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Supplemental Information

**H4K20 Methylation Is Differently Regulated
by Dilution and Demethylation in Proliferating
and Cell-Cycle-Arrested *Xenopus* Embryos**

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H4K20 methylation kinetics are differently regulated by dilution and demethylation in proliferating and cell-cycle arrested *Xenopus* embryos

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Summary

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Scientific editor: Bernadett Gaál, DPhil.

First round of review: Number of reviewers: 4
4 confidential, 0 signed
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Major changes anticipated
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Second round of review: Number of reviewers: 4
4 original, 0 new
4 confidential, 0 signed
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Data freely available: Yes
Code freely available: Yes

Editor's View

This paper is addressing the salient biological problem of whether the cell cycle plays an "active" role in shaping the histone modification landscape in proliferating cells, given that DNA replication "passively" (by default) dilutes histone modifications. The answer to this question will be of interest to biologists interested

in how histone methylation is maintained and regulated, particularly in rapidly dividing cells during embryonic development.

The problem addressed in this paper is a very nicely constrained one and proliferating vs. cell-cycle arrested *Xenopus* embryos offer an exceptionally well-suited *in vivo* model in which to address it. We also noted that the computational modelling here "an example of a well-placed model that's necessary for insight", that is, an exceptionally good example of good use of modelling. First, the succinct description of the model hypotheses that could describe H4K20 methylation kinetics in cycling and cell-cycle arrested cells gives a clear view of the problem at hand and of the space of possibilities. Using multi-start maximum likelihood optimization and quantitative model selection and the experimental data, the authors arrive at biological insights that would be difficult to attain relying solely on currently available experimental tools and without modelling.

We are always on the lookout for studies that address salient biological questions through a combination of well-designed experiments and good use of modeling, so this was not a difficult initial decision to make. During peer review we were therefore primarily looking to make sure that our assessment of the suitability of the experimental system and computational modelling was correct and that the experiments and analysis are robust and technically correct.

Accordingly, in my first decision letter I emphasized the need to address the questions the reviewers had about the robustness of the model and the key conclusions, and their concerns about whether the quantification of histone methylation was sufficiently accurate for the purposes of this work, and whether the HUA treatment arrested cells as expected and had no major off-target effects that could affect the results. The authors were able to address the concerns raised by the reviewers in one round of revisions.

This Transparent Peer Review Record is not systematically proofread, type-set, or edited. Special characters, formatting, and equations may fail to render properly. Standard procedural text within the editor's letters has been deleted for the sake of brevity, but all official correspondence specific to the manuscript has been preserved.

Editorial decision letter with reviewers' comments, first round of review

Dear Carsten,

I'm enclosing the comments that reviewers made on your paper, which I hope you will find useful and constructive. As you'll see, they express interest in the study, but they also have a number of criticisms and suggestions. Based on these comments, it seems premature to proceed with the paper in its current form; however, if it's possible to address the concerns raised with additional experiments and/or analysis, we'd be interested in considering a revised version of the manuscript.

As a matter of principle, I usually only invite a revision when I'm reasonably certain that the authors' work will align with the reviewers' concerns and produce a publishable manuscript. In the case of this manuscript, the first priority should be to thoroughly address the concerns the reviewers raise about some of the assumptions of the model, its robustness to these and those with respect to data quality and accuracy. I've highlighted some of the most important of these below.

1. Can we know whether the cell cycle duration function capture the heterogeneous cell cycle durations in the developing embryo or at least can we be confident that the results and conclusions are robust to this heterogeneity? (raised by Reviewer 1)
2. Are the results and conclusions robust to the way that the model handles H4K20 methylation dilution? (Reviewer 3 point 1)
3. Reviewer 4 is concerned about the potential for overfitting.
4. Given that high accuracy quantification of histone methylation is exceptionally important in this context, the technical concerns raised in this regard by Reviewer 1 are important to address.
5. Please provide demonstration that HUA treatment is acting as anticipated, as requested by Reviewer 2.

If it's technically possible to experimentally distinguish between the predictions of the models with and without demethylation, along the lines of what Reviewer 3 suggests (point 2), adding this would certainly strengthen the conclusions.

I've also highlighted portions of the reviews that strike me as particularly critical. I'd also like to be explicitly clear about an almost philosophical stance that we take at Cell Systems.

