stresses that are highest at the rear of the migrating LLP cells, it is not clear why the forces are strongest in the downward direction, as well as laterally. It is puzzling that the greatest forces are not along the direction of migration to push the collective of LLP cells forward. These findings might instead support some alternative explanation, e.g., that the forces are needed to months of a subset of cells, somewhat analogous to the claimed intercalation and forward movement of cells during normal crest cell migration.

6. Although the stress patterns are convincing, it is not clear what they mean in terms of the movement of the entire LLP. That is, do the authors think that the rear cells are actually pushing the more anterior cells forward, or do the more anterior cells differ in being able to migrate with very little stress and strain on the underlying basement membrane, perhaps because they face less resistance in squeezing between the skin and basement membrane than the thicker posterior portion of the LLP? The alternative explanation of the findings would be that the more anterior cells use lamellipodia and cell adhesions to both the overlying skin and the underlying basement membrane to squeeze a thinner LLP region forward, but that the cells at the rear are only pushing against the basement membrane because they must use actin flow and the molecular clutch rather than lamellipodia for forward locomotion of a thicker mass of cells facing more resistance because of the size of the LLP posterior. A possible means of clarifying these issues might be high-resolution imaging of the individual cells compared to their neighbors, as well as a more careful examination of the presence of adhesions to the skin versus the basement membrane (and ideally after removal of the skin), plus evaluations of lamellipodia.

7. The authors should re-check each of their conclusions within the text to verify their accuracy. For example, the text states "Itgb1b is required within the primordium for migration" but the effects are surprisingly modest after it's a mutation and even mutation of Palin and to integrations, which the authors describe four lines later as "the surprisingly mild defects" that do not seem to be explained by common founding maternal contributions in subsequent analyses. Kept towards the end of the manuscript, the authors claim that the mutated NPxY integration "failed to support efficient primordium migration" and concludes that the "integration/detail and complex is important for the primordium to move along its migratory route" but unless this reviewer is confused, the actual data seem to show that migration can still proceed at about 70% of the normal rate the authors need to be more precise in their descriptions and conclusions of the findings.

8. With the reservations enumerated above, the Abstract and overall conclusion appear reasonable in a paper with commendable experimental carefulness and good statistical analyses.

Minor points:

9. This novel method for quantifying tension/stress by strain analyses depends on the substrate being elastic rather than plastic. Can the authors show that the basement membrane snaps back to the same original pattern as verified quantitatively?

10. The estimates of stresses are a bit confusing. The text claims "stresses of around 1 kPa" but the

results show stresses of 64-600 Pa, with the largest being somewhat confusingly lateral or downward in Z.

11. The text states rather confusingly that the "cells on its side and rear push sideways and strongly backwards, consistent with theoretical predictions for adherence cell migration. However, that reference number 23 refers to integration-independent cell migration in cells that have virtually no adhesion to a substrate, so it is completely unclear how the present findings can be interpreted in light of the swimming motion of cells in environments with virtually no adhesion to the substrate. The large stresses reported in this paper seem to be consistent with strong focal adhesions, so the authors also need to consider why they can only observe nascent adhesions – perhaps because the cells make very strong but unusually transient adhesions to the basement membrane substrate.

12. The authors need to compare their findings on the role of Talin with the recently published effects of morpholino knockdown of Talin in PMID: 33096063.

13. There is a minor typo in line 263 in which "macrophages" has an extra "p."

In summary, this manuscript presents an impressive amount of high-quality data based on a novel and innovative approach to measuring in vivo traction forces, along with striking implication of the role of actin flow in vivo to highly efficient tissue translocation. Although these features of this impressively data-rich manuscript make this reviewer overall positive in terms of guarded optimism about the possibility of future acceptance for publication, the multiple seemingly major points raised in this review will need to be resolved directly.

Reviewer #3:
None

GUIDELINES FOR MANUSCRIPT SUBMISSION TO NATURE CELL BIOLOGY

READABILITY OF MANUSCRIPTS – Nature Cell Biology is read by cell biologists from diverse backgrounds, many of whom are not native English speakers. Authors should aim to communicate their findings clearly, explaining technical jargon that might be unfamiliar to non-specialists, and avoiding non-standard abbreviations. Titles and abstracts should concisely communicate the main findings of the study, and the background, rationale, results and conclusions should be clearly explained in the manuscript in a manner accessible to a broad cell biology audience. Nature Cell Biology uses British spelling.

MANUSCRIPT FORMAT – please follow the guidelines listed in our Guide to Authors regarding manuscript formats at Nature Cell Biology.

TITLE – should be no more than 100 characters including spaces, without punctuation and avoiding technical terms, abbreviations, and active verbs..

AUTHOR NAMES – should be given in full.

AUTHOR AFFILIATIONS – should be denoted with numerical superscripts (not symbols) preceding the names. Full addresses should be included, with US states in full and providing zip/post codes. The corresponding author is denoted by: "Correspondence should be addressed to [initials]."

ABSTRACT AND MAIN TEXT – please follow the guidelines that are specific to the format of your manuscript, as listed in our Guide to Authors (http://www.nature.com/ncb/pdf/ncb_gta.pdf) Briefly, Nature Cell Biology Articles, Resources and Technical Reports have 3500 words, including a 150 word abstract, and the main text is subdivided in Introduction, Results, and Discussion sections. Nature Cell Biology Letters have up to 2500 words, including a 180 word introductory paragraph (abstract), and the text is not subdivided in sections.

ACKNOWLEDGEMENTS – should be kept brief. Professional titles and affiliations are unnecessary. Grant numbers can be listed.

AUTHOR CONTRIBUTIONS – must be included after the Acknowledgements, detailing the contributions of each author to the paper (e.g. experimental work, project planning, data analysis etc.). Each author should be listed by his/her initials.

FINANCIAL AND NON-FINANCIAL COMPETING INTERESTS – the authors must include one of three declarations: (1) that they have no financial and non-financial competing interests; (2) that they have financial and non-financial competing interests; or (3) that they decline to respond, after the Author Contributions section. This statement will be published with the article, and in cases where financial and non-financial competing interests are declared, these will be itemized in a web supplement to the article. For further details please see https://www.nature.com/licenceforms/nrg/competing-interests.pdf.

REFERENCES – are limited to a total of 70 for Articles, Resources, Technical Reports; and 40 for Letters. This includes references in the main text and Methods combined. References must be numbered sequentially as they appear in the main text, tables and figure legends and Methods and must follow the precise style of Nature Cell Biology references. References only cited in the Methods should be numbered consecutively following the last reference cited in the main text. References only associated with Supplementary Information (e.g. in supplementary legends) do not count toward the total reference limit and do not need to be cited in numerical continuity with references in the main text. Only published papers can be cited, and each publication cited should be included in the numbered reference list, which should include the manuscript titles. Footnotes are not permitted.

METHODS – Nature Cell Biology publishes methods online. The methods section should be provided as a separate Word document, which will be copyedited and appended to the manuscript PDF, and incorporated within the HTML format of the paper.

Methods should be written concisely, but should contain all elements necessary to allow interpretation and replication of the results. As a guideline, Methods sections typically do not exceed 3,000 words.

The Methods should be divided into subsections listing reagents and techniques. When citing previous methods, accurate references should be provided and any alterations should be noted. Information must be provided about: antibody dilutions, company names, catalogue numbers and clone numbers for monoclonal antibodies; sequences of RNAi and cDNA probes/primers or company names and catalogue numbers if reagents are commercial; cell line names, sources and information on cell line identity and authentication. Animal studies and experiments involving human subjects must be reported in detail, identifying the committees approving the protocols. For studies involving human subjects/samples, a statement must be included confirming that informed consent was obtained. Statistical analyses and information on the reproducibility of experimental results should be provided in a section titled "Statistics and Reproducibility".

All Nature Cell Biology manuscripts submitted on or after March 21 2016 must include a Data availability statement as a separate section after Methods but before references, under the heading "Data Availability". . For Springer Nature policies on data availability see http://www.nature.com/authors/policies/availability.html; for more information on this particular policy see http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf. The Data availability statement should include:

• Accession codes for primary datasets (generated during the study under consideration and designated as "primary accessions") and secondary datasets (published datasets reanalysed during the study under consideration, designated as "referenced accessions"). For primary accessions data should be made public to coincide with publication of the manuscript. A list of data types for which submission to community-endorsed public repositories is mandated (including sequence, structure, microarray, deep sequencing data) can be found here http://www.nature.com/authors/policies/availability.html#data.

• Unique identifiers (accession codes, DOIs or other unique persistent identifier) and hyperlinks for datasets deposited in an approved repository, but for which data deposition is not mandated (see here for details http://www.nature.com/sdata/data-policies/repositories).

• At a minimum, please include a statement confirming that all relevant data are available from the authors, and/or are included with the manuscript (e.g. as source data or supplementary information), listing which data are included (e.g. by figure panels and data types) and mentioning any restrictions on availability.

• If a dataset has a Digital Object Identifier (DOI) as its unique identifier, we strongly encourage including this in the Reference list and citing the dataset in the Methods.

We recommend that you upload the step-by-step protocols used in this manuscript to the Protocol Exchange. More details can found at www.nature.com/protocolexchange/about.

DISPLAY ITEMS – main display items are limited to 6-8 main figures and/or main tables for Articles, Resources, Technical Reports; and 5 main figures and/or main tables for Letters. For Supplementary Information see below.

FIGURES – Colour figure publication costs $600 for the first, and $300 for each subsequent colour figure. All panels of a multi-panel figure must be logically connected and arranged as they would

appear in the final version. Unnecessary figures and figure panels should be avoided (e.g. data presented in small tables could be stated briefly in the text instead).

All imaging data should be accompanied by scale bars, which should be defined in the legend. Cropped images of gels/blots are acceptable, but need to be accompanied by size markers, and to retain visible background signal within the linear range (i.e. should not be saturated). The boundaries of panels with low background have to be demarked with black lines. Splicing of panels should only be considered if unavoidable, and must be clearly marked on the figure, and noted in the legend with a statement on whether the samples were obtained and processed simultaneously. Quantitative comparisons between samples on different gels/blots are discouraged; if this is unavoidable, it should only be performed for samples derived from the same experiment with gels/blots were processed in parallel, which needs to be stated in the legend.

Figures should be provided at approximately the size that they are to be printed at (single column is 86 mm, double column is 170 mm) and should not exceed an A4 page (8.5 x 11"). Reduction to the scale that will be used on the page is not necessary, but multi-panel figures should be sized so that the whole figure can be reduced by the same amount at the smallest size at which essential details in each panel are visible. In the interest of our colour-blind readers we ask that you avoid using red and green for contrast in figures. Replacing red with magenta and green with turquoise are two possible colour-safe alternatives. Lines with widths of less than 1 point should be avoided. Sans serif typefaces, such as Helvetica (preferred) or Arial should be used. All text that forms part of a figure should be rewritable and removable.

We accept files from the following graphics packages in either PC or Macintosh format:

- For line art, graphs, charts and schematics we prefer Adobe Illustrator (.AI), Encapsulated PostScript (.EPS) or Portable Document Format (.PDF). Files should be saved or exported as such directly from the application in which they were made, to allow us to restyle them according to our journal house style.

- We accept PowerPoint (.PPT) files if they are fully editable. However, please refrain from adding PowerPoint graphical effects to objects, as this results in them outputting poor quality raster art. Text used for PowerPoint figures should be Helvetica (preferred) or Arial.

- We do not recommend using Adobe Photoshop for designing figures, but we can accept Photoshop generated (.PSD or .TIFF) files only if each element included in the figure (text, labels, pictures, graphs, arrows and scale bars) are on separate layers. All text should be editable in ‘type layers’ and line-art such as graphs and other simple schematics should be preserved and embedded within 'vector smart objects’ - not flattened raster/bitmap graphics.

- Some programs can generate Postscript by 'printing to file' (found in the Print dialogue). If using an application not listed above, save the file in PostScript format or email our Art Editor, Allen Beattie for advice (a.beattie@nature.com).

