nature portfolio

Corresponding author(s): Associate Professor Anai Gonzalez-Cordero

Last updated by author(s): Mar 30, 2026

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	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 all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Confocal microscopy was performed on the Leica LASx image acquisition software. Flow cytometry data was collected on the BD FACsdiva software.
Data analysis	A Cellpose code has been developed for the analysis pipeline for IHC quantification and has been made available at: https://github.com/Stem-Cell-Medicine-Lab/Cellpose_pipeline-models. Statistical analysis of image quantification and flow cytometry was performed using Prism. Differential gene expression between RA conditions for a given cell type was calculated using FindMarkers from the Seurat package (v5.2.1). Gene set enrichment analysis for each cell type between RA conditions was conducted using the clusterProfiler package (v1.30.0) against the Gene Ontology Biological Process gene sets using the top 100 differentially expressed genes ranked by average log2FC. To assess the similarity in cell identity between cell types in the Zuo et al.21 foetal retina and the retinal organoids, cell-type specific differentially stable genes were independently calculated for each dataset using the Cepo package (v1.10.2). The CARD algorithm with default spot and gene filtering parameters was used for deconvolution of spatial data, keeping spatial locations where at least 100 counts were detected and including genes where at least expressed in 5 spots.

For annotation of STOMICs spatial data: each spot at 16 weeks was annotated using matching 16 weeks scRNA-seq as the reference for each condition. The Seurat transfer learning approach was applied to classify cells in the query scRNA-sequencing datasets using FindTransferAnchors() and TransferData(). To annotate the 26 weeks spatial data, previously generated in-house retinal organoids at 30 weeks derived from the UCLOOi017-A-1 (UCL) cell line was used to perform cell deconvolution with CARD.

Cell-cell communication signalling pathways were analysed against the curated repositories of ligand-receptor interactions from CellChatDB (v122, v223), which included NeuronDB, and CellPhoneDB (v4.1), including more than 3000 protein and non-protein interactions (e.g. metabolic and synaptic signaling).

Chord diagrams displaying the inferred cell-cell signalling pathways was generated using netVisual_chord_gene().

Separately, for scRNA-seq at 16 weeks,The inferred cell-cell signalling pathways was generated using netVisual_chord_gene(). netAnalysis_signalingRole_scatter() was used to visualise the total incoming and outgoing cell-cell interaction probabilities for each cell type across conditions. Information centrality was calculated using netAnalysis_computeCentrality() at pathway level, and the aggregated cell-cell communication network for each cell type using netAnalysis_signalingRole_heatmap().

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Single-cell RNA-seq and spatial transcriptomics data have been deposited at GEO (GSE293457, GSE293464, GSE293582) and are publicly available as of the date of publication. Flow cytometry data has been added to Source Data file. All microscopy images obtained for this study (over 68GB) will be shared by the corresponding author upon request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes of organoids were N=number of differentiation batches whilst n=number of technical replicates ie. amount of organoids analysed in each differentiation batch and/or the amount of images acquired.
Data exclusions	For image quantification, generated masks for quantification were manually screened against the original image being quantified for the confirmation of correct mask generation with obvious abnormalities being removed.
Replication	Reproducibility was achieved with in vitro experiments achieving similar results across 4 different pluripotent stem cell lines (both embryonic and induced pluripotent). Additionally experimental results were performed with ~3-5 biological replicates (different differentiation batches) and ~3-5 technical replicates (amount of organoids per differentiation batch).

Randomization	Random selection by dividing up organoids randomly into tissue culture plates before selecting wells for treatment.
Blinding	Quantification was automated and unsupervised apart from manual screening post quantification.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Primary used (1:400 Anti-ARR3 [Abcam; ab167507, ab189437)], 1:800 Anti-CRX [Abnova, H00001406-M02], 1:200 Anti-NRL [R&D, AF2945], 1:500 Anti-RBPMS [ThermoFisher, PA5-119676], 1:200 Anti-RXRG [Santa Cruz, sc-365252], 1:200 Anti-Chx10 [ExAlpha X1180P], 1:400 Anti-L/M-Opsin [Merck, ab5405], 1:200 Anti-S-Opsin [Merck, ab5407], 1:200 Anti-SOX9 [Abcam, ab18596], 1:1000 Anti-Rhodopsin [Merck, O4886], 1:100 Anti-ALDH1A1 [Abcam, ab52492], 1:100 Anti-ALDH1A3 [Abcam, ab129815], 1:200 Anti-NeuN [Merck, MAB377], 1:200 Anti-SOX2 [BD Biosciences, 561469], 1:200 Anti-PDE6H [Novus NBP2-68659], 1:200 Anti- PDE6A [Protein tech, 21200-1-AP], 1:200 Anti-Calbindin [Abcam, ab108404], 1:200 Anti-GNAT1 [Santa Cruz, SC-136143], 1:200 Anti-Ki67 [ThermoFisher, 14-5698-82, 1:200 Anti-mCherry [Abcam, ab167453], 1:200 Anti- CYP26A1 [Abcam, ab151968], 1:500 Anti-HUC/HUD [ThermoFisher, A21271], 1:200 Anti-PCP4 [ThermoFisher, PA5-52209])