We believe that understanding how approaches fail is fundamentally interesting: it provides critical insight into understanding how they work. We also believe that all approaches do fail and that it's unreasonable, even misleading, to expect otherwise. Accordingly, when papers are transparent and forthright about the limitations and crucial contingencies of their approaches, we consider that to be a great strength, not a weakness. Please keep this in mind when discussing what conclusions can and what cannot be drawn based on your analysis and approach.

As you address these concerns, it's important that you and I stay on the same page. I'm always happy to talk, either over email or by phone, if you'd like feedback about whether your efforts are moving the manuscript in a productive direction. We also appreciate that the COVID-19 pandemic challenges and limits what you and your lab can do, so if you would like to talk through your revision plan then let's schedule a Zoom call.

Do note that we generally consider papers through only one major round of revision, so the revised manuscript would be either accepted or rejected based on the next round of comments we receive from the reviewers. If you have any questions or concerns, please let me know. More technical information and advice about resubmission can be found below my signature. Please read it carefully, as it can save substantial time and effort later.

I look forward to seeing your revised manuscript.

All the best,

Bernadett

Bernadett Gaal, DPhil
Scientific Editor, Cell Systems

Reviewers' comments:

Reviewer #1: In this interesting manuscript, Schuh et al developed a computation model to determine how histone H4K20 methylation (me0/me1/me2/me3) states are integrated and regulated by the cell-cycle and *Xenopus laevis* embryogenesis. To build their model, the authors first use H4K20 methylation mass spectrometry of histone H4 from nuclei of differentiating *Xenopus* embryos and then use a variety of approaches to model the cell cycle and "methylation" rate constants. They then use these models to back-fit the cell cycle terms to derive a set of best-fit joint models. Using their model, the authors tested the question of whether or not demethylation is necessary to account for the observed dynamics of K20 methylation. Their model showed that demethylation is likely essential for explanation of the HUA-dependent H4K20 dynamics, but "redundant" in mock-treated embryos. This is an important observation that addresses some long-standing questions in the field. As this is just a model, the authors don't experimentally test this question. They propose, in the discussion, a fascinating biological consequence of this distinction that demethylases are only important as a trigger for differentiation. As this is likely the most important consequence of this manuscript, this result is begging for an experimental test. This may be beyond the scope of the current manuscript, but at least proposing how the authors would perform the test would be helpful (e.g. a PHF8 knockdown, a *Drosophila* screen of KDMs for cell-cycle elongation coupled differentiation defects, or something).

Overall, this manuscript is likely of interest to many in the developmental biology, chromatin, and molecular modeling fields. It will be of use to many in developing similar models for other histone and non-histone PTMs that may regulate the cell cycle. However, before I recommend publication, I suggest that the authors consider the critique above and the specific points below:

I'm confused by the statement at the bottom of page 3: "In HUA, methylation accumulates in the di- and tri-methylation states in comparison to mock. There, newly synthesized and unmodified histones are incorporated upon DNA replication, leading to an overall dilution of H4K20me and hence a higher proportion of lowly methylated un- and mono-methylated states." If HUA leads to an increase in di- and tri-methylation, and there is no DNA replication, that means that newly synthesized unmodified histones are NOT incorporated in HUA? This sentence is not written clearly.

Figure 1B - each time point of the H4K20me proportion should be subject to a t-test.

The difference in H4K20 methylation between mock and HUA is rather modest and less than one would predict from the simple hypothesis of cell cycle dependence. To truly demonstrate that this difference is

due to altered methyltransferase activities, a PR-Set7 or SUV420H morpholino injection would help strengthen this experiment. However, this could be challenging. To demonstrate that H4K20 methylation kinetics are truly altered upon HUA cell cycle arrest, perhaps an alternative experiment would be to perform this experiment in cell free extracts. The cell cycles are much faster than in post-MBT embryos and the consequences of altered H4K20 dynamics would be more robust.

The definition and derivation of the cell-cycle duration function $c(t)$ is complicated. The cell-cycle duration of pre- and post-MBT and gastrula cells are highly variable, going from exceedingly fast pre-MBT to very slow during gastrulation and neurulation. But do 3 functions (constant, linearly increasing, or gradually plateauing) account for the biological cell cycles observed in the very heterogeneous developing embryo? While the authors compared one of their top models with an "average cell-cycle duration" in neural progenitors, this still a conflation of a model derived from total embryo histone mass spectrometry to a heterogeneous population of cells. Some additional analysis of this question is warranted.