Regardless of format, all figures must be vector graphic compatible files, not supplied in a flattened raster/bitmap graphics format, but should be fully editable, allowing us to highlight/copy/paste all text and move individual parts of the figures (i.e. arrows, lines, x and y axes, graphs, tick marks, scale bars etc.). The only parts of the figure that should be in pixel raster/bitmap format are photographic

images or 3D rendered graphics/complex technical illustrations.

All placed images (i.e. a photo incorporated into a figure) should be on a separate layer and independent from any superimposed scale bars or text. Individual photographic images must be a minimum of 300+ DPI (at actual size) or kept constant from the original picture acquisition and not decreased in resolution post image acquisition. All colour artwork should be RGB format.

FIGURE LEGENDS – must not exceed 350 words for each figure to allow fit on a single printed NCB page together with the figure. They must include a brief title for the whole figure, and short descriptions of each panel with definitions of the symbols used, but without detailing methodology.

TABLES – main tables should be provided as individual Word files, together with a brief title and legend. For supplementary tables see below.

SUPPLEMENTARY INFORMATION – Supplementary information is material directly relevant to the conclusion of a paper, but which cannot be included in the printed version in order to keep the manuscript concise and accessible to the general reader. Supplementary information is an integral part of a Nature Cell Biology publication and should be prepared and presented with as much care as the main display item, but it must not include non-essential data or text, which may be removed at the editor's discretion. All supplementary material is fully peer-reviewed and published online as part of the HTML version of the manuscript. Supplementary Figures and Supplementary Notes are appended at the end of the main PDF of the published manuscript.

Supplementary items should relate to a main text figure, wherever possible, and should be mentioned sequentially in the main manuscript, designated as Supplementary Figure, Table, Video, or Note, and numbered continuously (e.g. Supplementary Figure 1, Supplementary Figure 2, Supplementary Table 1, Supplementary Table 2 etc.).

Unprocessed scans of all key data generated through electrophoretic separation techniques need to be presented in a supplementary figure that should be labelled and numbered as the final supplementary figure, and should be mentioned in every relevant figure legend. This figure does not count towards the total number of figures and is the only figure that can be displayed over multiple pages, but should be provided as a single file, in PDF or TIFF format. Data in this figure can be displayed in a relatively informal style, but size markers and the figures panels corresponding to the presented data must be indicated.

The total number of Supplementary Figures (not including the "unprocessed scans" Supplementary Figure) should not exceed the number of main display items (figures and/or tables (see our Guide to Authors and March 2012 editorial http://www.nature.com/ncb/authors/submit/index.html#suppinfo; http://www.nature.com/ncb/journal/v14/n3/index.html#ed). No restrictions apply to Supplementary Tables or Videos, but we advise authors to be selective in including supplemental data.

Each Supplementary Figure should be provided as a single page and as an individual file in one of our accepted figure formats and should be presented according to our figure guidelines (see above). Supplementary Tables should be provided as individual Excel files. Supplementary Videos should be provided as .avi or .mov files up to 50 MB in size. Supplementary Figures, Tables and Videos much be

accompanied by a separate Word document including titles and legends.

GUIDELINES FOR EXPERIMENTAL AND STATISTICAL REPORTING

REPORTING REQUIREMENTS – We are trying to improve the quality of methods and statistics reporting in our papers. To that end, we are now asking authors to complete a reporting summary that collects information on experimental design and reagents. The Reporting Summary can be found here https://www.nature.com/documents/nr-reporting-summary.pdf)If you would like to reference the guidance text as you complete the template, please access these flattened versions at http://www.nature.com/authors/policies/availability.html.

STATISTICS – Wherever statistics have been derived the legend needs to provide the n number (i.e. the sample size used to derive statistics) as a precise value (not a range), and define what this value represents. Error bars need to be defined in the legends (e.g. SD, SEM) together with a measure of centre (e.g. mean, median). Box plots need to be defined in terms of minima, maxima, centre, and percentiles. Ranges are more appropriate than standard errors for small data sets. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test used needs to be stated in the legend. Statistics such as error bars must not be derived from n<3. For sample sizes of n<5 please plot the individual data points rather than providing bar graphs. Deriving statistics from technical replicate samples, rather than biological replicates is strongly discouraged. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test stated in the legend.

Information on how many times each experiment was repeated independently with similar results needs to be provided in the legends and/or Methods for all experiments, and in particular wherever representative experiments are shown.

We strongly recommend the presentation of source data for graphical and statistical analyses as a separate Supplementary Table, and request that source data for all independent repeats are provided when representative experiments of multiple independent repeats, or averages of two independent experiments are presented. This supplementary table should be in Excel format, with data for different figures provided as different sheets within a single Excel file. It should be labelled and numbered as one of the supplementary tables, titled "Statistics Source Data", and mentioned in all relevant figure legends.

--------- Please don't hesitate to contact NCB@nature.com should you have queries about any of the above requirements ---------

Author Rebuttal to Initial comments

Response to reviewers' comments

We would like to sincerely thank the two reviewers for their thorough and thoughtful comments. We are detailing below our responses to the reviewers' suggestions and concerns.

Also, we would like to point out two additional changes to the manuscript. First, we assessed the contribution of Talin to primordium migration through Talin depletion in the entire primordium in addition to our initial clonal analysis. Consistent with the clonal analysis, this tissue-wide approach showed that removal of most Talin function in the primordium results in slower migration (new Fig. 4h–j), consistent with Talin's function as an adaptor protein between integrin and the actomyosin network. Second, we rearranged the text to better align the text with the figures (as recommended by the reviewers). We therefore now present the data on the stresses exerted by the primordium after discussing the role of the environment and integrins in primordium motility. For ease of assessing the changes to the manuscript, we indicate below which figure panels are "new" additions to this revised manuscript.

Reviewer #1 comments:

"The authors identify a rear based engine that drives the migration of primordial cells along the basement membrane. They use two approaches. First, imaging by TEM and fluorescent laminin (an exciting new tool). The imaging is clear and is of high quality. Second is Embryogram to measure the deformation of BM by primordium. Embryogram works by mapping fixed points on the basement membrane and then measuring how they change over time. This is used to construct a map of basement membrane deformation under the primordium as it moves. To derive traction force measurments, they measure the physical property of the substrate. By knowing how stiff the substrate is and how much it moves due to presence of the primordium, they can calculate traction force generated by the primordium.

Using Embryogram they identify a mode of migration that is characterized by increased traction force focused on the sides and back of the primordium. The front provides minimal traction stress on BM. There is compelling and reproducible (Embryogram measurements coupled with more conventional line scan showing LamC1 crumpling) to show the physical dynamics of primordium BM interaction.
The next sections describes the molecular details of how these forces are generated. The authors identify integrin and talin as the primary drivers of migration through their interaction with actin and the matrix. Using genetic approaches the authors show that Itgb1b and Tln1 form relatively transient adhesions with the basement membrane in vivo, and are required for tissue migration.
This study provides novel insight into the movement of cells/tissue in vivo. They identify that transient, rather than stable, adhesions are utilized. Further, they identify a "rear-engine" that drives motility. This observation, that traction forces are highest toward the rear and sides, is interesting, but more could be done to link this feature of tissue migration to the molecular components (integrin/talin) that produce the required force."

We thank Reviewer 1 for support and constructive suggestions. Major concerns:

1. “The authors note that cells of the primordium contribute equally to directed migration (Discussion) and hypothesize that the increased density of cells in the rear accounts for increased force production there rather than the front of the tissue. Can this be measured? This is a crux of the paper, but they don’t do experiments designed to ask whether the cells in the side/rear form adhesions differently than the front. It is conceivable that they do, which would open interesting questions about how this is done, or it is possible that adhesions are transient throughout and cell density is the driver of the “rear-engine”. The authors could assess stability of integrin/talin/actin using the existing

approaches in the paper (FRAP, actin flow measurement) and the chimeric primordium to look at cells in different regions that correspond to their traction force map.”

We agree that this is a key point of the manuscript. To address whether or not the cells in the front and back of the primordium are different we now assessed myosin II activity, actin flow, integrin levels, integrin engagement, and integrin-mediated stresses across the migrating primordium. These analyses revealed that:

1. non-muscle myosin II activity was increased in the rear and sides of the primordium compared to its front as determined by the localization of the myosin light chain 12.1 fused to the red fluorescent protein mScarlet (Myl12.1- mScarlet) expressed from the cxcr4b promoter in the primordium (new Fig. 8a, b).
2. actin flow was not statistically significantly across the primordium (new Fig. 8c, d).
3. Itgb1b-sfGFP levels were similar across the primordium as judged by quantification of the GFP fluorescence on the membrane of the primordium cells (new Fig. 8e).
4. Itgb1b clustering—an indication of integrin engagement with its extracellular ligands and the actin network—was similar across the primordium as determined by the fluorescence intensity changes on the membrane of Itgb1b- sfGFP over the talin-binding deficient Itgb1b□NPxY-sfGFP normalized by Itgb1b- tdTomato fluorescence intensity expressed from the other itgb1b allele in heteroallelic embryos (itgb1b:itgb1b-sfGFP/itgb1b:itgb1b-tdTomato and itgb1b:itgb1b□NPxY-sfGFP/itgb1b:itgb1b-tdTomato, new Fig. 8f).
5. Itgb1b mediated more stresses in the rear than in the front of the primordium as judged by comparing the stresses generated by primordia in wild-type and itgb1b mutant embryos (new Fig. 8g–i).

These data indicate that the cells in the rear of the primordium generate more force and

transmit more force through Integrin b1b to the basement membrane. We therefore adjusted our model and now suggest that a combination of cell number and force per cell generates the higher stresses in the rear than in the front of the primordium.

As Reviewer 1 suggested, we also assessed the stability of Itgb1b-sfGFP and the control Itgb1b□NPxY-sfGFP across the primordium by FRAP. We did not detect a difference in the mobility between Itgb1b-sfGFP and the control Itgb1b□NPxY-sfGFP across the primordium, suggesting that this approach is too

noisy to draw a conclusion. If the Reviewer 1 feels that these data should be included we are happy to do so.

2. “They initially introduce the chemokine CxCl12a gradient and Cxcr4b receptor as the means for directional movement. How does this work with the rear-engine (i.e. cells in the front sense directionality, but those in the rear produce majority of traction force)?”
This is a very good point that we failed to clarify in our initial submission. The primordium generates a Cxcl12a attractant gradient across itself. It does so by expressing the Cxcl12a clearance receptor Cxcr7b in its rear only. Cxcr7b outcompetes the guidance receptor Cxcr4b for the chemokine and sequesters and degrades Cxcl12a (Donà et al., 2013; Venkiteswaran et al., 2013). This generates a Cxcl12a gradient across the first 120 □m of the primordium that the cells in the front, middle and rear sense. At the very rear of the primordium the cells do not sense the Cxcl12a gradient and slow down and become deposited as sensory organs called neuromasts. Using a Cxcl12a signaling sensor based on Cxcl12a-induced Cxcr4b internalization we now include a panel that shows that the cells in the rear sense this self-generated gradient (new Extended Data Fig. 10d).

Specific concerns:

1. “Related to figure 1e-g: Do they have data that compares WT primordial migration (LamC1 +/+ and Cxcl12a +/+) with LamC1 +/+, Cxcl12a null larvae

injected with acta1:cxcl12a?

