

Peer Review File

Huntington's Disease Cellular Phenotypes are Rescued Non-Cell Autonomously by Healthy Cells in Mosaic Telencephalic Organoids



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Editorial Note: This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments and rebuttal letters for versions considered at *Nature Communications*. Mentions of the other journal have been redacted.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

This manuscript was submitted to [REDACTED] originally. Most of the reviewers' comments have been addressed with extra analysis and experiments or reasonable explanations, which strongly support their hypothesis and significantly increase the quality of this revised manuscript.

Overall, the authors used a novel mosaic organoids approach and demonstrated the disease-modifying effect of healthy cells in restoring ventral developmental programs. Their findings highlights the therapeutic potential of cell replacement and gene silencing strategies in HD, underscoring the beneficial non-cell autonomous effects of healthy cells on HD cells.

Reviewer #2 (Remarks to the Author):

The authors have carried out a substantial revision, which has satisfied most of my concerns. I have only two remaining minor points that the authors should address.

1. In their response to my point 2(a), they refer to their answer to Reviewer 1 (point 2). I do not see a specific answer to my point in that response. I would be grateful if the authors could clarify the original concern as to why cell death cannot be rescued in the mosaics.
2. I appreciate the authors' more balanced discussion in the revised manuscript. However, it would be helpful if they could write a brief paragraph that explicitly explains the limitations of their model.

Point-by-point response to the reviewers' comments

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We thank the reviewer for his/her time and efforts.

Reviewer #2 (Remarks to the Author):

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In our last revision process we have already commented this point in the discussion. We have now added a few more words to be clearer. Lines 446-450:

“Although we observed a near-full transcriptional recovery in HD cells grown with CTRL ones in mosaic organoids, some phenotypes, such as cell polarity in progenitors' organization and apoptosis, were not fully rescued. While further investigations are required, we speculate that the mechanism

driving these defects involves genes with cell-autonomous regulation, likely linked to the intrinsic toxicity of mHTT, and therefore unaffected and not rescued by interactions with control cells.”

2. I appreciate the authors’ more balanced discussion in the revised manuscript. However, it would be helpful if they could write a brief paragraph that explicitly explains the limitations of their model.

Ok, we thank the Reviewer and understand the point. We have now included this sentence in the discussion of the revised manuscript. Lines 451-457:

“One remaining limitation of this study lies in the model. Organoids are complex multicellular cultures that, compared to other culture systems, can produce a broader spectrum of cell types better reflecting the diversity of the endogenous brain. However, they are still not heterogeneous and oriented enough to fully mimic the brain. This limitation could impede investigations of more advanced phenotypes due to the lack of specific cell types or connections between areas. Despite this, in the absence of faithful experimental models for human neuronal biology, organoids remain one of the most valuable alternatives.”