For the histone mass spectrometry, it is not clear that in-gel trypsin digestion and recovery is adequately quantitative and representative of the entire histone pool. Similarly, it does not appear that missed cleavages are accounted for; while propionylation should eliminate this issue, the authors should at least include searches for missed cleavage. While the authors have published this approach before, some additional justification that this approach matches the recovery from RP-HPLC followed by propionylation/digestion is warranted in this study. This is particularly important as the model development needs high accuracy quantification to be valid.

"Methylation rate constants" should probably be renamed. Maybe "biological methylation rate constants". Rate constants have a specific biochemical meaning for each enzyme (e.g. k_{cat} for the turnover rate and K_m for the Michaelis-Menten constant representing substrate affinity). As the authors are not deriving actual enzymatic rates constants, the authors should ensure that readers are not misled.

What is the authors' proposed biological/biochemical reason for the Hill coefficient being not 1?

In Figure 4C and F, the annotation for the model ID (e.g. a-f) is confusing. The figure subpanels are also a-f.

Reviewer #2: This article by Schuh and colleagues addresses a fundamental puzzle in developmental cell biology: as cells proliferate, how do they maintain their epigenetic state and combat dilution of histone marks through DNA replication? Schuh and colleagues tackle this question using the multiple methylation states of H4K20 in developing *Xenopus laevis* embryos as a model. In this study, they examine the levels of un- mono- di- and tri-methylated H4K20 across a developmental time course, beginning at gastrulation stages (stage 11), when *X. laevis* embryos have re-acquired a full cell cycle with G1, S, G2 and M phases. The principle perturbation used in hydroxyurea/aphidicolin (HUA) which induces cell cycle arrest at the G1/S transition, and the authors use mathematical modeling to infer the kinetics of methylation and demethylation rates in normal and HUA-treated embryos. They find that three independent methylation rate constants are needed to generate each of the methylation states with the dynamics observed, and interestingly, predict that demethylation is dispensable in normally-cycling cells. The underlying fundamental question of how epigenetic states are maintained across a proliferating and heterogeneous

cell population is exciting and should be interesting to a broad audience. The application of this modeling approach to developing embryos has the potential to be broadly useful across many biological settings. Therefore I think the work is potentially appropriate for Cell Systems.

I have one major concern:

Given the very long incubation times with HUA, I think it's **essential to demonstrate that the HUA treatment is acting as anticipated**. This is especially since, by the authors' calculations, the number of cells should be increasing from 20k to 300k over this period.

In particular, the authors **should confirm that HUA is robustly introducing the expected G1/S cell cycle arrest throughout the embryo**.

I can imagine several experimental strategies for this, but the most obvious would be to use FACS together with DNA staining (Hoechst e.g.) to illustrate and quantify the degree of cell cycle arrest in HUA versus control embryos at each of the stages used.

I also think it would be prudent **to confirm that drastic off target effects are not being caused that might conflate the conclusions**; the most obvious of these to me would be cell death, which could be checked by TUNEL staining at each stage.

Minor comment:

In the Introduction, when discussing the roles of H4K20 modifications and the associated enzymatic activities that deposit them, the authors move rapidly between discussing results observed in cell culture and those obtained across a wide range of model organisms. I request that the authors specify the experimental system in which each result was obtained, because it's potentially misleading to assume that conclusions reached in human cell lines will also hold true in early *Xenopus* embryos.

Reviewer #3: In this manuscript the authors analyze the cell cycle dependent kinetics of H4K20 methylation. Their main result is that active demethylation is probably dispensable during active cell cycles but not in cases where the cell cycle has been arrested. In the former case, dilution at DNA replication is potentially sufficient for regulating H4K20me levels. In my view, this work is a useful contribution to the field, as quantitative characterization of histone PTM kinetics is rarely performed, despite the importance of such analyses. However, I have some difficulties with the analysis, both from a theoretical and experimental angle, as I detail below. Furthermore, one of the principle results, namely that active demethylation is less important for active cell cycles is already a known result for some other histone modifications, particularly H3K27me. This detracts somewhat from the manuscript's novelty.

* **The model handles dilution through an outflow of H4K20 states dependent on the cell cycle duration c.** However, in reality in a single cell, dilution is a discrete event. At a population level, this may not matter **but only if the cells are unsynchronized**. In the experiments presented here, the cells will start out synchronised by virtue of fertilisation at $t=0$. Given this synchrony, **it is not clear whether the desynchronization assumption is true at the later timepoints, between 14.75 and 40 hpf, when the average cell cycle is about 8 h long**. This issue needs to be discussed.