The comparisons shown adequately show that whatever level of Cxcl12a expression is achieved in muscle is capable of promoting migration in the presence of BM, but we do not know if this is equivalent to the WT context. Comparing migration distance between the genotypes above would provide a convincing control that the comparisons shown in Fig. 1g are replicating to some degree the normal chemokine environment.
Currently, the evidence that there is sufficient Cxcl12a to be “functional” is the receptor internalization data (Fig. 1h-j). But it still seems possible that they are undershooting the WT Cxcl12a expression. While not probable, it may be possible that more Cxcl12a could overcome the lack of BM in lamC1 -/-. Thus, they should show the Cxcl12a expression they achieve ectopically is close to WT level. Doing so by comparing migration distance with the genotypes above would be sufficient.”
We now have included data that show the migration distance of the primordium in lamC1+/+; cxcl12a-/- and lamC1+/+; cxcl12a+/+ embryos injected with acta1:cxcl12a DNA and uninjected lamC1+/+; cxcl12a+/+ embryos (new Extended Data Fig. 2h). As reported in our initial submission, expression of Cxcl12a from clones in the muscle restores primordium migration in lamC1+/+; cxcl12a-/- embryos but does not do so to the extent seen in lamC1+/+; cxcl12a+/+ embryos. The reason for this is that we are

unable to generate Cxcl12a-expressing clones along the entire migratory route of the primordium. However, we think that it is unlikely that we are undershooting the wild- type levels of Cxcl12a for three reasons. First, we express Cxcl12a from the acta1 promoter that is stronger than the endogenous cxcl12a promoter; We cannot detect Cxcl12a-GFP expressed from the endogenous locus while Cxcl12a-GFP expressed from the acta1 promoter is visible using a V16 Zeiss microscope, suggesting that Cxcl12a levels are higher than those secreted from the endogenous source tissue. Second, a small clone expressing Cxcl12a is sufficient to restore migration, suggesting that it is secreting enough Cxc12a to support primordium migration in its vicinity (Fig. 2g, h).

Third, ectopic expression of Cxcl12a from the muscle causes comparable Cxcr4b internalization to endogenous Cxcl12a (Fig. 2j and new Extended Data Fig. 2i). For these reasons, we feel that the acta1 promoter is probably producing more Cxcl12a than the endogenous cxcl12a promoter.

2. "Related to figure 1h-j: As noted, the purpose of this is to show that their muscle- specific Cxcl12a expression is capable of stimulating the primordium. This method of quantification seems very reliant on the plane of imaging (i.e. the Kate signal is still present but if you imaged lower into the cells you could achieve a higher RFP/GFP ratio even after Cxcl12a expression). The important part to this is not the intensity, but the localization of signal. Can authors show 3D representation of Z-stack to show internalization of Cxcr4b-Kate (and this movement away from memGFP? Line-scan of memGFP illustrating strong co-localization without Cxcl12a, but loss of co-localization with Cxcl12a would also work."
We fully agree that the internalization of Cxcr4b-Kate2 is the key readout for Cxcl12a binding to its receptor. We now include images that included an orthogonal section through the z-stack along the XZ-plane to more clearly show that Cxcl12a still induces Cxcr4b internalization in lamC1-/-; cxcl12a-/- embryos (new Extended Data Fig. 2i).

3. "Do the authors ever link the molecular force production machinery (Itgb1a and Tln1) to the traction stress measurements from the first half of the paper? For example, they show in great detail that higher traction stress is produced at the sides and back of the primordium as it moves forward. Does this coincide with more stable complexes in these locations? They describe the transient nature of integrin/talin interaction with actin in the primordium (mostly Extended data 8), but would these still be predicted to be more stable (i.e. less FRAP recovery) in areas where increased force generation occurs. Can the authors measure Itgb1b/Tln1/Actin interaction across the primordium (front/side/rear)?

Question: Where did they measure the integrin/talin/actin interactions? They note it is in the basal portion of the primordium cell membrane, but where in relation to the front/side/rear?

Again, this goes back to the general question I have related to how the cells in the rear and sides are producing greater force. Is it simply that there are more of them, or is it that they are capable of forming more mature adhesions to produce greater force?"
We describe our experiments to address this concern in detail above (Reviewer 1's major concern 1). Briefly, we found that non-muscle myosin II activity is increased in the rear of the primordium (new Fig. 8a, b) and that the primordium's rear relies more on Integrin b1b-mediated traction stress generation than its front (new Fig. 8g–i). This is consistent with the idea that the rear cells in the primordium produce greater force.
However, while the traction stresses are more strongly reduced in the rear than in the front in itgb1b mutant embryos, the rear still generates more traction stresses than the front in this genetic scenario. This is consistent with the idea that the higher number of cells in the rear also contributes to the difference in traction stress generation across the primordium.

4. "Related to Extended data 6k: They mention forming the chimeric primordium in which Talin depletion was concentrated in cells toward the front of the primordium. It is difficult to assess whether that was the case, but would they expect this to actually affect migration more-so than talin depletion in the rear of the primordium given that additional force is required in the rear?
It is believable that talin depletion generally would be expected to reduce migration, but if the authors are capable of finding chimeric primordia with enriched depletion in the front vs. back, it would be interesting to see if one of these affected migration more than the other. The same could be said for Itgb1b expression.

This is a related experiment to the thought above about linking their traction force measurements to the molecular components that produce such force."
We agree with Reviewer 1 that removal of Talin or Integrin activity in different parts of the primordium would be an excellent approach to correlate traction stresses and force coupling across the primordium. We have tried this approach but grouping our chimeric primordia according to the position of the clones with reduced Talin activity (front versus back of the primordium) did not yield significant differences. This is expected since primordia with depleted Talin in all cells still traverse 70% of the migratory route (new Fig. 4h–j), offering only a small range for differences in phenotype between talin depletion in the back versus front cells. The phenotype of depleting most Talin activity in all the cells of the primordium is a recent observation that we did not know about during our initial submission of the manuscript. However, inactivation of the guidance receptor Cxcr4 in the primordium completely blocks its migration (David et al., 2002) and thus offers a much more sensitive assay to ask how each cell in the primordium contributes to the migration of the primordium depending on the cell's position. We have previously used this approach and shown that front and back cells in the primordium both contribute to its migration—in fact the distance that the primordium travels is a fairly linear function of the number of cells in the primordium that can perceive the guidance cue Cxcl12a (Fig. 2E in (Colak- Champollion et al.,

2019)). Since the direction of the force generating machinery in the primordium should be randomized in Cxcr4-deficient cells—Cxcl12a affects directionality but not motility, all cells in the primordium should contribute to the motility of the primordium. This is consistent with the idea that the differences in the traction stresses that we observed between the front and the back are in part a result of the difference in the number of cells (see also response to major concern 1).

5. "Related to figure 4i-k: The authors assess actin flow as an indirect measure of the stability of the adhesions primordium cells are making as they migrate.

Increased actin retrograde flow and polymerization rate in Itgb1b -/- indicates that integrins (and talin) are needed to produce the traction force for migration. However, it would be more impactful if the authors could assess cells located within different regions of the primordium as described above and link this to the traction forces they have measured.
This would indicate whether cells at different regions of the primordium produce fundamentally different types of adhesions, thus accounting for the increased traction force (and providing some cool additional biological questions) or whether the increased traction force is somehow a product of cell density or regional differences in BM stiffness, etc.
This raises an overall question, that should be at least discussed more in the text- related to the question above is: How are cells sensing a chemokine gradient in the front, but responding primarily with force generation toward the back? It is most intuitive to think of the leader cell reading the chemical environment and forming physical adhesions to pull the tissue forward. The interesting aspect of this paper is the "rear-engine". The authors identify well the molecular machinery that produces the migratory force, but they don't address how this machinery seems to preferentially produce force in the rear vs. the front."
This question summarizes most of the questions that Reviewer 1 raised above. We briefly summarize here the main points discussed in more detail above.
Force generation and traction stress

We find that the rear of the primordium has increased non-muscle myosin II activity, slightly increased actin flow and enhance Integrin-b1b-mediated force coupling to the basement membrane (new Fig. 8a–d, g–i). Together with the higher cell number in the primordium's rear, this explains—at least in part—the high traction stresses in the primordium's rear and provides a molecular and cellular explanation for the rear-engine design that we observed (see also above Reviewer 1's major concern 1).
Chemokine gradient sensing

The primordium generates a Cxcl12a attractant gradient across itself through selective uptake and degradation of Cxcl12a in its back (Donà et al., 2013; Venkiteswaran et al., 2013). This gradient stretches from the front of the primordium to its rear (about 120

m, new Extended Data Fig. 10d) and thus provides directional information to most of the cells in the primordium (the very rear cells do not experience a Cxcl12a gradient and slow down and become deposited as neuromasts). This gradient is sensed by most of the cells in the primordium; most of the primordium cells extend protrusions towards the front, sense the Cxcl12a gradient, and contribute to the overall speed of migration. When interfering with the directionality of cells in the front, the back cells frequently push the front cells forward (Colak-Champollion et al., 2019). Together, this suggests a model in which all cells in the primordium sense the attractant gradient, generate a basal traction force, and contribute to overall tissue movement with the rear pulling stronger on the basement membrane, probably to provide the necessary forces to wedge the skin and muscle apart and push the more epithelial-like rear of the primordium forward. We now briefly introduce this model in the discussion section.

Minor concerns:

1. "The organization of figures and extended data makes the paper very hard to follow. There is a lot of mixing data across figures and going backward to reference data from previous figures that had not been previously discussed. Requiring the reader to bounce back and forth between figures unnecessarily affects comprehension."
We agree and have rearranged the text such that we now discuss the results of the traction stress analysis towards the end of the manuscript and reordered the figures such that each result section mostly corresponds to one specific figure.

2. "Text in the figures, specifically labels on data plots, is often very small and difficult to read. Text within image panels is often much larger and therefore easy

to see. There is plenty of unused space within figures that should allow for making all the text larger and more easily readable."
We now have adjusted the size of the labels of the data plots to make them more legible.

3. "I don't see a reason for listing "data not shown". This seems to be in reference to the ability of Itgb1b and Tln1 constructs to restore viability in mutant backgrounds. The authors should show any relevant data or remove the text that it refers to."
We now included the rescue data for the itgb1b:itgb1b-sfGFP knock-in line and the tln1:tln1-YPet BAC transgenic line (Fig. 4g and main text).

Reviewer #2 comments:

“This clearly presented manuscript establishes a novel method for quantifying stress forces in vivo. The authors examine in detail the mechanical forces involved in zebrafish posterior lateral line primordium migration. They establish that the collective migration of this population of cells occurs on a basement membrane substrate using transient rather than the more prolonged adhesion to substrates reported for cells in tissue culture. They then establish that these cells deform the underlying basement membrane during cell migration with a “maximal stress close to 1 kPa.” This adhesive force is similar to those described for other integrin-based adhesions. This integrin-dependent adhesion is transient and is coupled to the stalling of retrograde actin flow that is suggested to result in forward movement.
The nature of the cell-substrate traction or stresses is downward, sideways, and backwards, thereby transmitting stresses along the sides of this tissue at the rear of the mass of migrating cells. The authors conclude that this mechanism can be summarized as a novel tissue migration system in which a “rear-engine drives

adherent tissue migration in vivo”.

This manuscript is carefully documented and is of generally quite high scientific quality, with excellent applications of zebrafish genetic approaches and large sample sizes. It describes a novel, ingenious, and useful new approach for evaluating tissue stresses in vivo without needing to incorporate foreign objects into the animal. It involves the clever approach of photobleaching a hexagonal pattern of dots in the basement membrane labeled with a chimeric fluorescent protein. The movement of cells in a tissue over this pre-marked substrate allows the authors to estimate stresses on the basement membrane. This approach appears to be a valuable advance for a relatively straightforward procedure for evaluating forces in vivo, and as such deserves publication. Nevertheless, there are several substantive concerns that would need to be resolved before this otherwise outstanding manuscript can be considered further for publication.”
We thank Reviewer 2 for support and constructive suggestions.

