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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	Count matrices for snMultiome data were generated using cellranger-arc (v2.0.2; 10X Genomics). Count matrices for snRNA-seq data from Singleron were generated by Singleron using CeleScope scopeV3.0.1 (kit V2) technology. Bulk RNA-seq count matrices were generated with the pipelines nf-core/rnaseq (v.3.18.0; doi:10.5281/zenodo.1400710) or AccuraCode (v1.2.0; for Singleron data). Imaging data were acquired with the Leica Application Suite X, or the FV31S-SW Fluoview software.
Data analysis	Sequencing data were analysed in an R environment (R v4.3.3), using the Seurat single cell analysis package v5.1.0, with the Signac extension (v1.13.0) for basic analyses. sccomp (v1.7.15) and MiloR (v1.10.0) were used for compositional analyses. DESeq2 v1.42.1 and Nebula (v1.5.3) were used for differential gene expression analyses. limma (v3.58.1) was used for batch correction of expression matrices. clusterProfiler v4.10.1 with annotations from DOSE(v 3.28.2) and org.Hs.eg.db (v3.18.0) were used for functional enrichment analyses. BSgenome.Hsapiens.UCSC.hg38 (v1.4.5) and EnsDb.Hsapiens.v86 (v2.99.0) were used for ATAC-seq mapping and annotations. scMEGA v1.0.2 was used for gene regulatory network analyses, using binding motifs from the JASPAR2024 package, v0.99.6. Protein-protein-interactions from the BioGRID database (v4.4.233) were retrieved using the BioGRID API via the R packages jsonlite (v1.8.8) and httr (v1.4.7). tidyverse (v2.0.0) with ggplot (v3.5.1), pheatmap(v1.0.12) and the ggraph package (v2.2.1) were used for general data analyses and visualisations. FIJI/ ImageJ v1.53q was used for processing and analysing immunofluorescence images, using R packages above for downstream quantifcation. The used analysis pipelines based on these software tools will be made available on https://github.com/lattkem1/Down_Syndrome_Multiome

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

Raw and processed sequencing data generated for this study will be made available under GEO accessions GSEXXX. The fetal tissue dataset will also be available as interactive online resource on the DISCO platform (https://www.immunesinglecell.org/tool/integration/ res/Down_syndrome). Other data will be provided by the authors upon reasonable 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	Samples from both sexes (based on Karyotyping provided by the HDBR) have been included in this study and are reported in Supplementary Table 1. Preliminary analyses did not identify major sex-specific differences, therefore no sex-specific analyses are reported in the manuscript.
Reporting on race, ethnicity, or other socially relevant groupings	As the material is from banked foetal tissue, no information such information is available for the analysed samples.
Population characteristics	Samples were derived from foetal tissue from post-conceptional weeks 10 to 20. The estimated stage is reported in Suppl. Table 1. Beside the sex (see above), no other population characteristics were available.
Recruitment	Samples were donated by individuals undergoing surgical terminations of pregnancy in UK clinics to the Human Developmental Biology Resource (https://www.hdbr.org/).
Ethics oversight	The HDBR provides tissue under an umbrella licence covered by ethics approvals (London/Newcastle; REC approval 18/LO/0822 and 18/NE/0290). The individual project whose results are reported here have been approved by the HDBR (HDBR Project 200585).

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	The study used 20 CON and 19 DS fetal brain samples. The study aimed to cover human foetal samples from post-conceptional weeks 10-20 with each 5 CON and DS samples for weeks 10-12, 13-16 and 17-20. However, Sample sizes for human foetal tissue were determined and limited by the availability of this precious donated tissue, mainly for weeks 12 and 13. Therefore we acknowledge that our study has limited power to assess changes before PCW11 and after PCW14. For in vitro experiments derived from a trisomic iPSC line and a isogenic control line were used, with at least 3 technical replicates per group. This is not powered to comprehensively detect differences, but sufficient to validate a large proportion of differences detected in the fetal samples. To assess independence of detected changes from inter-experimental and genetic variability, the basic characterization was repeated with multiple different cell preparations and a second pair of isogenic trisomic and control iPSC lines as outlined in the manuscript. 8 CON and 4 DS graft samples derived from a trisomic iPSC line and a isogenic control line were obtained to assess the ability of these grafts to model human fetal development and DS phenotypes. While this design reduces variability due to genetic background variability, it cannot account for changes that may be influenced by the diverse genetic backgrounds of the human population.
Data exclusions	As the donated fetal tissue was of very variable quality, and various other technical and biological parameters affected data quality (e.g. the RNase inhibitor used affected RNA library quality, regional identity), we have excluded 5 Control and 4 DS samples from our analyses, as well as low quality libraries and cells as described in detail in the manuscript (Suppl. Table 1). We based exclusion criteria on histological assessments of tissue quality, cell and sample level QC measures established in the field (e.g. transcript counts/cell, enrichment of ATAC reads in transcriptional start sites, ...), mapping of data to regions of a developing brain reference atlas and on the ability of the combined datasets to distinguish cell subtypes expected to be present at these stages (Suppl. Table 1). Similar inclusion/exclusion criteria were applied to the human graft analysis (Suppl. Table 6). We also excluded one tissue bulk RNA-seq library which showed a transcriptome globally very strongly diverging from all 11 other samples, identified by principal component analysis

(Extended Data Fig. 6c). This sample also showed poorer QC measures and an apparent lack of expression of a subset of Chr21 genes (not shown), which were very consistently upregulated in the 5 other DS samples.

