nature portfolio

Peer Review File

Efficient control of enterochromaffin versus islet differentiation from human pluripotent stem cell-derived pancreatic progenitors

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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 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The revised manuscript from Misra et al. has been significantly strengthened by the inclusion of data from additional hPSC lines, comparisons with other published differentiation protocols, and functional analyses of the product generated using their enhanced β-cell protocol. Although some experimental evidence is necessarily limited in scope, the amount of work performed is already considerable and well aligned with the expectations for Nature Communications, especially given that the study was originally conceived for a higher-tier venue. Overall, these additions substantially improve the rigor and generalizability of the study.
I have only two minor comments that could be addressed straightforwardly and would further enhance the manuscript.
1. In Supplemental Figure 1d, the newly added data clearly confirm that LatA treatment improves NKX6-1 induction. It would be helpful to know whether LatA also impacts early definitive endoderm (DE) induction. If such an effect is observed, the authors may consider briefly discussing that LatA treatment could reduce variability in DE formation arising from differences in seeding density or cell line–specific behavior (in discussion section).
2. I continue to believe that applying the new β-cell patterning strategy to additional experimental settings or differentiation protocols could be broadly beneficial to the field. That said, the current manuscript already represents a substantial body of work, and I leave it to the editorial board to decide whether inclusion of such data is necessary at this stage.
Overall, this is a strong and carefully executed study, and the authors should be commended for the significant effort involved in repeating experiments across multiple cell lines and benchmarking their protocol against existing approaches.

Reviewer #2

(Remarks to the Author)
The authors have satisfactorily addressed all my concerns.
In particular, the authors have greatly streamlined the manuscript by removing some of the data not directly relevant to the main scope of the paper and expanded the characterization of the obtained cultures.
This revision has significantly improved the manuscript, which is now acceptable for publication in Nature Communications.

We are very grateful to the Editorial Board and the reviewers for their positive response and for providing us with an opportunity to improve our manuscript.

Below you will find our response to the concerns raised. Additionally, editorial changes have been included to format the manuscript according to Nature Communications guidelines.

All the new changes made in the manuscript are made using track changes in purple. A clean copy is also included in the resubmission. Our responses to the reviewers' comments are explained below in blue.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

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RESPONSE: Indeed our studies suggest that LatA impacts early definitive endoderm induction, as stated in the results section in the following lines:

Lines 79-82: *At day 3 of differentiation, LatA-treated high-density hPSC cultures generated similar frequencies of CXCR4$^+$KIT$^+$cells (Supplemental Fig. 1a), but significantly higher frequencies of FOXA2$^+$SOX17$^+$ DE-like cells (Fig. 1e-f).*

Lines 95-98: *Taken together, our findings demonstrate a role for LatA in broadly supporting early differentiation of hPSCs into primitive streak and downstream mesodermal and endodermal lineages. Given these results, all subsequent experiments were performed using LatA on day 0 of differentiation.*

We agree with the reviewer that this point is worth highlighting in the discussion. To this end, we have added a new paragraph near the end of our discussion (lines 454-468):

Finally, it is worth noting that our dissection of the molecular processes regulating endocrine lineage diversification in hPSC-PPs required strong early endodermal commitment to prevent confounding contributions from off-target lineages. Previous studies have established that high hPSC seeding density at day 0 of differentiation can reduce the efficiency of DE differentiation[18, 77, 78]. Based on findings from one study that identified a connection between high-density cultures, actin stress-fiber formation, and impaired differentiation into BRA$^+$ primitive streak[20], we speculated that cytoskeletal disruption might negate some of this variability and provide an effective "low-density" signaling environment irrespective of actual cell density. Indeed, treating high-density hPSCs with the actin destabilizer LatA restored efficient progression to BRA$^+$ primitive streak, thereby improving subsequent directed differentiation into both KDR$^+$PDGFRA$^+$ mesoderm and FOXA2$^+$SOX17$^+$ DE. Notably, LatA's effect on improved DE differentiation at high starting cell density resulted in improved pancreatic differentiation across multiple cell lines, and also improved differentiation towards NKX2-1$^+$ lung progenitors. These findings support the use of LatA treatment to reduce variability in pancreatic differentiation related to initial seeding density.

2. I continue to believe that applying the new β-cell patterning strategy to additional experimental settings or differentiation protocols could be broadly beneficial to the field. That said, the current manuscript already represents a substantial body of work, and I leave it to the editorial board to decide whether inclusion of such data is necessary at this stage.

The editorial board indicated that these experiments are not required.

Overall, this is a strong and carefully executed study, and the authors should be commended for the significant effort involved in repeating experiments across multiple cell lines and benchmarking their protocol against existing approaches.

Thank you.

Reviewer #2 (Remarks to the Author):

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characterization of the obtained cultures.
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Thank you.