Major concerns:

1. A question about novelty will need to be resolved. A 2018 paper in Science 2018 (PMID: 30337409) titled: “Supracellular contraction at the rear of neural crest cell groups drives collective chemotaxis” established the concept of a rear-wheel drive for tissue migration, which seems to be at least superficially analogous to the authors' term "rear-engine." It will be important to establish how the current paper differs from the following abstract conclusion besides just a different cell type: “Studying Xenopus and zebrafish, we have shown that the neural crest exhibits a tensile actomyosin ring at the edge of the migratory cell group that contracts in a supracellular fashion. This contractility is

polarized during collective cell chemotaxis: It is inhibited at the front but persists at the rear of the cell cluster. The differential contractility drives directed collective cell migration ex vivo and in vivo through the intercalation of rear cells. Thus, in neural crest cells,

collective chemotaxis works by rear-wheel drive."
As suggested by Reviewer 2, we now have more closely tested whether the primordium uses the same strategy as the cranial neural crest in Xenopus to propel itself forward.
The cranial neural crest front cells are attracted by Cxcl12 and the rear cells form a supracellular actomyosin cable across Cdh2-rich adherens junctions around the back of this tissue. This cable contracts periodically and "squeezes" the rear cells forward. This displaces front cells sideways and then backwards such that the cells in the tissue constantly cycle from a rear position to a front position and back again (Extended Data Fig. 10e, (Shellard et al., 2018)). In contrast to the cranial neural crest, the primordium generates its own Cxcl12a attractant gradient that stretches across most of the primordium and is sensed not only by the cells in the front but by all the primordium cells (Extended Data Fig. 10d, (Donà et al., 2013; Venkiteswaran et al., 2013)). Using a reporter for non-muscle myosin II activity that we generated (cxcr4b:Myl12.1-mScarlet) and Cdh2 tagged with mCherry expressed from a transgenic BAC line (cdh2:cdh2- mCherry), we fail to detect a supracellular actomyosin cable across adherens junctions in the back of the primordium (new Fig. 8a, new Extended Data Fig. 10h). Similarly, tracking the cell junction lengths in the rear of the primordium and assessing the cumulative migration distance shows that the primordium neither contracts its rear nor moves in a pulsatile manner but rather migrates at a constant speed (new Extended Data Fig. 10f, g and (Colak-Champollion et al., 2019; Haas and Gilmour, 2006; Nogare et al., 2017)). Also, the cells of the primordium maintain their neighbor-neighbor relationship during their migration and do not move from the rear to the front and back again (new Extended Data Fig. 10g., Video 1, and (Colak-Champollion et al., 2019; Haas and Gilmour, 2006; Nogare et al., 2017)). Thus, the cranial neural crest and the primordium are both powered by a rear engine but the molecular and cellular basis of propulsion is different. We now have included these observations and conclusions in the revised manuscript.

2. Because the paper in point #1 goes further than the current paper in providing a

cellular mechanism for how this rear propulsion system supposedly works, the authors of the current paper need to stain for the actomyosin cytoskeleton to establish whether there is a similar or a different mechanism. In addition, it would be very helpful to know whether lateral line cell migration involves the intercalation process established by the Mayor lab.
We agree with Reviewer 2 that these experiments were missing in our initial submission and now have included them. They are detailed in our response to Reviewer 2's major concern 1 above so we are only briefly outlining them here. We have assessed the actomyosin network (with Myl12.1, a non-muscle myosin II subunit, new Fig. 8a, b), adherens junction localization (with mCherry tagged Cdh2, new

Extended Data Fig. 10h), rear contraction (cell junction length measurement, new Extended Data Fig. 10f), and cell intercalation in the primordium (nuclear tracking, new Extended Data Fig. 10g).
These observations indicate that the primordium does not rely on rear contraction by a supracellular actomyosin cable for propulsion as observed for the cranial neural crest but rather relies on integrin-mediated actin flow coupling to the basement membrane.

3. "A recent paper in Developmental Biology studying what appears to be the same zebrafish lateral line developmental system comes to rather different conclusions, including claims of the existence of focal adhesions and importance of interactions with the overlying skin: PMID: 33096063 "Lamellipodia-like protrusions and focal adhesions contribute to collective cell migration in zebrafish" "…our results suggest a model where the coordinated dynamics of lamellipodia-like protrusions, making contact with either the overlying skin or the extracellular matrix through focal adhesions, promotes migration of pLLP cells." It appears important that the authors determine whether the LLP cells make functionally significant adhesions to the skin, rather than having their migration driven solely by interactions with the basement membrane. A theoretical alternative explanation is that the tension forces that they describe are needed to break adhesions with the skin, rather than being the primary motive force For LLP

cell migration, which ideally needs to be rolled out. A possible approach might be to characterize stress patterns during cell migration after removal of the skin if the process can still continue under those conditions. Until this point is resolved, it does not appear to be safe to state "This suggests that the BM serves as the primordium's substrate for migration."
A second point of comparison needs to involve whether the current study reporting what appear to be nascent adhesions somehow missed what the authors of the Developmental Biology paper claim to be focal adhesions. In particular, the authors need to clarify what types of cell adhesions exist between the LLP and the overlying skin. That is, are there any apical cell adhesions?"
The role of the skin in primordium propulsion

We agree with Reviewer 2 that the skin could also serve as a substrate for the migrating primordium. Unfortunately, surgical removal of the skin impairs the primordium cells' polarity and stalls the primordium (Nogare et al., 2020), such that we cannot assess traction stresses in the absence of the skin. We therefore assessed the contribution of the skin to primordium migration in two less invasive ways:
1. To propel itself forward, the primordium could adhere to a basement membrane on its apical side. This apical basement membrane would then be anchored to the skin such that there would be a "cell-basement membrane-cell bridge" that would couple the primordium to the skin. We therefore reassessed the distribution of the basement membrane marker LamC1 between the skin and the primordium. We detect little to no LamC1-GFP by confocal microscopy between the skin and the

primordium (new Extended Data Fig. 1j), consistent with our initial failure to detect a basement membrane between the skin and the primordium by TEM (Extended Data Fig. 1b–d). This suggests that the skin and the primordium are neither separated by a basement membrane nor coupled to each other through a shared basement membrane. Consistent with this, we find that the speed of actin flow in the superficial cells—the primordium cells on the

primordium's apical side that face the skin (Nogare et al., 2020; Olson and Nechiporuk, 2020)—is fast (7 □m/min), and not slowed down when Integrin b1b activity is removed from these cells (new Fig. 5b, d, Extended Data Fig. 7g, Video 6 (bottom)). This suggests that the primordium does not use integrins to couple actin flow to the skin for propulsion.

2. Alternatively, the primordium could propel itself forward by adhering directly to the skin through cell-cell adhesion. We therefore determined whether cadherin- mediated cell-cell adhesion between the primordium and the skin is required for primordium migration. The primordium expresses the cell adhesion receptors N- Cadherin and E-Cadherin and the skin expresses E-Cadherin (Colak-Champollion et al., 2019; Matsuda and Chitnis, 2010; Revenu et al., 2014; Romero-Carvajal et al., 2015) (new Fig. 1c and new Extended Data Fig. 10h). To remove Cadherin- mediated cell-cell adhesion, we depleted □-Catenin specifically in the migrating primordium using a protein degron approach. Because □-Catenin is an essential linker between cadherins and the actin cytoskeleton, its depletion should compromise all cadherin-mediated cell-cell adhesion of the primordium cells. Although □-Catenin depletion impairs neuromast deposition by the primordium and reduces directionality of the primordium cells (new Fig. 2a, b (Colak- Champollion et al., 2019)), it does not affect the velocity of the primordium cells (new Fig. 2c, new Video 1). Consistent with this, we found that F-actin is enriched on the basal rather than the apical sides of the primordium cells (new Fig. 1c). This suggests that the primordium does not use cadherin-mediated adhesion to the skin for its migration.

Breakage of adhesions with the skin

We agree that the primordium needs to break the adhesion between the skin and the basement membrane and also push itself forward. When we decompose the stresses along the Z-axis (apical-basal axis) and the XY-axes (plane of migration), we find that the stresses along the Z-axis are about twice as high as the stresses along the XY-axes (Fig. 6j). Since the stress to separate the skin from the basement membrane needs to be

perpendicular to the plane of the skin/basement membrane, the stress along the Z-axis reflects the stress that separates the skin from the basement membrane. In contrast, the stresses along the XY-axes are in the plane of the basement membrane and skin and should not contribute much to the breakage of the basement membrane from the skin but reflect the pushing and pulling of the migrating primordium. In our revision we confirmed this prediction by assessing the stresses exerted by the primordium on the basement membrane in integrin b1b mutant embryos. In the absence of Integrin b1b, the primordium exerts lower stresses along the XY-axes than in wild-type embryo while the

stresses along the Z-axis did not change significantly (new Fig. 8i). This suggests that the stresses to propel the primordium forward require Integrin b1b but that the stresses needed to separate the skin from the basement membrane do not, in line with the idea that the stresses along the XY-axes mostly reflect the stresses exerted by the primordium for propulsion.
Cell adhesion types between the primordium and the overlying skin

In their paper on lamellipodia and focal adhesions during primordium migration, the authors used an antibody against phosphorylated Paxillin to identify focal adhesions (Olson and Nechiporuk, 2020). They observed phosphorylated Paxillin in small dots that line the interface between the skin and the primordium. Due to the spatial resolution limit of light microscopy they could not decipher whether the dots of phosphorylated Paxillin were in the superficial cells of the primordium or in the basal layer of the overlying skin. To clarify this issue, we now generated chimeric primordia with single cells expressing Itgb1b-sfGFP in embryos that otherwise express only untagged Itgb1b to image integrin clustering on the apical side of the primordium cells. This analysis showed that Itgb1b-sfGFP does form short-lived cluster on the apical side of the primordium cells facing the overlying skin (new Extended Data Fig. 4a). However, these integrin cluster probably do not engage with extracellular ligands because we cannot detect a basement membrane between the apical side of the primordium and the skin (new Extended Data Fig. 1j). Additionally, we find that actin flow on the apical side is not slowed down in itgb1b mutant primordium cells compared to wild-type primordium

cells (new Fig. 5d), indicating that these integrin clusters do not couple actin flow on the apical side of the primordium to the skin. In summary, these observations suggest that integrins form transient clusters on the apical side of the primordium cells but that these clusters do not engage in force transmission.

4. “There is confusion about the interpretation of the photo bleaching data to determine dynamics. When evaluating the stability of laminin, the authors indicate that its half-life of 12.3 minutes with mobile fraction = 20.1% indicates a stable system for optical landmarks, yet the authors state that “the mobility of Itgb1b-sfGFP is high” with parameters of a half-life of 11.4 seconds and mobile fraction = 33.6%, which to this reviewer does not seem high. It is also not at all clear that these differences are particularly significant, and the authors should be careful in their claims. Instead, the only safe comparison appears to be after experimental treatments that indicate a change in mobility, not absolute conclusions about mobility.”
We agree and now have describe the change in mobility in different scenarios rather than drawing absolute conclusions about protein mobility.

5. Although the authors convincingly establish an intriguing pattern of substrate stresses that are highest at the rear of the migrating LLP cells, it is not clear why the forces are strongest in the downward direction, as well as laterally. It is puzzling that the greatest forces are not along the direction of migration to push the collective of LLP cells forward. These findings might instead support some alternative explanation, e.g., that the forces are needed to months of a subset of cells, somewhat analogous to the claimed intercalation and forward movement of cells during normal crest cell migration.

We agree with Reviewer 2 that the pattern of stresses is surprising. We now have addressed possible reasons for the molecular and cellular underpinnings of the

observed stress pattern.

Stresses in the rear and sides of the primordium

The higher stresses in the rear and sides of the primordium correlate with higher non- muscle myosin II activity in these areas of the primordium (new Fig. 8a). Additionally, the stresses in the rear depend to large part on Integrin b1b activity. In the absence of Integrin b1b, the primordium exerts less stress on the basement membrane and this reduction is largest in the middle and rear (new Fig. 8i, Video 10). Together, this suggests that increased force generation and force transmission pull more on the basement membrane on the sides and rear of the primordium.

Stresses along the Z-axis/apical-basal axis

The stresses along the Z-axis/apical-basal axis are largest and do not depend on Integrin b1b activity (new Fig. 8i, Video 10). This suggests that these stresses are exerted on the basement in a cell-substrate independent manner, probably through the cortical actin of the cells in primordium's middle and rear which are higher/extend more along the apical-basal axis and need to push the skin and the muscle apart in a wedge-like manner for the primordium to move forward.