Replication

While a replication of the fetal sequencing study with other samples was not feasible, we replicated key analyses with different parameter settings and/or computational tools, and focused on findings that were reproducible between different analyses. For example, exclusion of lower quality samples retained the reduction of RORB/FOXP1 neurons and a large fraction of the DEGs. Using an alternative differential expression analysis approach (Nebula) or published data (Extended Data Fig. 5), or considering developmental stage as covariate (not shown), confirmed the detected DEGs, and detected many other DEGs, but these additional DEGs were less consistent between different analyses. We replicated many changes in the in vitro model, using multiple cell preparations and an additional pair of isogenic cell lines, confirming robustness of the models (Fig. 4, Extended Data Fig. 9).

Randomization

We matched sex and developmental stage between CON and DS syndrome groups, as far as sample availability allowed (see Suppl. Table 1). As mentioned above, gene expression changes were largely retained if sex and developmental stage were considered as covariates, suggesting the absence of major biases.
For in vitro and graft studies, cells derived from isogenic pairs of cell lines were used to avoid bias due to different genetic backgrounds.

Blinding

As the study is based mainly on systematic global computational analyses without strong initial hypotheses, no systematic blinding was required.

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

IF: Primary antibodies used were mouse anti-SATB2 (Abcam, ab51502, fresh frozen sections), rabbit anti-PAX6 (Biolegend #901301), rat anti-CTIP2 (Abcam, ab18465), mouse anti-SATB2 (Santa Cruz Biotechnology, sc-81376, paraffin embedded sections), rabbit anti-FOXP1 (Abcam, ab16645). Western Blot: antibodies used were mouse anti-Beta-Actin (Santa Cruz Biotechnology, sc-47778, 1:100), mouse anti-BACH1 (Abcam, ab128486, 1:2000), rabbit anti-PKNOX1 (ThermoFisher, PA5-30244, 1:750), mouse anti-GABPA (ThermoFisher, MA5-15419, 1:1000), goat anti-Rabbit-HRP (ThermoFisher, 31460, 1:5000) and goat anti-Mouse-HRP (ThermoFisher, 31430, 1:5000).

Validation

Mouse anti-SATB2 (Abcam, ab51502; 1:100) has been KO-validated by the supplier for applications including ICC/IHC (note that the antibody cross-reacts with SATB1). Mouse anti-SATB2 (Santa Cruz Biotechnology, sc-81376; 1:400) according to the supplier website has been validated for IF. Rabbit anti-PAX6 (Biolegend #901301; 1:200) according to the supplier website has been validated for IHC and reported to be also suitable for ICC. Rat anti-CTIP2 (Abcam, ab18465; 1:500) according to the supplier website has been validated for IHC-P and ICC/IF. Rabbit anti-FOXP1 (Abcam, ab16645; 1:200) has been validated by the supplier for mouse Foxp1 staining in IHC-P and IHC-FoFr and is predicted to similarly bind the homologous human FOXP1. According to the manufacturer's website it has also been used in numerous publications for human tissue.
All antibodies showed staining consistent with their expected region-specific expression.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The neural progenitors from the isogenic C9 (CON) and C13 (DS) iPSC lines have been provided by the laboratory that has generated and tested these lines (see Murray et al., 2015, Alic et al., 2021). The isogenic DS2U (CON) and DS1 (DS) iPSC lines have been purchased from WiCell.

Authentication

WiCell provides extensive batch-specific characterization of the DS2U and DS1 iPSC lines.
The ability of all cell lines to differentiate into neurons has been verified. The increased expression of Chr21 genes in DS iPSC-derived neural cells in our study (Fig. 4, Extended Data Fig. 9) further confirms the retained trisomy of the DS cells used in our study.

Mycoplasma contamination

The cells have not been systematically tested for mycoplasma.

Commonly misidentified lines (See ICLAC register)	NA

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Immunodeficient mice (JAX™ NSG, Charles River) aged between 3-5 months
Wild animals	NA
Reporting on sex	Both male and female mice were used. As only human-derived cells were analysed, no mouse-sex-specific analyses were performed.
Field-collected samples	NA
Ethics oversight	All mouse experimental procedures were approved by the Animal Care and Use Committee (IACUC) at Duke-NUS, Singapore (Ref. nr. 2022/SHS/1766), and ethics approved by the Institutional Review Board (NUS-IRB-2022-149), as well as by the Ministry of Health (MOH Ref: RR-2023/01) under the Human Biomedical Research Act guidance.

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

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.*