nature portfolio

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Last updated by author(s): Feb 12, 2025

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	No software was used for the purpose of data collection.
Data analysis	The following software was used for data analysis: R (versions 4.1.0 or 4.3.1) Seurat (v4, v5) Monocle3 (v1.3.4) ggpubr (v0.6.0) EnrichR (v3.2) ggplot2 (v3.4.3) PIPseeker (v02.01.04, v3) bcl2fastq (v2.20.0.422) FIJI (RRID:SCR_002285) Imaris (v10, RRID:SCR_007370) Slingshot (v2.7.0) WGCNA (v1.72.5) factoextra dynamicTreeCut data.table morpheus R package (v1.0-4) reticulate (v1.36.1)

scanorama
pySCENIC (v0.12.1)
cistargetDB
DoubletFinder (v2.0.4)
plink (v1.9)
samtools (v1.21)
Vireo (run via Demuxafy v3)
cellranger-arc (v2.2.0)
Signac (v1.14)

Custom code was used in this paper and is publicly available at Github: https://github.com/BhaduriLab/dev-ctx-meta-atlas

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

Our developmental and adult meta-atlases are browsable in the UCSC Genome Browser: (https://dev-ctx-meta-atlas.cells.ucsc.edu and https://adult-ctx-meta-atlas.cells.ucsc.edu), in which our meta-atlas is also available as a Seurat object for download. Original datasets for the developmental meta-atlas are accessible as follows: Bhaduri et al., 2021 (UCSC Cells - Neocortex: https://dev-brain-regions.cells.ucsc.edu), Fan et al., 2018 (GEO Series GSE103723), Fan et al., 2020 (GEO Series GSE120046), Nowakowski et al., 2017 (UCSC Cells - Cortex Development: https://cortex-dev.cells.ucsc.edu), Polioudakis et al., 2019 (http://solo.bmap.ucla.edu/shiny/webapp/), Smith et al., 2021 (Figshare - Dataset: https://figshare.com/s/64b648891e4817efb123), and Zhong et al., 2018 (GEO Series GSE104276).

Original datasets for the adult meta-atlas are accessible as follows: Bakken et al., 2021 (Human M1 10x: https://portal.brain-map.org/atlases-and-data/rnaseq/human-m1-10x), Caglayan et al., 2023 (GEO Series GSE192773), Gandal et al., 2022 (DOI: 10.1038/s41586-022-05377-7), Herring et al., 2022 (GEO Series GSE168408), Jorstad et al., 2023 (Human Multiple Cortical Areas Smart-Seq: https://portal.brain-map.org/atlases-and-data/rnaseq/human-multiple-cortical-areas-smart-seq), Lake et al., 2017 (GEO Series GSE97942), Ma et al., 2022 (GSE207334), Mathys et al., 2023 (Aging Brain Data: http://compbio.mit.edu/ad_aging_brain/), Morabito et al., 2021 (DOI: 10.1038/s41588-021-00894-z), Nagy et al., 2020 (GEO Series GSE144136), Pfisterer et al., 2020 (Epilepsy19: https://kkh.bric.ku.dk/Epilepsy19/; metadata from: https://github.com/khodosevichlab/Epilepsy19), Ramos et al., 2022 (DOI: 10.1038/s41467-022-34975-2), Schirmer et al., 2019 (DOI: 10.1038/s41586-019-1404-z), Siletti et al., 2023 (CellxGene Collection: https://cellxgene.cziscience.com/collections/283d65eb-dd53-496d-adb7-7570c7caa443), Velmeshev et al., 2023 (Pre-Postnatal Cortex RNA: https://pre-postnatal-cortex.cells.ucsc.edu), and Zhu et al., 2023 (Cellxgene Collection: https://cellxgene.cziscience.com/collections/ceb895f4-ff9f-403a-b7c3-187a9657ac2c).

Raw data, count matrices, and relevant meta-data for the single-cell profiles of FEZF2 / TSHZ3 KD organoids (GSE242779) and chimeroids (GSE278573) were deposited in the NCBI Gene Expression Omnibus.

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	Human data was used from publicly available sources and metadata can be retrieved from source datasets if available.
Reporting on race, ethnicity, or other socially relevant groupings	No reporting on race, ethnicity or other socially relevant groupings were used.
Population characteristics	No population characteristics were used.
Recruitment	No participants were recruited.
Ethics oversight	All brain samples and iPSC cell lines collected for analysis in this study were obtained with consent and donor anonymization. All of the procedures were reviewed and approved by the UCLA IBC and hPSCRO (UCLA Institutional Biosafety Committee approval number BUA-2020-091-025-A; UCLA Human Pluripotent Stem Cell Research Oversight Committee approval number 2020-008-05).