* The author's favorite model is the one without any active demethylation. However, a model with active demethylation still fits equally well (Fig 2D), although is structurally more complex. The authors say in the abstract that active demethylation is redundant. I think that is going too far: to rule out active demethylation, what seems to be required is measurement of H4K20me at earlier time points (<10 hpf), where the model predictions with/without active demethylation diverge (Fig 2E). In my view, it is essential

to perform these experiments to validate the model prediction. It would also be useful to plot the prediction from the best active demethylation model to the data of Fig 2F,G to see how it compares.

* In the methods, it is mentioned that $me0_0$ is set to 0.1 for structural identifiability. Do other initial conditions lead to different answers? If so, I would be concerned about the robustness of the methodology.

* I think there may also be an error in the equations for $d/dt(me0)$ for the cases where the cell cycle time is itself a function of time. For the case where $c(t)=a+bt$, then $d/dt(me0)$ should have a term which is $(\ln 2 a)/(a+bt)^2 \times (me0+me1+me2+me3)$. A similar issue occurs in the Hill function cases. If so, the analysis would have to be redone with potentially different conclusions.

Reviewer #4: Schuh et al. present a study on computational modeling of H4K20 methylation. On the whole this is an interesting angle: modeling methylation rates on the basis of quantitative mass-spec measurements. It is a manuscript with a relatively narrow, but well-specified focus. It is well-written and the results are clearly presented. The code to reproduce the analyses is available: good.

I have mostly one major concern which has to do with the number of measurements in relation to the number of models tested. For instance, the authors fit 180 models to the normally developing embryos (mock-treated). The data consists of 16 measurements (four methylation states at four stages). To me this sounds like this has a huge potential for overfitting. By fitting this large number of models I'm not sure this actually is certain to result in something biologically meaningful in the end. Could not any combination of 16 measurements be fitted to some degree? In the end, the models and fitted parameters can be biologically explained, but how trustworthy are these models in the end? This concern is not alleviated by the results in the manuscript, as a model fitted on the mock-treated embryos cannot explain the data of the HUA embryos. In this case, a demethylation rate is necessary. How useful is a model if it cannot predict the effect of perturbations? In my view, a perturbation experiment would really be necessary to validate a model. Here, the assumption would be that a good model could predict the effect of a perturbation. For instance, inhibition or knock-out of a methyltransferase or demethylase. However, due to functional redundancy and different specificities, this is likely not a trivial experiment. Added to that, I am hesitant to require further experiments in these uncertain times.

In conclusion, I think the manuscript is interesting and original. However, due to the combined experimental and computational setup, I'm not sure how valid the fitted models are and to what extent these can be interpreted biologically.

Other remarks:

* Abstract: "suggesting that cell-cycle mediated dilution of chromatin marks is an essential regulatory component for shaping the epigenetic landscape during early embryonic development." This too broad of a statement. The manuscript described H4K20 methylation, which has been linked to the cell cycle. This may or may not hold for other chromatin marks, but this is outside the scope of this manuscript.

* It is stated that the model retrieves correct cell-cycle durations. This is not surprising, as a specific model is chosen to agree with the cell-cycle durations: "only a constrained scaled Hill function with Hill coefficient 1 and offset 0.5 gives a cell-cycle duration in the expected range." If you choose a model that fits the data, it is not unexpected that this model yields correct predictions.

* Why were these four stages chosen? The earliest measured stage is late gastrula (NF 13), however, establishment of H4K20 methylation would be interesting to measure. In *Xenopus*, a large fraction of the

epigenome is established at or around MBT (blastula stage), therefore it would be interesting to include several stages that also cover blastula and early and mid-gastrula.

Authors' response to the reviewers' first round comments

Attached.

Editorial decision letter with reviewers' comments, second round of review

Dear Carsten,

I'm very pleased to let you know that the reviews of your revised manuscript are back, the peer-review process is complete, and only a few minor, editorially-guided changes are needed to move forward towards publication.