Validation

All antibodies were commercially acquired and have been used in previous publications examining the retina:
West, E.L., Majumder, P., Naeem, A., Fernando, M., O'Hara-Wright, M., Lanning, E., Kloc, M., Ribeiro, J., Ovando-Roche, P., Shum, I.O. and Jumbu, N., 2022. Antioxidant and lipid supplementation improve the development of photoreceptor outer segments in pluripotent stem cell-derived retinal organoids. Stem cell reports, 17(4), pp.775-788.

Gonzalez-Cordero, A., Kruczek, K., Naeem, A., Fernando, M., Kloc, M., Ribeiro, J., Goh, D., Duran, Y., Blackford, S.J., Abelleira-Hervas, L. and Sampson, R.D., 2017. Recapitulation of human retinal development from human pluripotent stem cells generates transplantable populations of cone photoreceptors. Stem cell reports, 9(3), pp.820-837.

Dorgau, B., Collin, J., Rozanska, A., Zerti, D., Unsworth, A., Crosier, M., Hussain, R., Coxhead, J., Dhanaseelan, T., Patel, A. and Sowden, J.C., 2024. Single-cell analyses reveal transient retinal progenitor cells in the ciliary margin of developing human retina. Nature communications, 15(1), p.3567.

Fernando, M., Lee, S., Wark, J.R., Xiao, D., Lim, B.Y., O'Hara-Wright, M., Kim, H.J., Smith, G.C., Wong, T., Teber, E.T. and Ali, R.R., 2022. Differentiation of brain and retinal organoids from confluent cultures of pluripotent stem cells connected by nerve-like axonal projections of optic origin. Stem Cell Reports, 17(6), pp.1476-1492.

Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T. and Sasai, Y., 2011. Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature, 472(7341), pp.51-56.

Yamasaki, S., Tu, H.Y., Matsuyama, T., Horiuchi, M., Hashiguchi, T., Sho, J., Kuwahara, A., Kishino, A., Kimura, T., Takahashi, M. and Mandai, M., 2022. A Genetic modification that reduces ON-bipolar cells in hESC-derived retinas enhances functional integration after transplantation. Iscience, 25(1).

Datta, P., Allamargot, C., Hudson, J.S., Andersen, E.K., Bhattarai, S., Drack, A.V., Sheffield, V.C. and Seo, S., 2015. Accumulation of non-outer segment proteins in the outer segment underlies photoreceptor degeneration in Bardet–Biedl syndrome. Proceedings of the National Academy of Sciences, 112(32), pp.E4400-E4409.

Or have had validation performed by manufacturer:
Anti-RBPMS [ThermoFisher, PA5-119676],
ELISA analysis using RBPMS Polyclonal Antibody (Product # PA5-119676). Coating Antigens: free peptide. Coating Concentration: 2 µg/mL, 100 µL / well. Coating Buffer: Phosphate Buffered Saline, pH7.4. Secondary Antibody: Goat Anti-Guinea pig IgG (H+L), HRP Conjugated (1:1000).

This Antibody was verified by Knockout to ensure that the antibody binds to the antigen stated.

Ki67 [ThermoFisher, 14-5698-82],
Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in Ki-67 KO cell line compared to control cell line using Ki-67 Monoclonal Antibody (SolA15), eBioscience™ (Product # 14-5698-82). Knockout validation info.

SOX9 [EPR14335-78] [Abcam, ab18596],
is a rabbit monoclonal antibody detecting SOX9 in Western Blot, Flow Cytometry (Intra), Flow Cytometry, IHC-P, ICC/IF. Suitable for Human, Mouse, Rat.
- Clone EPR14335-78 is the most cited clone to SOX9
- Biophysical QC for unrivalled batch-batch consistency
- Over 240 publications

ALDH1A1 antibody [EP1933Y] - C-terminal [Abcam, ab52492],
is a rabbit monoclonal antibody detecting ALDH1A1 in Western Blot, Flow Cytometry (Intra), Flow Cytometry, IP, IHC-P, ICC/IF. Suitable for Human, Mouse.
- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 180 publications
- Trusted since 2007

ALDH1A3 antibody [Abcam, ab129815],
is a rabbit polyclonal antibody detecting ALDH1A3 in Western Blot, IHC-P, ICC/IF. Suitable for Human, Mouse, Rat.
- Over 30 publications