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	No sample size calculation was performed for meta-atlas generation and analysis due to the use of publicly available datasets. As is a typical standard in the field, all experiments were replicated in biological duplicate or triplicate where possible and immunostainings were performed in duplicate based upon human tissue availability, with one tissue piece used for each age represented. For single-cell transcriptomic/multiomic experiments, the basis of outputs on single cells restricted our analysis to the number of high-quality cells obtained per capture.
Data exclusions	Developmental meta-atlas: For all datasets but the Polioudakis et al., 20196, Bhaduri et al., 20219, Nowakwoski et al., 20175, gene count matrices for individual cells and corresponding metadata were downloaded from the Gene Expression Omnibus, and cells from the same individual were combined. Polioudakis et al data was downloaded from the browser within the publication (see below) and Bhaduri et al and Nowakowski et al were received via personal communication, but could also be downloaded from the UCSC Cell Browser84. In the case of Smith et al., 202116, directories containing the 10X-provided matrix.mtx, genes.tsv, and barcodes.tsv were downloaded from https://figshare.com/s/64b648891e4817efb123 as originally described16. We then processed the 10X-derived count matrices and directories from each individual with a standard pipeline to generate Seurat objects (Seurat v426), conducting a normalization of the counts as needed and filtering out cells with < 500 genes detected and > 5% of UMIs mapping to mitochondrial genes. Genes detected in < 3 cells were omitted. In cases where the original publication used more stringent criteria for these quality control measures, we defaulted to the original publication's settings (Table S1). In the case of Polioudakis et al., 2019, after downloading raw counts (UMI) gene expression matrix and metadata from the original paper's indicated data browser, we adapted the quality control measures described by the authors, selecting for cells with the 95th percentile of total UMIs with < 250 UMIs mapping to the mouse genome, and < 5% of UMIs mapping to mitochondrial genes. For consistency with the remainder of our meta-atlas, we then applied a stricter minimum for the number of genes detected per cell (increasing the minimum from 200 in the original paper to 500), and we did not place a maximum on the genes detected per cell, as the biologically relevant range of the number of genes per cell will vary between cell types. We then removed genes detected in < 3 cells. Adult meta-atlas: With the exception of datasets for Gandal et al., 202220, Ramos et al., 202215, and Schirmer et al., 201943, Seurat objects or gene counts matrices for individual cells and corresponding metadata for each dataset were downloaded from the Gene Expression Omnibus or as otherwise instructed in the original publication (Table S13). Seurat objects for the Gandal et al., 2022, Ramos et al., 2022, and Schirmer et al., 2019 datasets were obtained via personal communication from M. Gandal, S. Ramos, and A. Zulji, respectively. All datasets for adult meta-atlas are single-nuclei profiles. Seurat objects for each individual were generated as described for the developmental meta-atlas (Seurat v5), with the exception that the additional filtering based on the percent of mitochondrial genes per cell was applied to only a few datasets given the relatively low mitochondrial RNA detected single-nuclei sequencing (Table S13). In the case of Siletti et al., 2023, area-specific Seurat objects that corresponded to cortical areas were downloaded and additional modifications were required for computation feasibility. For two individuals (H19.30.001 and H19.30.002), individual-level Seurat objects were generated by first extracting the counts matrix for each individual from each area-specific Seurat object. Features in the resulting matrices were converting Ensembl IDs to Gene Symbols, and the feature counts were summarized across genes to collapse data from different transcripts of the same gene. Seurat objects were generated from the resulting individual-level, area-specific counts matrix, and the 5 area-specific Seurat objects for were merged into one object representing a single individual. The features in the resulting Seurat objects were filtered to retain just the genes that overlapped with the H18.30.001 in the Bakken et al., 2021 dataset. These objects were then randomly subset into 8 objects. The H18.30.002 individual was subset by first randomly grouping the cell barcodes belonging to this individual into 11 subsets, which were then used as a guide to generate Seurat objects as detailed above. Features from the counts matrices of the Zhu et al., 2023 datasets were also converted from Ensembl ID to Gene Symbols and counts matrices were filtered to retain just the features that overlap with the Bakken et al., 2021 dataset for consistency with the remainder of the meta-atlas.
Replication	All experiments were replicated in duplicate or triplicate where possible, immunostainings were performed in duplicate based upon human tissue availability, with one tissue piece used for each age represented.
Randomization	Randomization was not applicable for this study as it was imperative to keep track of which experimental samples were which to ensure sample integrity.
Blinding	Blinding was not possible for experiments given the use of different fluorescent reporters to demarcate conditions and the need to ensure provenance of publically available datasets used in the meta-atlas. Immunofluorescent stainings were conducted to validate results from computational analyses, and conclusions were restricted to binary conclusions rather than subtle, graded comparisons between conditions.

