

DDX18 coordinates nucleolus phase separation and nuclear organization to control the pluripotency of human embryonic stem cells

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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 #1

(Remarks to the Author)

I think the revised manuscript is significantly improved and I think the authors for carefully addressing all my previous comments. I think this paper should be published as is.

Reviewer #2

(Remarks to the Author)

Shi et al. Revision

The authors provide a thorough response to the critiques of three reviewers which overlapped to a considerable degree. They have buttressed some conclusions with additional data, which has clarified the biology underlying the differentiation of DDX18 knockdown cells, the role of nucleolar integrity in changing patterns of gene expression, the significance of changes in HOX relocation and gene expression, and the role of helicase activity. This additional work adds a good deal of clarity to the original submission. The study now presents an interesting foray into a new perspective on regulation of pluripotency and cell state transitions.

Reviewer #3

(Remarks to the Author)

I thank the authors for their extensive work addressing the reviewers' concerns. In my opinion, the manuscript is suitable for publication now.

Page 9: It's well documented that the nucleolus does not have a simple "core-shell" structure like some other nuclear condensates, but more complex three distinct layers/phases form by the outward flux of pre-rRNA molecules [doi.org/10.1038/s41580-020-0272-6; DOI: 10.1007/s00418-024-02297-7]. Therefore, the authors should change the wording on page 9 to: "nicely recapitulating the 'core-shell' architecture of nucleoli" to mimic the three-layered structure of the nucleolus.

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Point-by-point responses to the remaining issues

Reviewer #1 (Remarks to the Author):

I think the revised manuscript is significantly improved and I think the authors for carefully addressing all my previous comments. I think this paper should be published as is.

Response: We thank this reviewer for his/her positive feedback from reviewing our revised manuscript and finding that our manuscript is greatly improved and can be published as is.

Reviewer #2 (Remarks to the Author): Shi et al. Revision

The authors provide a thorough response to the critiques of three reviewers which overlapped to a considerable degree. They have buttressed some conclusions with additional data, which has clarified the biology underlying the differentiation of DDX18 knockdown cells, the role of nucleolar integrity in changing patterns of gene expression, the significance of changes in HOX relocation and gene expression, and the role of helicase activity. This additional work adds a good deal of clarity to the original submission. The study now presents an interesting foray into a new perspective on regulation of pluripotency and cell state transitions.

Response: We are pleased that this reviewer found that our additional data has clarified our findings and strengthened the manuscript, voicing his/her support for the publication of our study.

Reviewer #3 (Remarks to the Author):

I thank the authors for their extensive work addressing the reviewers' concerns. In my opinion, the manuscript is suitable for publication now.

Response: We are pleased to have this reviewer's positive comments and support.

Page 9: It's well documented that the nucleolus does not have a simple "core-shell" structure like some other nuclear condensates, but more complex three distinct layers/phases form by the outward flux of pre-rRNA molecules [doi.org/10.1038/s41580-020-0272-6; DOI: 10.1007/s00418-024-02297-7]. Therefore, the authors should change the wording on page 9 to: "nicely recapitulating the 'core-shell' architecture of nucleoli" to mimic the three-layered structure of the nucleolus.

Response: This point is well taken. Per this reviewer's suggestion, we have updated the wording on page 9 to reflect a more accurate description of the nucleolus.