Calbindin antibody [EP3478] [Abcam, ab108404],
is a rabbit monoclonal antibody detecting Calbindin in Western Blot, Flow Cytometry (Intra), Flow Cytometry, IHC-P, IHC-Fr, ICC/IF, mIHC. Suitable for Human, Mouse, Rat.
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

mCherry antibody [Abcam, ab167453],
is a rabbit polyclonal antibody detecting mCherry in Western Blot, ICC/IF.
- Over 470 publications

CYP26A1 antibody [Abcam, ab151968],
Suitable for IHC-P, WB and reacts with Mouse, Human samples. Cited in 4 publications. Immunogen corresponding to Recombinant Fragment Protein within Human CYP26A1 aa 200 to C-terminus.

PCP4 Antibody [ThermoFisher, PA5-52209],
This Antibody was verified by Relative expression to ensure that the antibody binds to the antigen stated.
Relative expression in different tissues in IHC: Detection of differential expression levels of PCP4 demonstrates antibody specificity. Immunohistochemical analysis of PCP4 using anti-PCP4 Polyclonal Antibody (Product # PA5-52209), shows significant staining of PCP4 in human cerebral cortex and shows minimal or weak staining in human liver tissues. The relative expression levels of PCP4 within each tissue is shown using RNA-Seq. Relative expression validation info.

Not Validated (These antibodies have not been previously validated, but were validated within this study via co-localisation to other validated markers as well as cell morphology:
ARR3 [Abcam; ab167507]
Mouse Polyclonal Arrestin C antibody. Suitable for WB and reacts with Human samples. Immunogen corresponding to Recombinant Full Length Protein corresponding to Human ARR3.

ARR3 [Abcam; ab189437]
Rabbit Polyclonal Arrestin C antibody. C-terminal. Suitable for WB, IHC-P and reacts with Human samples. Immunogen corresponding to Synthetic Peptide within Human ARR3 aa 300 to C-terminus.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Experiments described in this study have been performed across four PSC lines including two WiCell human embryonic stem cell lines (WA09, CRX.tdTomato and WA07, A81.BRN3B-P2AmCherry) and two human iPSC lines (HPSI0314i-hoik_1 from the European Collection of Authenticated Cell Cultures (ECACC) and 149Br.CRX.mcherry). The 149br.CRX.mcherry line was generated by insertion of an IRES-mCherry reporter at the c-terminus of CRX (exon 4) using CRISPR/Cas9 mediated HDR in the 149br iPSC cell line, which was originally reprogrammed from 149BR (ECACC 90011807) fibroblasts.
Authentication	STR cell line identification has been performed by CellBank Australia and was used for validation of each of these cell lines.
Mycoplasma contamination	All cell lines tested negative for Mycoplasma. Mycoplasma is perfomed once a month in our laboratory via Luminescence Assay and at CellBank Australia via PCR-based assay.

Commonly misidentified lines (See ICLAC register)	N/A. All our cell lines are identified correctly in Cellosaurus.org

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	H7 A81.BRN3b.mCherry and 149br.CRX.mCherry/WA09, CRX.tdTomato PSC-derived organoids were dissociated at various time points of culture into a single cell suspension using a Neurosphere Dissociation Kit. Five to six organoids were collected for each condition in a 1.5 ml tube and washed twice with PBS. Retinal organoids were dissociated into single cell suspension using the Neurosphere Dissociation Kit (P) (Miltenyi Biotec; 130-095-943). Enzymatic digestion was performed as per manufacturer protocol for 10 minutes at 37 ◦C with intermittent agitation, followed by gentle mechanical dissociation, and a further incubation for 5 minutes at 37 ◦C. The cell suspension was further dissociated into single cells by pipetting before the enzymatic reaction was stopped by washing with HBSS. The cell suspension was filtered through MACS SmartStrainer 30µm (Miltenyi Biotec, 130-041-407) before being pelleted by centrifugation at 300g for 10 minutes at room temperature. The cell pellet was resuspended in ALT90 and maintained on ice.
Instrument	BD LSR II Flow Cytometer
Software	BD FacsDiva
Cell population abundance	Cell population abundances of relevant cells are described in text.
Gating strategy	Cells were analysed using FACSDiva software (BD Biosciences). Background fluorescence was measured using age matched organoids derived from a control PSC line. These cells were used to set gating parameters between positive and negative populations whilst unstained controls were used to set gating parameters for Sytox staining. Small debris, cell fragments and aggregates were excluded from analysis by forward and side scatter (measuring cell size and granularity, respectively) whilst doublets were gated out using forward scatter area vs height. 15,000 events were captured on average per experiment.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.