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 antibodies: FEZF2(ab214186, abcam, Lot 1006049, Clone N/A); TSHZ3(N/A, Lot N/A, Clone N/A: Non-commercial guinea-pig anti-TSHZ3 antibody produced in-house by Alistair Garratt's laboratory was raised against mouse amino acids 557-664, cloned as a His-tagged fusion protein in pET14b); CTIP2(ab18465, abcam, Lot 2101034078, Clone 25B6); SATB2(ab51502, abcam, Lot GR3281721-13, Clone SATBA4B10); HS6ST2(ab122220, abcam, Lot GR148036-25, Clone N/A); ADAM33(sc-514055, Santa Cruz Biotechnology, Lot E1716, Clone A-3); ZBTB16/PLZF(sc-28319, Santa Cruz Biotechnology, Lot I1022, Clone D-9); VGAT(AB5062P, Millipore Sigma, Lot 3974593, Clone N/A); QKI(MABN624, Millipore Sigma, Lot 3506608, Clone N147/6); PDLIM5/ENH(388800, Thermo Fisher, Lot RC234862, Clone N/A); SOX2(sc-365823, Santa Cruz Biotechnology, Lot C2523, Clone E-4); VSNL1(UM870034, OriGene, Lot W001, Clone UMAB115); SALL1(PA5-62057, Thermo Fisher, Lot ZG4382825A, Clone N/A), c-FOS(sc-16694, Santa Cruz Biotechnology, Lot C2523, Clone E-8), and ZFP36L1/2/BRF1(12306-1-AP, Cell Signaling Technology, Lot 00082509, Clone N/A).

Polyclonal AlexaFluor secondary antibodies from Thermo Fisher used: mouse (488: # A-31570, A-11001; 555: # A-31572, A-11005; 568: # A-31573, A-11004; 647: # A-31574, A-11029); rat (488: # A-21208, A-11007; 555: # A-21209, A-11009; 568: # A-21210, A-11008; 647: # A-21211, A-11037); guinea pig (488: # A-11073, A-11075; 555: # A-21449, A-21450; 568: # A-11075, A-11073; 647: # A-21450, A-21449); rabbit (488: # A-11008, A-11034; 555: # A-21428, A-21207; 568: # A-11011, A-21245; 647: # A-21245, A-21207); goat (488: # A-11055, A-11034; 555: # A-21432, A-11039; 568: # A-11056, A-11040; 647: # A-21435, A-11041).

Validation

In-house TSHZ3 antibody was described and validated extensively in Caubit, X. et al. TSHZ3 deletion causes an autism syndrome and defects in cortical projection neurons. Nat Genet 48, 1359-1369, doi:10.1038/ng.3681 (2016).

Validation statements for all commercial antibodies listed above can be found through the following links to the manufacturer's website:

Primary Antibodies:
FEZF2 (ab214186, Abcam): https://www.abcam.com/fezf2-antibody-ab214186.html
CTIP2 (ab18465, Abcam): https://www.abcam.com/ctip2-antibody-ab18465.html
SATB2 (ab51502, Abcam): https://www.abcam.com/satb2-antibody-ab51502.html
HS6ST2 (ab122220, Abcam): https://www.abcam.com/hs6st2-antibody-ab122220.html
ADAM33 (sc-514055, Santa Cruz Biotechnology): https://www.scbt.com/p/adam33-antibody-a-3
ZBTB16/PLZF (sc-28319, Santa Cruz Biotechnology): https://www.scbt.com/p/plzf-antibody-d-9
VGAT (AB5062P, Millipore Sigma): https://www.sigmaaldrich.com/catalog/product/sigma/ab5062p
QKI (MABN624, Millipore Sigma): https://www.sigmaaldrich.com/catalog/product/sigma/mabn624
PDLIM5/ENH (388800, Thermo Fisher): https://www.thermofisher.com/order/catalog/product/PA5-62057
SOX2 (sc-365823, Santa Cruz Biotechnology): https://www.scbt.com/p/sox2-antibody-e-4
VSNL1 (UM870034, OriGene): https://www.origene.com/catalog/antibodies/primary-antibodies/um870034/vsnl1-antibody-umab115
SALL1 (PA5-62057, Thermo Fisher): https://www.thermofisher.com/order/catalog/product/PA5-62057
c-FOS (sc-16694, Santa Cruz Biotechnology): https://www.scbt.com/p/c-fos-antibody-e-8
ZFP36L1/2/BRF1 (12306-1-AP, Cell Signaling Technology): https://www.cellsignal.com/products/antibodies/zfp36l1-2-brf1-antibody-12306