I've made some suggestions about your manuscript within the "Editorial Notes" section, below. Please consider my editorial suggestions carefully, ask any questions of me that you need, make all warranted changes, and then upload your final files into Editorial Manager. ***We hope to receive your files within 5 business days, but we recognize that the COVID-19 pandemic may challenge and limit what you can do. Please email me directly if this timing is a problem or you're facing extenuating circumstances.***

I'm looking forward to going through these last steps with you. More technical information can be found below my signature, and please let me know if you have any questions.

All the best,
Bernadett

Bernadett Gaal, DPhil
Scientific Editor, Cell Systems

Editorial Notes

Transparent Peer Review: Thank you for electing to make your manuscript's peer review process transparent. As part of our approach to Transparent Peer Review, we ask that you add the following sentence to the end of your abstract: "A record of this paper's Transparent Peer Review process is included in the Supplemental Information." Note that this ***doesn't*** count towards your 150 word total!

Also, if you've deposited your work on a preprint server, that's great! Please drop me a quick email with your preprint's DOI and I'll make sure it's properly credited within your Transparent Peer Review record.

Abstract: I've gone over your abstract with the goals of making it more succinct and concrete, largely by reworking and recombining parts of your original version. See what you think. Please feel free to revert anything that you don't like or that you feel distorts your meaning! I apologize if there are instances of the latter.

"DNA replication during cell division leads to dilution of histone modifications and can thus affect chromatin-mediated gene regulation, raising the question of how the cell-cycle shapes the histone modification landscape, particularly during embryogenesis. We tackled this problem by manipulating the cell-cycle during early *Xenopus laevis* embryogenesis and analysing in vivo histone H4K20 methylation kinetics. The global distribution of un-, mono- di- and tri-methylated histone H4K20 was measured by mass spectrometry in normal and cell-cycle arrested embryos over time. Using multi-start maximum likelihood optimization and quantitative model selection, we found that three specific biological methylation rate constants were required to explain the measured H4K20 methylation state kinetics. While demethylation is essential for regulating H4K20 methylation kinetics in non-cycling cells, demethylation is very likely dispensable in rapidly dividing cells of early embryos, suggesting that cell-cycle mediated dilution of H4K20 methylation is an essential regulatory component for shaping its epigenetic landscape during early development."

Manuscript Text: The text is compelling and clear, but the Discussion seems rather too long. We favour slim Discussions that do not reiterate what's found in the Results beyond a brief transitional summary and are limited to around four medium sized paragraphs. There are important points in the Discussion, including those that you included in response to Reviewers 1 and 3. Please focus on these and limit reiteration of the results to what's necessary to convey these points. Please also remove subheadings from the Discussion section. Please feel free to ignore the strict character limits on the main text listed in the checklist that I had sent you some time ago, but please do work on making the Discussion shorter and more succinct.

Also:

- House style disallows editorializing within the text (e.g. strikingly, surprisingly, importantly, etc.), especially the Results section and in the Summary. These terms are a distraction and they aren't needed—your excellent observations are certainly impactful enough to stand on their own. Please remove these words and others like them. “Notably” is suitably neutral to use once or twice if absolutely necessary.
- We don't allow “priority claims” (e.g. new, novel, first, etc.). For a discussion of why, read: <http://crosstalk.cell.com/blog/getting-priorities-right-with-novelty-claims>, <http://crosstalk.cell.com/blog/novel-insights-into-priority-claims>.

Figures and Legends:

Please ensure that all figures included in your point-by-point response to the reviewers' comments are present within the final version of the paper, either within the main text or within the Supplemental Information.

STAR Methods:

Please replace your Materials and Methods section with STAR Methods and please follow the STAR Methods format for reporting experimental procedures, methods, and analysis, including the Key Resources Table. Cell Press introduced the STAR Methods format to help improve the rigour in reporting methods and resources for reproducibility. This section replaces the Experimental Procedures and Supplemental Experimental Procedures sections. For detailed instructions on STAR Methods and a template for the Key Resources Table, see our [STAR Methods](#) webpage. Please contact me if you have any questions about restructuring your manuscript using the STAR Methods format.

Cell Press has recently changed the way it approaches "availability" statements for the sake of ease and clarity. Please revise the first section of your STAR Methods as follows, noting that the particular examples used might not pertain to your study.

RESOURCE AVAILABILITY

Lead Contact: Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jane Doe (janedoe@qwerty.com).

Materials Availability: This study did not generate new materials. *-OR-* Plasmids generated in this study have been deposited at [Addgene, name and catalog number]. *-OR-* etc.