Intercalation and stresses

As detailed in our response to Reviewer 2's major concern 1, we have now assessed the direction and trajectories of the nuclei of primordium cells and do not see cell intercalation (new Extended Data Fig. 10g, Video 1), consistent with previous reports (Colak-Champollion et al., 2019; Nogare et al., 2017).

6. Although the stress patterns are convincing, it is not clear what they mean in terms of the movement of the entire LLP. That is, do the authors think that the rear cells are actually pushing the more anterior cells forward, or do the more anterior cells differ in being able to migrate with very little stress and strain on the underlying basement membrane, perhaps because they face less resistance in

squeezing between the skin and basement membrane than the thicker posterior portion of the LLP? The alternative explanation of the findings would be that the more anterior cells use lamellipodia and cell adhesions to both the overlying skin and the underlying basement membrane to squeeze a thinner LLP region forward, but that the cells at the rear are only pushing against the basement membrane because they must use actin flow and the molecular clutch rather than lamellipodia for forward locomotion of a thicker mass of cells facing more resistance because of the size of the LLP posterior. A possible means of clarifying these issues might be high-resolution imaging of the individual cells compared to their neighbors, as well as a more careful examination of the presence of adhesions to the skin versus the basement membrane (and ideally after removal of the skin), plus evaluations of lamellipodia.
We agree and have done the experiments suggested by Reviewer 2. High resolution imaging
High resolution imaging of primordium cell nuclei indicates that all cells move at fairly constant speed and directionality (new Extended Data Fig. 10g, new Video 1 (Colak- Champollion et al., 2019; Nogare et al., 2017)), suggesting that the rear cells do not push the front cells forward. However, we previously showed that when the front cells have a reduced sense of directionality, they repeatedly slow down and seem to be pushed forward by the rear cells, resulting in a more saltatory rather than a smooth and continuous migration pattern of the cells (Colak-Champollion et al., 2019).
Primordium adhesion to the skin

Examination of adhesion of the primordium to the skin indicates that there is very little to no LamC1 between the two tissues (new Extended Data Fig. 1j), suggesting that the skin and the primordium are not separated by a very thin basement membrane that cannot be detected by transmission electron microscopy. Although we do detect transient clustering of Integrin b1b on the apical sides of primordium cells (new Extended Data Fig. 4a), actin flow is fast in the primordium cells facing the skin and does

not depend on the presence of Integrin b1b (new Fig. 5d), suggesting that while Integrin b1b forms transient clusters it does not couple actin flow to the outside. Another possibility for force transmission from the primordium to the skin is direct cell-cell adhesion. Consistent with this idea, the skin expresses high levels of Cadherin-1 and the primordium lower levels of Cadherin-1 and Cadherin-2 (E-Cadherin and N-cadherin, new Fig. 1c and new Extended Data Fig. 10h), suggesting that cadherin-mediated cell-cell adhesion might provide coupling of the primordium to the skin. However, when we depleted □-Catenin—an essential linker between cadherins and the actin network— specifically in the primordium, the primordium shed off cells from its rear rather than depositing clusters of sensory organs (new Fig. 2a) and the cells migrated less directional (new Fig. 2b and new Video 1) as reported (Colak-Champollion et al., 2019) but the speed of the cells was not affected (new Fig. 2c), indicating that cadherin-mediated cell adhesion is not required for primordium motility.
Differential force generation across the primordium

We found that non-muscle myosin II activity is higher in the rear and on the sides of the primordium (new Fig. 8a), suggesting that the rear and sides of the primordium generate greater force than its front.
Evaluations of lamellipodia

We and others previously reported that cells in the front, middle and rear of the primordium extend protrusions in the direction of migration (Colak-Champollion et al., 2019; Haas and Gilmour, 2006; Nogare et al., 2020; Olson and Nechiporuk, 2020), suggesting that all the cells in the primordium use protrusions and contribute to the forward movement of the primordium.
Together, these observations support a model in which the front and rear cells couple to the basement membrane through forward directed protrusions but that the front cells face likely less resistance in wedging between the skin and the muscle than the rear cells, which therefore would have to generate larger forces to move forward.

7. The authors should re-check each of their conclusions within the text to verify their accuracy. For example, the text states “Itgb1b is required within the primordium for migration” but the effects are surprisingly modest after it’s a mutation and even mutation of Palin and to integrations, which the authors describe four lines later as “the surprisingly mild defects” that do not seem to be explained by common founding maternal contributions in subsequent analyses. Kept towards the end of the manuscript, the authors claim that the mutated NPxY integration “failed to support efficient primordium migration” and concludes that the “integration/detail and complex is important for the primordium to move along its migratory route” but unless this reviewer is confused, the actual data seem to show that migration can still proceed at about 70% of the normal rate the authors need to be more precise in their descriptions and conclusions of the findings.
We thank Reviewer 2 for pointing this out. We now have rechecked and adjusted our conclusions about the effect of loss of Integrin b1b and Talin function on the migration of the primordium to clearly indicate that in both scenarios the primordium migrates slower but does not stall. We would like to clarify that our statement about “the surprisingly mild defects” was in reference to the overall phenotype of zygotic talin triple mutant (tln1-/-; tln2a-/-; tln2b-/-) embryos and not about the migration defect of the primordium in these embryos (migration is not affected)—we apologize for the confusion this might have created. We were surprised because talin-1 mutant mice arrest during gastrulation (Monkley et al., 2000) and inactivation of talin-1 and talin-2 in different mouse tissues results in strong phenotypes (Conti et al., 2009; Monkley et al., 2011).

8. With the reservations enumerated above, the Abstract and overall conclusion appear reasonable in a paper with commendable experimental carefulness and good statistical analyses.
We would like to thank Reviewer 2 for the appreciation of our approach and study.

9. This novel method for quantifying tension/stress by strain analyses depends on the substrate being elastic rather than plastic. Can the authors show that the basement membrane snaps back to the same original pattern as verified quantitatively?
We agree and now have included a careful analysis of movement of bleached marks on the basement membrane before, during, and after the primordium traversed the marks. Consistent with our initial observation that the skin reversibly deforms the basement membrane over tens of seconds (new Extended Data Fig. 9a-e), this analysis confirms that the basement membrane also snaps back to its original position after tens of minutes (new Extended Data Fig. 9f, g), suggesting that the basement membrane is largely elastic over these time scales and degrees of deformations.

10. The estimates of stresses are a bit confusing. The text claims “stresses of around 1 kPa” but the results show stresses of 64-600 Pa, with the largest being somewhat confusingly lateral or downward in Z.
We agree and thank Reviewer 2 for pointing this out. We have now corrected this and state that the maximal stresses we observe are around 600 Pa.

11. The text states rather confusingly that the “cells on its side and rear push sideways and strongly backwards, consistent with theoretical predictions for adherence cell migration. However, that reference number 23 refers to integration-independent cell migration in cells that have virtually no adhesion to a substrate, so it is completely unclear how the present findings can be interpreted in light of the swimming motion of cells in environments with virtually no adhesion to the substrate. The large stresses reported in this paper seem to be consistent with strong focal adhesions, so the authors also need to consider why

they can only observe nascent adhesions – perhaps because the cells make very strong but unusually transient adhesions to the basement membrane substrate.
We cited the work by Paluch and colleagues because the authors provided a theoretical prediction for the traction stress pattern for a migrating cell using friction versus adhesion to couple to the substrate (Fig. 4b in (Bergert et al., 2015)), and we found that the traction stress pattern generated by the primordium is similar to the authors’ prediction for “adherent walkers”. We now state this more clearly in the revised manuscript.
We agree with Reviewer 2 that the strong stresses we observed below the migrating primordium suggest that there should be mature and long-lasting focal adhesions.
However, we do not detect focal adhesion-like structures unless we place the primordium cells on slides (Extended Data Fig. 4l-n). To the best of our knowledge there is no clear report of nascent or focal adhesions in migrating cells in vivo. In contrast to migrating cells cultured on 2D substrates, focal adhesions were initially not observed in cells migrating in 3D culture (Fraley et al., 2010) but later

detected by lowering the expression levels of GFP-tagged paxillin (Doyle et al., 2015; Kubow and Horwitz, 2012). This prompted us to tag Integrin b1b with GFP and tdTomato at the endogenous itgb1b locus and observe the distribution of Integrin b1b at endogenous levels. Although we cannot exclude that focal adhesions form very transiently, we feel that it is unlikely that we missed focal adhesions during our spinning disk confocal time lapse recordings with an exposure time of 500 milliseconds and a time interval of 30 seconds. We therefore tend to believe that migrating cells in vivo do not mature their nascent adhesion-like structures into focal adhesions. One possible reason for this is that the basement membrane is much softer than the substrate stiffness used for culturing migrating cells. Since focal adhesions are larger with increasing substrate stiffness, this might explain the discrepancy between observations in the animal and in cell culture.

12. The authors need to compare their findings on the role of Talin with the recently published effects of morpholino knockdown of Talin in PMID: 33096063.

For our study we opted not to use morpholino-mediated knock-down of Talin activity because this approach often generates artifacts (Kok et al., 2015), especially in the migrating primordium (Aman et al., 2011), which can confound the interpretation of the observed phenotype. Consistent with this worry, in contrast to morpholino knock-down of tln1 (Olson and Nechiporuk, 2020), the primordium does not migrate slower in tln1 mutant embryos. Even removing tln1, tln2a and tln2b zygotically does not slow primordium migration (Extended Fig.6b, c). Only when we also remove the maternal contribution of tln1 and tln2b do we observe slowed primordium migration (new Fig. 4h-j). This suggests that tln1 knock-down by morpholinos impairs primordium migration non-specifically, probably by inducing cell death in the primordium which has been shown to slow primordium migration (Aman et al., 2011). We therefore feel that genetically impairing Talin function is reliable approach to study Talin.

13. There is a minor typo in line 263 in which “macrophages” has an extra “p.”

We now have fixed the typo.

In summary, this manuscript presents an impressive amount of high-quality data based on a novel and innovative approach to measuring in vivo traction forces, along with striking implication of the role of actin flow in vivo to highly efficient tissue translocation. Although these features of this impressively data-rich manuscript make this reviewer overall positive in terms of guarded optimism about the

possibility of future acceptance for publication, the multiple seemingly major points raised in this review will need to be resolved directly.
We thank Reviewer 2 for this encouraging and appreciative comment on our manuscript.

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Decision Letter, first revision:

Dear Dr Knaut,

I hope you are well. I'm Daryl; now part of the editorial team at Nature Cell Biology and I am looking forward to working with you. I am happy to be handling the further peer review of your manuscript.

Your revised manuscript, "A rear-engine drives adherent tissue migration in vivo", has now been seen by our 2 referees, who are experts in cell migration, development (referee 1); cell migration, biophysics (referee 2).

As you will see, while the referees acknowledge the additional experiments performed, and Reviewer 1 offers positive comments, Reviewer #2 continues to have persisting concerns with regard to the proposed rear-engine model, and we believe that their remaining concerns should be addressed before we can consider publication in Nature Cell Biology. We are willing to allow one more round of revision to address the referees' concerns. Please be aware that further reconsideration of this manuscript will be conditional on the referees being fully satisfied with the extent of the revisions, as we generally do not encourage multiple review rounds at this journal.

Nature Cell Biology editors discuss the referee reports in detail within the editorial team, including the chief editor, to identify key referee points that should be addressed with priority, and requests that are overruled as being beyond the scope of the current study. To guide the scope of the revisions, I have listed these points below. We are committed to providing a fair and constructive peer-review process, so please feel free to contact me if you would like to discuss any of the referee comments further.