Secondary Antibodies:

Mouse Antibodies
A-31570: https://www.thermofisher.com/order/catalog/product/A-31570
A-11001: https://www.thermofisher.com/order/catalog/product/A-11001
A-31572: https://www.thermofisher.com/order/catalog/product/A-31572
A-11005: https://www.thermofisher.com/order/catalog/product/A-11005
A-31573: https://www.thermofisher.com/order/catalog/product/A-31573
A-11004: https://www.thermofisher.com/order/catalog/product/A-11004
A-31574: https://www.thermofisher.com/order/catalog/product/A-31574
A-11029: https://www.thermofisher.com/order/catalog/product/A-11029

Rat Antibodies
A-21208: https://www.thermofisher.com/order/catalog/product/A-21208
A-11007: https://www.thermofisher.com/order/catalog/product/A-11007
A-21209: https://www.thermofisher.com/order/catalog/product/A-21209
A-11009: https://www.thermofisher.com/order/catalog/product/A-11009
A-21210: https://www.thermofisher.com/order/catalog/product/A-21210
A-11008: https://www.thermofisher.com/order/catalog/product/A-11008

A-21211: https://www.thermofisher.com/order/catalog/product/A-21211
A-11037: https://www.thermofisher.com/order/catalog/product/A-11037

Guinea Pig Antibodies
A-11073: https://www.thermofisher.com/order/catalog/product/A-11073
A-11075: https://www.thermofisher.com/order/catalog/product/A-11075
A-21449: https://www.thermofisher.com/order/catalog/product/A-21449
A-21450: https://www.thermofisher.com/order/catalog/product/A-21450
A-11075: https://www.thermofisher.com/order/catalog/product/A-11075
A-11073: https://www.thermofisher.com/order/catalog/product/A-11073
A-21450: https://www.thermofisher.com/order/catalog/product/A-21450
A-21449: https://www.thermofisher.com/order/catalog/product/A-21449

Rabbit Antibodies
A-11008: https://www.thermofisher.com/order/catalog/product/A-11008
A-11034: https://www.thermofisher.com/order/catalog/product/A-11034
A-21428: https://www.thermofisher.com/order/catalog/product/A-21428
A-21207: https://www.thermofisher.com/order/catalog/product/A-21207
A-11011: https://www.thermofisher.com/order/catalog/product/A-11011
A-21245: https://www.thermofisher.com/order/catalog/product/A-21245
A-21245: https://www.thermofisher.com/order/catalog/product/A-21245
A-21207: https://www.thermofisher.com/order/catalog/product/A-21207

Goat Antibodies
A-11055: https://www.thermofisher.com/order/catalog/product/A-11055
A-11034: https://www.thermofisher.com/order/catalog/product/A-11034
A-21432: https://www.thermofisher.com/order/catalog/product/A-21432
A-11039: https://www.thermofisher.com/order/catalog/product/A-11039
A-11056: https://www.thermofisher.com/order/catalog/product/A-11056
A-11040: https://www.thermofisher.com/order/catalog/product/A-11040
A-21435: https://www.thermofisher.com/order/catalog/product/A-21435
A-11041: https://www.thermofisher.com/order/catalog/product/A-11041

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Lines UCLA1 (female, aka NIHhESC-10-0058) and UCLA6 (male, aka NIHhESC-11-0089) were obtained from the UCLA Stem Cell Core, CIRM 20107 (female) was obtained from the CIRM Fujifilm repository, and KOLF was obtained from Jackson Laboratories. HEK-293T cells were purchased from Takara (Lenti-X™ 293T Cell Line, Cat # 632180).
Authentication	Each pluripotent cell line was karyotyped prior to use in the lab and was validated by the UCLA Stem Cell Core or the CIRM Repository prior to distribution for authenticity. Lenti-X™ 293T Cell Lines are validated as per manufacturer website: https://www.takarabio.com/documents/Certificate%20of%20Analysis/632180/632180-120716.pdf.
Mycoplasma contamination	All lines tested negative for mycoplasma and all newly generated data used in this study was also aligned to a mycoplasma genome to verify the lack of contamination.
Commonly misidentified lines (See ICLAC register)	None

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*