nature portfolio

Peer Review File

The G1/S transition in mammalian stem cells in vivo is autonomously regulated by cell size

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
In this manuscript, Jan Skotheim and his team demonstrate for the first time that cell size autonomously controls the timing of the G1 to S phase transition in vivo. The work is a technical tour-the-force, and because they investigate multiple tissue types and different species (i.e. mice and fish), they make a strong case that cell size is a fundamental cell cycle control principle in metazoan tissues.

The work conceptually builds forth on their previous work (e.g. PMID: 32703881, PMID: 32109398), but the extension to in vivo tissue analysis represents a significant advancement. I had previously reviewed this manuscript for Nature, and the authors have already addressed my concerns adequately in an initial round of revision.

I have no further concerns. This revised manuscript is an exciting and thorough piece of work, and I fully support publication in Nature Communications.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
I want to thank the authors for their careful reply to my comments.

Response to point 1: I appreciate the inclusion of maximum projections. A robust signal for the FUCCI channel is now convincingly demonstrated.

Response to point 4: I understand that demonstrating Rb concentration dependence across different tissues is time-consuming. Therefore, the conclusions should be limited to the ear skin epithelium. This limitation should be clearly stated in both the manuscript text and the abstract.

Response to point 5: If the claims cannot be extended beyond the ear skin, it is important to explicitly state in the manuscript and abstract that the findings presented in Fig. 2a–d, and related data, are not generalizable to other skin types.

Response to point 6: If the analysis and feature extraction cannot be extended to other datasets, it is difficult to claim that volume is the sole determinant of the decision to divide in tissues. This limitation should be clearly acknowledged in the manuscript and abstract, specifying that the conclusion applies only to the ear skin.

Response to point 7 : The validation of Rb levels performed in an in vitro culture setting (which does not adhere to this size/division control mechanism) are not sufficient to support the in vivo conclusions. This limitation should be stated in the

text.

Given the experimental constraints and the significant limitations in expanding the data analysis, as indicated by the authors, I do not find sufficient evidence to support a general mechanism of G1/S transition controlled by cell size. Therefore, I believe that claims of a unifying mechanism should be omitted from the manuscript. As a result, I find it difficult to identify the advances of this study over the previously published work by the same group, which already demonstrated that cell size coordinates division in the mouse epidermis (Xie et al., 2020, Current Biology). I leave it to the editor to make a final decision on this matter.

(Remarks on code availability)

Reviewer #2 (Remarks to the Author):

In this manuscript, Jan Skotheim and his team demonstrate for the first time that cell size autonomously controls the timing of the G1 to S phase transition in vivo. The work is a technical tour-the-force, and because they investigate multiple tissue types and different species (i.e. mice and fish), they make a strong case that cell size is a fundamental cell cycle control principle in metazoan tissues.

The work conceptually builds forth on their previous work (e.g. PMID: 32703881, PMID: 32109398), but the extension to in vivo tissue analysis represents a significant advancement. I had previously reviewed this manuscript for Nature, and the authors have already addressed my concerns adequately in an initial round of revision.

I have no further concerns. This revised manuscript is an exciting and thorough piece of work, and I fully support publication in Nature Communications.

We thank the reviewer for their time, effort, and comments throughout the review process. We are pleased that they recognize the significance of this work.

Reviewer #3 (Remarks to the Author):

I want to thank the authors for their careful reply to my comments.

We thank the reviewer for their input and comments throughout the review process. We are thankful for the time and effort that they have put in to provide such extensive feedback.

Reviewer 3: “I appreciate the inclusion of maximum projections. A robust signal for the FUCCI channel is now convincingly demonstrated.”

We thank the reviewer for help in improving our data visualization.

Reviewer 3: “I understand that demonstrating Rb concentration dependence across different tissues is time-consuming. Therefore, the conclusions should be limited to the ear skin epithelium. This limitation should be clearly stated in both the manuscript text and the abstract.”

We thank the reviewer on this point. We have amended the abstract to the following: “Here, we address this question by tracking single epidermal stem cells growing in the mouse ear.” In the main results section, we now state: “In addition, our analysis of mouse ear skin shows that the RB pathway is crucial for cell-autonomous size control *in vivo*.”

Reviewer 3: “If the claims cannot be extended beyond the ear skin, it is important to explicitly state in the manuscript and abstract that the findings presented in Fig. 2a–d, and related data, are not generalizable to other skin types.”

We thank the reviewer for their comments, but we do not believe that an explicit statement of this kind is standard practice in the field. We state clearly how the experiment was done and believe the readers should be free to draw their own conclusions.

Reviewer 3: “If the analysis and feature extraction cannot be extended to other datasets, it is difficult to claim that volume is the sole determinant of the decision to divide in tissues. This limitation should be clearly acknowledged in the manuscript and abstract, specifying that the conclusion applies only to the ear skin.”

Similarly to the above comment, we believe that it is not standard practice to make such exceptionally limiting statements. Future work will no doubt extend or refine our conclusions.

Response 3: “The validation of Rb levels performed in an in vitro culture setting (which does not adhere to this size/division control mechanism) are not sufficient to support the in vivo conclusions. This limitation should be stated in the text.”

We thank the reviewer for this comment. We had incorporated this point into our previous revisions, in the results section: “While our genetic analysis shows that both Rb1 and Rbl1 function are required for effective coupling between cell size and G1/S progression, future work is needed to demonstrate that the *in vivo* G1/S transition is quantitatively sensitive to the concentration of these Rb-family members and that their concentration decreases with increasing cell size *in vivo*.”

Response 3: “Given the experimental constraints and the significant limitations in expanding the data analysis, as indicated by the authors, I do not find sufficient evidence to support a general mechanism of G1/S transition controlled by cell size. Therefore, I believe that claims of a unifying mechanism should be omitted from the manuscript. As a result, I find it difficult to identify the advances of this study over the previously published work by the same group, which already demonstrated that cell size coordinates division in the mouse epidermis (Xie et al., 2020, Current Biology). I leave it to the editor to make a final decision on this matter.”

We thank the reviewer for their time and effort in reviewing our manuscript. However, we believe there is a *lot* of data in this manuscript. It is more like 2 or 3 manuscripts worth of data covering mouse skin, intestinal organoids, and fish osteoblasts. We therefore are going to have to agree to disagree on this point. We think that our main conclusions are strongly supported by our data and that our interpretations are in line with the standard practices of our field.