In particular, it would be essential to:

A) Describe the integration of traction forces, cell adhesion, and cell force propagation and how these will contribute to a proposed "rear-engine" drive (Reviewer #2)

B) Assess subcellular localization of cell adhesion molecules in vivo (Reviewer #2)

C) All other referee concerns pertaining to strengthening existing data, providing controls, methodological details, clarifications and textual changes, should also be addressed.

D) Finally please pay close attention to our guidelines on statistical and methodological reporting (listed below) as failure to do so may delay the reconsideration of the revised manuscript. In particular please provide:

- a Supplementary Figure including unprocessed images of all gels/blots in the form of a multi-page pdf file. Please ensure that blots/gels are labeled and the sections presented in the figures are clearly indicated.

- a Supplementary Table including all numerical source data in Excel format, with data for different figures provided as different sheets within a single Excel file. The file should include source data giving rise to graphical representations and statistical descriptions in the paper and for all instances where the figures present representative experiments of multiple independent repeats, the source data of all repeats should be provided.

We would be happy to consider a revised manuscript that would satisfactorily address these points, unless a similar paper is published elsewhere, or is accepted for publication in Nature Cell Biology in the meantime.

When revising the manuscript please:

- ensure that it conforms to our format instructions and publication policies (see below and https://www.nature.com/nature/for-authors).

- provide a point-by-point rebuttal to the full referee reports verbatim, as provided at the end of this letter.

- provide the completed Reporting Summary (found here https://www.nature.com/documents/nr-reporting-summary.pdf). This is essential for reconsideration of the manuscript will be available to editors and referees in the event of peer review. For more information see http://www.nature.com/authors/policies/availability.html or contact me.

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Nature Cell Biology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

This journal strongly supports public availability of data. Please place the data used in your paper into a public data repository, or alternatively, present the data as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories appears below.

Please submit the revised manuscript files and the point-by-point rebuttal to the referee comments using this link:

[REDACTED]

*This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We would like to receive a revised submission within six months.

We hope that you will find our referees' comments, and editorial guidance helpful. Please do not hesitate to contact me if there is anything you would like to discuss.

Best wishes,

Daryl Jason David

Daryl J.V. David, PhD

Senior Editor, Nature Cell Biology
Consulting Editor, Nature Communications
Nature Portfolio

Heidelberger Platz 3, 14197 Berlin, Germany
Email: daryl.david@nature.com
ORCID: https://orcid.org/0000-0002-9253-4805

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
This paper is a real tour de force. I recommend acceptance without any further revision.

Reviewer #2:
Remarks to the Author:
In this resubmitted manuscript revised in response to the previous reviewers, the authors have done an excellent job of resolving most of my concerns. Their toning down or clarifying of some statements was quite helpful. Nevertheless, two significant reservations remain.

1. As noted in my original review, it is still not clear (at least to me as a very confused reader) exactly how the described traction forces, myosin, and integrins, as well as cell-cell force propagation within the primordium, can be shown to produce migration using the proposed 'rear engine' drive. Specifically, the authors need to provide a clear diagrammatic model or diagrams to explain to readers exactly how their system actually works from the point of view of the whole primordium and also for individual cells at the back and the front. That might allow them to explain the unusual Z-direction forces, the nature of adhesive interactions, and exactly how the localized myosin forces result in movement of the entire primordium, especially because the authors report no differences in individual cell motility at the front vs. back. That is, please explain clearly exactly how this system works, and why it is a 'rear-engine' considering point #2 below.

2. The authors' response states: "Together, these observations support a model in which the front and rear cells couple to the basement membrane through forward directed protrusions but that the front cells face likely less resistance in wedging between the skin and the muscle than the rear cells, which therefore would have to generate larger forces to move forward." This does not sound at all like a 'rear-engine' that can drive the primordium forward from the rear, but instead merely a requirement for migrating rear cells to exert more force by themselves to be able to squeeze their own cell bodies through greater resistance. That is simply not the same as a rear-engine drive for the primordium as a whole, but just more work for rear cells to deal with their local environment than the front cells. That is, are there front engines and rear engines, and just the latter need to work harder?

3. A second concern raised in my original review involved focal or other adhesions. The authors seem to denigrate the accuracy of the Olson and Nechiporuk 2020 paper by suggesting that the phospho-paxillin staining was in epidermal cells by stating "they could not decipher whether the dots of phosphorylated Paxillin were in the superficial cells of the primordium or in the basal layer of the overlying skin." Yet the authors do see apical integrin clusters that elsewhere seem to be interpreted as adhesions, though with altered actin flow. This sounds like the now-discredited paper from Fraley et al. published in NCB where there were claims of apparent cytoskeletal functions in the absence of cell adhesions – which turned out to be due to technical problems (e.g., see PMID 21173800 and later work). Please use your better optics to resolve this question by doing the phosphorylated paxillin staining yourselves.
Additionally, even though one might well not see standard focal adhesions in this system, shouldn't there be some evidence for altered cell adhesions at sites of greater force transmission at the rear of

the migrating primordium? Specifically, at a minimum, the authors need to similarly test phospho-paxillin staining (and ideally other adhesion markers including anti-paxillin protein) to resolve the issues with the 2020 paper and to explain how substantial traction forces can be propagated to the basement membrane without the formation of more frequent, larger, or more stabilized cell adhesions. The integrin and myosin analyses were a useful start, but one worries about chimera expression artifacts – why not just perform standard immunofluorescence to confirm (i) the claimed absence of apical cell adhesions, (ii) more (or altered) adhesions at the rear or not, and (iii) no supracellular actomyosin cable?

4. A minor point is that even though the authors seem to be making a remarkable claim that there is no basement membrane under skin (which sounds quite remarkable compared to other species) because there is no BM between the skin and the migrating primordium, is there no other type of matrix to which cells might adhere? Why are there apical integrin clusters?

This fine paper still seems to be weakened by a few significant ongoing issues, which this reviewer hopes can be resolved sufficiently definitively.

GUIDELINES FOR MANUSCRIPT SUBMISSION TO NATURE CELL BIOLOGY

READABILITY OF MANUSCRIPTS – Nature Cell Biology is read by cell biologists from diverse backgrounds, many of whom are not native English speakers. Authors should aim to communicate their findings clearly, explaining technical jargon that might be unfamiliar to non-specialists, and avoiding non-standard abbreviations. Titles and abstracts should concisely communicate the main findings of the study, and the background, rationale, results and conclusions should be clearly explained in the manuscript in a manner accessible to a broad cell biology audience. Nature Cell Biology uses British spelling.

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ACKNOWLEDGEMENTS – should be kept brief. Professional titles and affiliations are unnecessary. Grant numbers can be listed.

AUTHOR CONTRIBUTIONS – must be included after the Acknowledgements, detailing the contributions of each author to the paper (e.g. experimental work, project planning, data analysis etc.). Each author should be listed by his/her initials.

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Methods should be written concisely, but should contain all elements necessary to allow interpretation and replication of the results. As a guideline, Methods sections typically do not exceed 3,000 words. The Methods should be divided into subsections listing reagents and techniques. When citing previous methods, accurate references should be provided and any alterations should be noted. Information must be provided about: antibody dilutions, company names, catalogue numbers and clone numbers for monoclonal antibodies; sequences of RNAi and cDNA probes/primers or company names and catalogue numbers if reagents are commercial; cell line names, sources and information on cell line identity and authentication. Animal studies and experiments involving human subjects must be reported in detail, identifying the committees approving the protocols. For studies involving human subjects/samples, a statement must be included confirming that informed consent was obtained. Statistical analyses and information on the reproducibility of experimental results should be provided in a section titled "Statistics and Reproducibility".

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• Accession codes for primary datasets (generated during the study under consideration and designated as "primary accessions") and secondary datasets (published datasets reanalysed during the study under consideration, designated as "referenced accessions"). For primary accessions data should be made public to coincide with publication of the manuscript. A list of data types for which submission to community-endorsed public repositories is mandated (including sequence, structure, microarray, deep sequencing data) can be found here http://www.nature.com/authors/policies/availability.html#data.

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• At a minimum, please include a statement confirming that all relevant data are available from the authors, and/or are included with the manuscript (e.g. as source data or supplementary information), listing which data are included (e.g. by figure panels and data types) and mentioning any restrictions on availability.

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We recommend that you upload the step-by-step protocols used in this manuscript to the Protocol Exchange. More details can found at www.nature.com/protocolexchange/about.

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only be performed for samples derived from the same experiment with gels/blots were processed in parallel, which needs to be stated in the legend.

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- We do not recommend using Adobe Photoshop for designing figures, but we can accept Photoshop generated (.PSD or .TIFF) files only if each element included in the figure (text, labels, pictures, graphs, arrows and scale bars) are on separate layers. All text should be editable in 'type layers' and line-art such as graphs and other simple schematics should be preserved and embedded within 'vector smart objects' - not flattened raster/bitmap graphics.

- Some programs can generate Postscript by 'printing to file' (found in the Print dialogue). If using an application not listed above, save the file in PostScript format or email our Art Editor, Allen Beattie for advice (a.beattie@nature.com).

Regardless of format, all figures must be vector graphic compatible files, not supplied in a flattened raster/bitmap graphics format, but should be fully editable, allowing us to highlight/copy/paste all text and move individual parts of the figures (i.e. arrows, lines, x and y axes, graphs, tick marks, scale bars etc.). The only parts of the figure that should be in pixel raster/bitmap format are photographic images or 3D rendered graphics/complex technical illustrations.

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descriptions of each panel with definitions of the symbols used, but without detailing methodology.

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Unprocessed scans of all key data generated through electrophoretic separation techniques need to be presented in a supplementary figure that should be labelled and numbered as the final supplementary figure, and should be mentioned in every relevant figure legend. This figure does not count towards the total number of figures and is the only figure that can be displayed over multiple pages, but should be provided as a single file, in PDF or TIFF format. Data in this figure can be displayed in a relatively informal style, but size markers and the figures panels corresponding to the presented data must be indicated.

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Each Supplementary Figure should be provided as a single page and as an individual file in one of our accepted figure formats and should be presented according to our figure guidelines (see above). Supplementary Tables should be provided as individual Excel files. Supplementary Videos should be provided as .avi or .mov files up to 50 MB in size. Supplementary Figures, Tables and Videos much be accompanied by a separate Word document including titles and legends.

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Information on how many times each experiment was repeated independently with similar results needs to be provided in the legends and/or Methods for all experiments, and in particular wherever representative experiments are shown.

We strongly recommend the presentation of source data for graphical and statistical analyses as a separate Supplementary Table, and request that source data for all independent repeats are provided when representative experiments of multiple independent repeats, or averages of two independent experiments are presented. This supplementary table should be in Excel format, with data for different figures provided as different sheets within a single Excel file. It should be labelled and numbered as one of the supplementary tables, titled "Statistics Source Data", and mentioned in all relevant figure legends.

--------- Please don't hesitate to contact NCB@nature.com should you have queries about any of the above requirements ---------

Author Rebuttal, first revision:

Response to reviewers' comments

Reviewer #1 comments:

"This paper is a real tour de force. I recommend acceptance without any further revision."
We thank Reviewer 1 for the appreciation and continued support of our study.

Reviewer #2 comments:

"In this resubmitted manuscript revised in response to the previous reviewers, the authors have done an excellent job of resolving most of my concerns. Their toning down or clarifying of some statements was quite helpful. Nevertheless, two significant reservations remain."
We thank Reviewer 2 for the continued support and thoughtful suggestions.

1. "As noted in my original review, it is still not clear (at least to me as a very confused reader) exactly how the described traction forces, myosin, and integrins, as well as cell-cell force propagation within the primordium, can be shown to produce migration using the proposed 'rear engine' drive. Specifically, the authors need to provide a clear diagrammatic model or diagrams to explain to readers exactly how their system actually works from the point of view of the whole primordium and also for individual cells at the back and the front. That might allow them to explain the unusual Z-direction forces, the nature of adhesive interactions, and exactly how the localized myosin forces result in movement of the entire primordium, especially because the authors report no differences in individual cell motility at the front vs. back. That is, please explain clearly exactly how this system works, and why it is a 'rear-engine' considering point #2 below."