Data and Code Availability:

- **Source data statement** (described below)
- **Code statement** (described below)
- **Scripts statement** (described below)
- Any additional information required to reproduce this work is available from the Lead Contact.

Starting in August of 2020, Cell Systems papers will need to contain a comprehensive and structured "Data and Code Availability" statement. These statements will exceed standard STAR Methods requirements, so please note that **the instructions below supersede the instructions found [here](#).**

Data and Code Availability statements pertain to the source data and original code reported in the study. In this context, **source data** is defined as the collection of individual, unprocessed observations used to generate the figures reported in the paper. Examples include scRNA-seq and proteomic datasets, but also CSV spreadsheets used to generate graphs, and original micrographs in TIFF format. **Code** is defined as any computationally implemented program, algorithm, or pipeline necessary to reproduce the

analysis or conclusions reported in a paper. Smaller **scripts** that have been used to visualize data and generate figures should also be included in the statement, as described below.

Data and Code Availability statements are reported in the first section of the STAR Methods. **They have four parts and each part must be present. Each part should be listed as a bullet point, as indicated above. For convenience, a .docx template for Data and Code availability statements can be downloaded [here](#).**

Part 1 pertains to source data. Examples can be used in any number or combination, making sensible modifications as necessary:

- [Data-type] source data have been deposited at [data-type-specific repository] and are publicly available under the accession numbers: [Insert].
- [Data-type] source data have been deposited at [general repository] and are publicly available at [insert DOI].
- [Data-type] source data are available in the paper's Supplemental Information.
- The [data-type] source data reported in this study have not been deposited in a publicly available repository because [reason why data are not public] . They have been archived locally [insert archiving plan]. To request access [insert instructions].
- This paper analyzes existing, publicly available data. These datasets' accession numbers are provided in the Key Resource Table.
- Source data are not provided in this paper but are available from the Lead Contact on request. *(Note: Cell Systems discourages this practice. If you need to make this statement, please discuss it with your editor first.)*

Part 2 pertains to original code. Examples can be used in any number or combination, making sensible modifications as necessary:

- [Adjective] original code is publicly available at [repository name and DOI].
- [Adjective] original code is available in this paper's Supplemental Information.
- The original code reported in this study is not publicly available repository because [reason why data are not public]. Original code has been archived locally [insert archiving plan]. To request access [insert instructions].
- This paper does not report original code.

Part 3 pertains to scripts used to generate figures. Examples to be used in any number or combination:

- The scripts used to generate the figures reported in this paper are available at [repository name and DOI].
- The scripts used to generate the figures reported in this paper are available in this paper's Supplemental Information.

- The scripts used to generate the figures reported in this paper are available in the [name software package, with version, and provide reference or URL] and their use is described in the STAR Methods.
- Scripts were not used to generate the figures reported in this paper.
- Scripts used to generate the figures presented in this paper are not provided in this paper but are available from the Lead Contact on request. *(Note: Cell Systems discourages this practice. If you need to make this statement, please discuss it with your editor first.)*

Part 4 is a statement: “Any additional information required to reproduce this work is available from the Lead Contact.”

Please ensure that the large datasets generated in this paper has been archived in at least one publicly accessible repository (e.g. GEO, PRIDE, etc.). If there is no community-approved repository for your large-scale data, we recommend that you deposit them on Mendeley Data. Please provide your datasets' DOIs within both the Data and Software Availability section of the STAR Methods and the Key Resources Table. Thank you!

Please ensure that custom code has been archived in at least one publicly accessible repository (e.g. GitHub, Zenodo, etc) and that a DOI is provided within both the Data and Software Availability section of the STAR Methods and the Key Resources Table. Thank you!

You do not currently have a **Key Resources Table** (KRT). The KRT highlights key reagents and resources used in the paper. The table need not list every item used or generated in the study and does **not** replace the detailed explanation of the methods and materials used in the study in the STAR Methods text. For details, please refer to the [Table Template](#) or feel free to ask me for help.

All datasets and code generated or used in this paper must be listed, with their unique identifiers, within appropriate sections of the Key Resources Table. For the work generated in this paper, please indicate "This work" as the source.

Thank you!

Reviewer comments:

Reviewer #1: The authors have satisfactorily addressed my critique.

Reviewer #2: The authors have satisfactorily addressed my comments.

Reviewer #3: The authors have satisfactorily addressed my questions.

Reviewer #4: The authors have sufficiently addressed my concerns.