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Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

Describe how sample size was determined.

Where possible, we used 3 biological replicates as standard practice (see Replication section for details).

2. Data exclusions

Describe any data exclusions.

For single-cell RNA-sequencing analysis, we removed low quality cells (expressing less than 1000 human genes or more than 300 mouse genes) from downstream analysis.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

We used three replicates for all experiments where possible. Results were reproduced between biological replicates. For iN cells day-3 conditions, we used 2 replicates (given the limited nature of the analysis of this condition). For electrophysiology measurements, we used 6 cells, but we do not perform any statistical tests on these results; they are included to demonstrate the feasibility of this approach. For genome editing experiments, 2 biological replicates of 3D tissues were used (per condition). For the targeting gRNA, DNA from two biological replicates was used for NGS analysis. For the non-targeting control, DNA from two biological replicates was pooled for NGS analysis. From each tissue, 1000 mCherry+ cells were isolated for analysis of genome editing efficiency. For qPCR comparison of human astrocytic cells to primary human astrocytes, for the control (primary human astrocytes), all 3 replicates were derived from a single pool of astrocytes. For 3D co-cultures of iN cells with human astrocytic cells and with human primary astrocytes experiments, each 3D culture/co-culture had triplicates and a population of 1000 mCherry+ iN cells was collected from each disassociated 3D tissue.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

This is not relevant to this study. Samples were not randomized.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

This is not relevant to this study. Samples were not blinded.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Graphpad Prism 7 was used to generate plots for qPCR, electrophysiology, and imaging experiments, gene ontology analyses, and representing indel percentages for genome-editing experiments. Data for characterization of mechanical properties of hydrogels were analyzed by using TA instruments TRIOS software and Graphpad Prism 7. Bulk RNA-seq analyses were performed using DESeq2 package and R. Gene Set Enrichment Analysis (GSEA) was performed using GSEA software v2.2.3. Cellranger was used to map fastq files generated by single-cell RNA-seq to the joint hg19 and mm10 transcriptome. Single-cell RNA-seq analysis was performed using Seurat v1.3 in R.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No restrictions.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

We used commercially available antibodies.

Mouse anti-Map2 (M4403, Sigma) and citing articles : http://www.sigmaaldrich.com/catalog/product/sigma/m4403?lang=en®ion=US

Rabbit anti-Pax6 (901301, BioLegend) and citing articles: https://www.biolegend.com/en-us/products/purified-anti-pax-6-antibody-11511

Chicken anti-GFAP (ab4674, Abcam) and citing articles: http://www.abcam.com/gfap-antibody-ab4674-references.html

Mouse anti-S100β (ab11178, Abcam) and citing articles: https://www.citeab.com/antibodies/779833-ab11178-anti-s100-beta-antibody-sh-b1/publications

Rabbit anti-Vimentin (5741, Cell Signaling) and citing articles: https://www.cellsignal.com/products/primary-antibodies/vimentin-d21h3-xp-rabbit-mab/5741

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Human Embryonic Stem Cell (hESC) line (HUES66) was obtained from the Harvard Stem Cell Institute (https://ipscore.hsci.harvard.edu) and used with Embryonic Stem Cell Research Oversight committee (ESCRO) approval from the Broad Institute (BRR #32); human embryonic kidney 293FT (HEK293FT) cells were obtained from ATCC.

b. Describe the method of cell line authentication used.

No authentication method was used.

c. Report whether the cell lines were tested for mycoplasma contamination.

Not tested.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Newborn C57 mice were used in the study to isolate glial cells from their cortex in procedures carried out in accordance with Animal Care and Use regulations at the Broad Institute, with protocol (0008-06-14) approved by the Broad Institute's Institutional Animal Care and Use Committee (IACUC).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.