2. "The authors' response states: "Together, these observations support a model in which the front and rear cells couple to the basement membrane through forward directed protrusions but that the front cells face likely less resistance in wedging between the skin and the muscle than the rear cells, which therefore would have to generate larger forces to move forward." This does not sound at all like a 'rear-engine' that can drive the primordium forward from the rear, but instead merely a requirement for migrating rear cells to exert more force by themselves to be able to squeeze their own cell bodies through greater resistance. That is simply not the same as a rear-engine drive for the primordium as a whole, but just more work for rear cells to deal with their local environment than the front cells. That is, are there front engines and rear engines, and just the latter need to work harder?"

Rear-engine analogy
We thank Reviewer 2 for his thoughtful comments on the rear-engine idea, and we are very sorry for the confusion we created using this analogy. Maybe the confusion about the rear-engine analogy stems from a difference in what Reviewer 2 and we mean by a rear-engine design. In a strict definition a rear-engine is a propulsion system where only the rear contributes to locomotion and the front does not. This is similar to what drives an old VW van forward, an engine in the back and none in the front. This is clearly not the case for the primordium. In a loose definition, a rear-engine is a propulsion system where the rear contributes most to the locomotion and the front contributes little. This is what we postulate for how the primordium is propelled forward. We have shown that the front cells pull on the basement membrane in fairly random directions and generate little stresses (Fig. 6i, j, Video 10). In contrast, the rear cells pull on the basement membrane mostly sideways and rearwards and generate large stresses (Fig. 6i, j, Video 10). We therefore fully agree with Reviewer 2's summary of these observations that

there are front engines and rear engines, and that the latter need to work harder. We speculate that the reason for why the rear cells need to work harder is that they have to

push the front cells forward. The reasons for this speculation are that:

- the only difference in the local environment between the front and rear cells are the front cells in front of the rear cells—the rear cells move to the same location that the front cells occupied 60 min ago, and it is therefore likely that the environment is otherwise very similar,
- the front cells push and pull on the basement membrane in fairly random directions which should not support directed motion on its own (Fig. 6i, Video 10) but might require the pushing by the rear cells for forward directed movement, and
- severing the front cells from the rear cells using laser cutting (Nogare et al., 2014) or genetics (Colak-Champollion et al., 2019) stalls the front cells until the rear cells unite with the front cells again, suggesting that the front cells need the rear cells to move forward.

This is why we postulate that the primordium pushes itself mainly—but not solely— forward with its rear, akin to a rear-engine in the looser sense. As suggested, we have now depicted this idea in a diagrammatic model in Figure 8k. If Reviewer 2 feels that the use of the analogy of a "rear-engine" is inappropriate for how the primordium pushes itself forward, we are happy to use the "continuous breast-stroke" analogy instead and also welcome any suggestions by Reviewer 2.

Stresses in z-direction

We agree with Reviewer 2 that the observed stresses in the z-direction are surprising. We believe that the cause for these stresses is the squeezing motion of the primordium. The primordium needs to wedge itself between the skin and the basement membrane on top of the muscle. To do this, it needs to push the skin and basement membrane/muscle in opposite directions; the skin is pushed outwards and the basement membrane and muscle are pushed inwards by the primordium (towards the notochord). This squeezing is likely the cause for the stresses along the z-direction. We envision this process to be similar to pushing a wedge between to elastic sheets. Since

we find that the stresses in z-direction are independent of Integrin b1b activity (Fig. 8i), the source of the stresses is probably not the actin flow on the basal sides of the cells but likely the cortical actomyosin network in the cells of the primordium. We will test this supposition once we have finished building the tools to manipulate the cortical actomyosin network in the primordium. Importantly, stresses in the z-direction/against the substrate have also been observed in migrating cultured cells (Li et al., 2021, Legant et al., 2013, Delanoë-Ayari et al., 2010).

3. "A second concern raised in my original review involved focal or other adhesions. The authors seem to denigrate the accuracy of the Olson and Nechiporuk 2020 paper by suggesting that the phospho-paxillin staining was in epidermal cells by stating "they could not decipher whether the dots of phosphorylated Paxillin were in the superficial cells of the primordium or in the basal layer of the

overlying skin.“ Yet the authors do see apical integrin clusters that elsewhere seem to be interpreted as adhesions, though with altered actin flow. This sounds like the now-discredited paper from Fraley et al. published in NCB where there were claims of apparent cytoskeletal functions in the absence of cell adhesions – which turned out to be due to technical problems (e.g., see PMID 21173800 and later work). Please use your better optics to resolve this question by doing the phosphorylated paxillin staining yourselves.
Additionally, even though one might well not see standard focal adhesions in this system, shouldn't there be some evidence for altered cell adhesions at sites of greater force transmission at the rear of the migrating primordium? Specifically, at a minimum, the authors need to similarly test phospho-paxillin staining (and ideally other adhesion markers including anti-paxillin protein) to resolve the issues with the 2020 paper and to explain how substantial traction forces can be propagated to the basement membrane without the formation of more frequent, larger, or more stabilized cell adhesions. The integrin and myosin analyses were a useful start, but one worries about chimera expression artifacts – why not just

perform standard immunofluorescence to confirm (i) the claimed absence of apical cell adhesions, (ii) more (or altered) adhesions at the rear or not, and (iii) no supracellular actomyosin cable?”
We fully agree with Reviewer 2 that fusions to fluorescent proteins can impair the distribution and function of proteins. We have therefore tested and confirmed that Itgb1b-sfGFP, Itgb1b-tdTomato, and Tln1-YPet replace the function of the endogenous proteins (Fig. 4f-h and Methods). In our initial submission. we did not perform antibody staining against focal adhesion proteins and the actomyosin network for two reasons. First, fixation often distorts the tissues and the distribution of proteins, a caveat that is circumvented by tagging the proteins fluorescently and assessing the fusion protein distribution in live animals. This is nicely exemplified by the initial failure to detect focal adhesion-like structures in fly embryos by antibody staining (Narasimha and Brown, 2004) but not by live imaging (Goodwin et al., 2016). Second, the skin above and the muscle below the primordium express focal adhesion proteins, F-actin and myosin II, such that it is difficult to unambiguously assign the localization of proteins to the skin, muscle or primordium by light microscopy. This is why we generated primordium- specific F-actin and Myosin II reporter lines. Also, this is why we transplanted cells expressing Itgb1b-sfGFP, Tln1-YPet or F-Tractin-mCherry into unlabeled host embryos. However, we agree with Reviewer 2 that it is worthwhile to perform antibody staining against focal adhesion proteins, F-actin, and myosin II. We have now performed these staining experiments and summarize our observations and conclusions below.

Paxillin and phosphorylated paxillin staining
As suggested by Reviewer 2 we have stained embryos for Paxillin and phosphorylated Paxillin (p-Paxillin) protein using antibodies. We also analyzed the localization of Paxillin- mScarlet in the primordium expressed from the cxcr4b promoter on a BAC transgene. As expected, Paxillin, p-Paxillin and Paxillin-mScarlet localize to the myotendinous junctions in the muscle (arrows in Response Letter Fig. 1f). In the

primordium, Paxillin-mScarlet is cytoplasmic and not enriched at the membrane while Paxillin is both cytoplasmic and

localized to the membrane (Response Letter Fig. 1b, c). This difference in localization between Paxillin-mScarlet and Paxillin is likely due to the fact that the cxcr4b promoter expresses higher levels of Paxillin-mScarlet than the endogenous promoter which probably mask the endogenous localization pattern. p-Paxillin localizes to dots on the membrane of the primordium (Response Letter Fig. 1a) as was reported by Nechiporuk and colleagues (Olson and Nechiporuk, 2020). Interestingly, the levels of p-Paxillin are higher in the front than in the rear of the primordium while Paxillin levels are uniform across the front-rear axis of the primordium (Fig. 8j). Since p-Paxillin is thought to mark disassembling focal adhesions (Zaidel-Bar et al., 2006), this suggests that front cells adhere less to the basement membrane than the rear cells, consistent with the observation that the front cells exert smaller stresses than the rear cells on the basement membrane (Fig. 6j). We have now included this observation in the revised manuscript (Fig. 8j) and would like to sincerely thank Reviewer 2 for this helpful suggestion. We would have not thought of looking at Paxillin and p-Paxillin due to the caveats associated with antibody staining that we detailed above.

Vinculin and p-FAK staining
We have stained embryos for Vinculin and phosphorylated FAK (p-FAK). As expected, Vinculin and p-FAK localize to the myotendinous junctions but we did not see any differences in localization of Vinculin or p-FAK in the primordium (Response Letter Fig. 1d, e). We therefore did not include these experiments in the revised manuscript.

Actomyosin network
We have now stained embryos in which we labeled the primordium with membrane- tethered GFP (cxcr4b:EGFP-CaaX) for endogenous, untagged non-muscle myosin IIA (NMIIA), phospho-myosin light chain (pMLC) and F-actin using antibodies and phalloidin. As expected, the anti-NMIIA antibody labeled the myotendinous junctions. However, it also labeled all nuclei in the zebrafish embryo, making it impossible to draw conclusions about the localization of NMIIA in the primordium (Response Letter Fig. 2). In contrast,

the staining for pMLC and F-actin did not show unspecific staining. F-actin labeled by phalloidin localizes to the cell cortex and was enriched on the basal sides of the cells in the primordium (Extended Data Fig. 10i, left) and phosphorylated myosin II labeled by anti-pMLC antibody staining localized to the apical constrictions and to dots in the primordium cells (Extended Data Fig. 10i, right). This is consistent with our analysis of the F-actin and Myosin II distribution in live embryos using fluorescently tagged F- Tractin and Myl12.1 (Fig. 1c, 7a, 8a), and suggests that there is no actomyosin cable around the rear of the primordium. We have now included these observations in the revised manuscript (Extended Data Fig. 10i).

4. “A minor point is that even though the authors seem to be making a remarkable claim that there is no basement membrane under skin (which sounds quite remarkable compared to other species) because there is no BM between the skin and the migrating primordium, is there no other type of matrix to which cells might adhere? Why are there apical integrin clusters?”

We are probably at fault for creating a confusion here. We do not claim that there is no basement membrane under the skin in zebrafish embryos. Our transmission electron and confocal microscopy analysis shows that there is a LamC1-labeled basement membrane sandwiched between the skin and the muscle (Fig. 1a, b) as reported previously (Berg et al., 2019). Unexpectedly, however, we found that the primordium wedges between the skin and the basement membrane, and displaces the skin apically and the basement membrane and muscle basally (Fig. 1a, b). Once the primordium has passed, the skin reattaches to the basement membrane on top of the muscle; So only at the position of the primordium is the skin not attached to a basement membrane for a short period of time. We have now added a sentence to clearly state that the skin is attached to the basement membrane everywhere but at the primordium position.

To address the possibility that there are other extracellular matrix components localized

between the skin and the primordium we stained embryos for Fibronectin and chondroitin sulfate. This analysis showed that fibronectin is enriched underneath the migrating primordium similar to LamC1-sfGFP (Fig. 1a, b). However, in contrast to LamC1-sfGFP, Fibronectin also labels the membranes of the primordium, suggesting that it is present between the primordium cells (Extended Data Fig. 1k). Chondroitin sulfate is expressed by the primordium and the skin (Extended Data Fig. 1l). In contrast to Laminin C1, neither Fibronectin nor chondroitin sulfate formed a continuous layer along the migratory route. Thus, Fibronectin and proteoglycans (labeled by chondroitin sulfate) might be the ligands responsible for the transient integrin clusters we observe on the apical side of the primordium cells. We have now included these observations in our revised manuscript (Extended Data Fig. 1k, l).

“This fine paper still seems to be weakened by a few significant ongoing issues, which this reviewer hopes can be resolved sufficiently definitively.”

We thank Reviewer 2 for his support and the thoughtful comments and we hope that we now have addressed the concerns that were not addressed sufficiently in our first revised version.

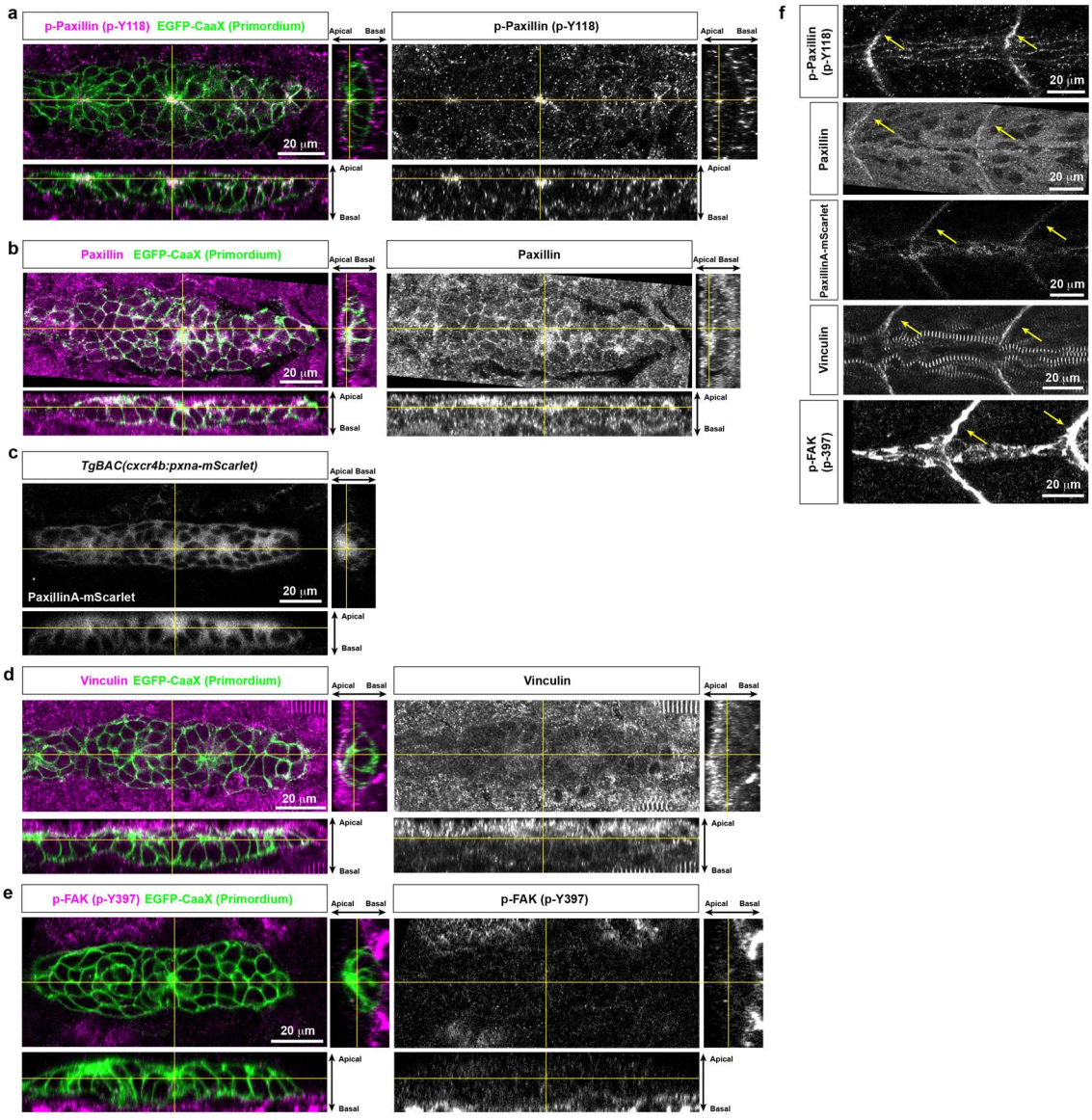

Fig. 1. Distribution of Paxillin, phospho-Paxillin, Paxillin-mScarlet, Vinculin and phospho-FAK in the primordium.

a. Immunofluorescent staining against phospho-Paxillin (p-Y118) and membrane-tethered EGFP in 32 hpf cxcr4b:EGFP-CaaX embryos. Images are single slices from a z-stack at the indicated position (yellow lines) shown as orthogonal views. (Left) Merged images showing p-Paxillin (p-Y118) in magenta and membrane-tethered EGFP in green. (Right) images showing p-Paxillin (p-Y118) only. Scale bar = 20 □m.

b. Immunofluorescent staining against Paxillin and membrane-tethered EGFP in 32 hpf cxcr4b:EGFP-CaaX embryos. Images are single slices from a z-stack at the indicated position (yellow lines) shown as orthogonal views. (Left) Merged images showing Paxillin in magenta and membrane-tethered EGFP in green. (Right) images showing Paxillin only. Scale bar = 20 m.

c. Live imaging of a cxcr4b:paxillinA-mScarlet 32 hpf embryo. Images are single slices from a z-stack at the indicated position (yellow lines) shown as orthogonal views. Scale bar = 20 m.

d. Immunofluorescent staining against Vinculin and membrane-tethered EGFP in 30 hpf cxcr4b:EGFP-CaaX embryos. Images are single slices from a z-stack at the indicated position (yellow lines) shown as orthogonal views. (Left) Merged images showing Vinculin in magenta and membrane-tethered EGFP in green. (Right) images showing Vinculin only. Scale bar = 20 m.

e. Immunofluorescent staining against p-FAK and membrane-tethered EGFP in 32 hpf cxcr4b:EGFP-CaaX embryos. Images are single slices from a z-stack at the indicated position (yellow lines) shown as orthogonal views. (Left) Merged images showing p-FAK in magenta and membrane-tethered EGFP in green. (Right) images showing p-FAK only. Scale bar = 20 m.

f. phospho-Paxillin, Paxillin, PaxillinA-mScarlet, Vinculin and p-FAK localize to the myotendinous junction in 32 hpf embryos. Images are single slices from the z-stacks shown in a for phospho-Paxillin (p-Y118), b for Paxillin, c for PaxillinA-mScarlet, d for Vinculin, and e for p-FAK. Scale bar = 20 m. Note, the cxcr4b promoter drives strong expression in the primordium and dim expression in the muscle.

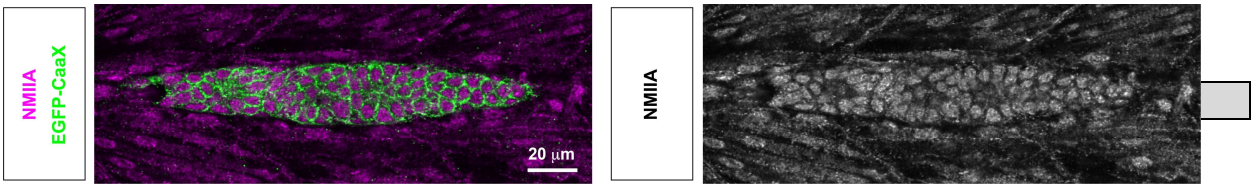

Fig. 2. Immunofluorescent staining against NMIIA.
EGFP-CaaX was expressed from the primordium-specific cxcr4b promoter. Images are single slices from a z-stack. (Left) Composite image showing NMIIA signal in magenta and GFP signal in green. (Right) NMIIA signal only. Scale bar = 20 m.

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Decision Letter, Second Revision:

4th November 2021

Dear Dr. Knaut,

Thank you for submitting your revised manuscript "A rear-engine drives adherent tissue migration in vivo" (NCB-K45545B). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Cell Biology, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

As the current version of your manuscript is in a PDF format, please email us a copy of the file in an editable format (Microsoft Word or LaTex)-- we can not proceed with PDFs at this stage.

Furthermore, while we agree that a rear-based “engine” may be a useful analogy moreso than any “continuous breast stroke” analogy, we would require any mention of rear-based engine to be removed from the title to avoid any potential confusion to our readers. We suggest a title to be “Rear cells contribute to adherent tissue migration (OR collective cell migration) in vivo”. Furthermore, in the text, we suggest any mentions of a “rear-engine design” be changed to a “rear-engine-like design”.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Cell Biology. Please do not hesitate to contact me if you have any questions.

Sincerely,

Daryl J.V. David, PhD

Senior Editor, Nature Cell Biology
Consulting Editor, Nature Communications
Nature Portfolio

Heidelberger Platz 3, 14197 Berlin, Germany
Email: daryl.david@nature.com
ORCID: https://orcid.org/0000-0002-9253-4805

Reviewer #2 (Remarks to the Author):

In this resubmitted manuscript, the authors have responded quite conscientiously and effectively to my remaining concerns. I fully agree with their characterization of types of engines in their response to reviewers, including that a rear engine cannot provide propulsion to the front, just as in a VW. Consequently, their proposed solution of changing their title from "A rear-engine" to "A continuous breast-stroke" or related wording would fully resolve my concerns, of course assuming that they clarify the breast-stroke analogy involves some anterior propulsion, but with more forward driving force coming from the rear. Their other revisions, and especially the summary diagram, were very helpful for understanding their conclusions and placing them in the context of the prior literature. I am therefore happy to recommend publication assuming they will correct their title to be more accurate, especially given other papers referring more correctly to a rear wheel drive, etc.

29th November 2021

Dear Dr. Knaut,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Cell Biology manuscript, "A rear-engine drives adherent tissue migration in vivo" (NCB-K45545B). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Please also check and comment on any additional marked-up edits we have proposed within the text. Ensuring that each point is addressed

will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within one week). Please get in contact with us if you anticipate delays.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

If you have not done so already, please alert us to any related manuscripts from your group that are under consideration or in press at other journals, or are being written up for submission to other journals (see: https://www.nature.com/nature-research/editorial-policies/plagiarism#policy-on-duplicate-publication for details).

In recognition of the time and expertise our reviewers provide to Nature Cell Biology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "A rear-engine drives adherent tissue migration in vivo". For those reviewers who give their assent, we will be publishing their names alongside the published article.

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If you have any further questions, please feel free to contact me.

Best regards,

Nyx Hills
Staff
Nature Cell Biology

On behalf of

Daryl J.V. David, PhD

Senior Editor, Nature Cell Biology
Consulting Editor, Nature Communications

Nature Portfolio

Heidelberger Platz 3, 14197 Berlin, Germany
Email: daryl.david@nature.com
ORCID: https://orcid.org/0000-0002-9253-4805

Reviewer #2:
Remarks to the Author:
In this resubmitted manuscript, the authors have responded quite conscientiously and effectively to my remaining concerns. I fully agree with their characterization of types of engines in their response to reviewers, including that a rear engine cannot provide propulsion to the front, just as in a VW. Consequently, their proposed solution of changing their title from "A rear-engine" to "A continuous breast-stroke" or related wording would fully resolve my concerns, of course assuming that they clarify the breast-stroke analogy involves some anterior propulsion, but with more forward driving force coming from the rear. Their other revisions, and especially the summary diagram, were very helpful for understanding their conclusions and placing them in the context of the prior literature. I am therefore happy to recommend publication assuming they will correct their title to be more accurate, especially given other papers referring more correctly to a rear wheel drive, etc.

Final Decision Letter:

Dear Dr Knaut,

I am pleased to inform you that your manuscript, "Rear traction forces drive adherent tissue migration in vivo", has now been accepted for publication in Nature Cell Biology.

Thank you for sending us the final manuscript files to be processed for print and online production, and for returning the manuscript checklists and other forms. Your manuscript will now be passed to our production team who will be in contact with you if there are any questions with the production quality of supplied figures and text.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Cell Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

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Please feel free to contact us if you have any questions.

With kind regards,

Daryl J.V. David, PhD

Senior Editor, Nature Cell Biology
Consulting Editor, Nature Communications
Nature Portfolio

Heidelberger Platz 3, 14197 Berlin, Germany
Email: daryl.david@nature.com
ORCID: https://orcid.org/0000-0002-9253-4805