nature portfolio

Corresponding author(s): Deepak Srivastava & Sanjeev Ranade

Last updated by author(s): 08/25/2025

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.

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.

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 software and code

Data collection: Lightsheet imaging data collected on SmartSPIM Light Sheet Microscope (LifeCanvas Technologies, Cambridge).
All single cell datasets collected through 10X Genomics Chromium instrument.MicroCT collected on µCT 50 cabinet microCT scanner.
Flow cytometry performed with BD LSRFortessa X-20 .

Data analysis: All data provided openly at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE271447
All scripts provided at: https://github.com/sanjeevRJMU1/DownSyndrome
NFcore for cut and run analysis: version 3.2.2
microCT: 3D Slicer 5.2.2 and OsiriX MD v. 14.0
Flow cytometry data were analyzed with: FlowJo (v.9.2)
Lightsheet data were analyzed: Imaris 10.1.1, ImarisStitcher 10.1.1, ImarisFileConverter 10.2.0

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

GEO: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE271447. Human references: Hg38. Mouse references: Mm10.

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	n/a
Data exclusions	n/a
Replication	n/a
Randomization	n/a
Blinding	n/a

Behavioural & social sciences study design

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

Study description	n/a
Research sample	n/a
Sampling strategy	n/a
Data collection	n/a
Timing	n/a
Data exclusions	n/a
Non-participation	n/a
Randomization	n/a

Ecological, evolutionary & environmental sciences study design

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

Study description	n/a
Research sample	n/a
Sampling strategy	n/a
Data collection	n/a
Timing and spatial scale	n/a
Data exclusions	n/a
Reproducibility	n/a
Randomization	n/a
Blinding	n/a

Did the study involve field work? ☐ Yes ☑ No

Field work, collection and transport

Field conditions	n/a
Location	n/a
Access & import/export	n/a
Disturbance	n/a

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	Flag (Monoclonal ANTI-FLAG M2 antibody (Sigma-Aldrich, F1804-200 µG, 1:200 dilution) H3K4me3 Antibody (Epicypher 13-0060, 1:200 dilution)
Validation	H3K4me3 antibody verified by vendor in K562 cells against a panel of histone modifications. Flag antibody validation data include sensitivity benchmarks, recommended usage protocols, a broad range of validated applications, and citations supporting reproducibility from vendor (Sigma).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	WiCell (DS2U, DS1, WC-24-02-DS-B and WC-24-02-DS-M). HEK293T (ATCC, CRL-3216)
Authentication	Karyotyping analysis, described in methods and data shown in Extended Data Fig S1
Mycoplasma contamination	Tested negative at regular intervals
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study

Palaeontology and Archaeology

Specimen provenance	
Specimen deposition	
Dating methods	

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	

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

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	C57Bl6/J, Dp1Tyb and Hmgn1 mouse models. All animals were at least 8 weeks of age for breeding. Embryonic timepoints were used for scRNAseq (E11.5) and microCT (E15.5)
Wild animals	No wild animals were used in this study
Reporting on sex	Both males and females were used in the study
Field-collected samples	No field collected samples were used in this study
Ethics oversight	Use of all animals were approved by UCSF IACUC (described in detail in methods section)

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	n/a
Study protocol	n/a
Data collection	n/a
Outcomes	n/a

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
X	☐	Public health
X	☐	National security
X	☐	Crops and/or livestock
X	☐	Ecosystems
X	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
X	☐	Demonstrate how to render a vaccine ineffective
X	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
X	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
X	☐	Increase transmissibility of a pathogen
X	☐	Alter the host range of a pathogen
X	☐	Enable evasion of diagnostic/detection modalities
X	☐	Enable the weaponization of a biological agent or toxin
X	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

ChIP-seq

Data deposition

☑ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☑ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	GEO: GSE271447, token: wbydumsobnovrkx
Files in database submission	Fastq and bigwig
Genome browser session (e.g. UCSC)	No longer applicable for the final submission

Methodology

Replicates	Peaks were called from minimum of n=2 replicates
Sequencing depth	Cut&Run sequencing performed to 5-10 million reads as recommended
Antibodies	Flag (Monoclonal ANTI-FLAG M2 antibody (Sigma-Aldrich, F1804-200 µG)
Peak calling parameters	SEACR peak calling
Data quality	All data analyzed through NFcore pipelines, described in methods section

Software: NFcore

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: hiPSCs were dissociated with Accutase, neutralized and resuspended in FACS buffer

Instrument: BD LSRFortessa X-20

Software: BD FACSDiva software

Cell population abundance: hiPSCs infected with CROP-seq vector were screened for presence of mCherry activity. Cells were 10.4% positive after initial infection and 98% positive after puromycin selection

Gating strategy: Unifected hiPSCs were used as negative controls for establishing gates (Ext. Data Fig. S4d). Boundries between the positive and negative populations were based on mCherry expression.

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

Magnetic resonance imaging

Experimental design

Design type: n/a

Design specifications: n/a

Behavioral performance measures: n/a

Imaging type(s): n/a

Field strength: n/a

Sequence & imaging parameters: n/a

Area of acquisition: n/a

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software: n/a

Normalization: n/a

Normalization template: n/a

Noise and artifact removal: n/a

Volume censoring: n/a

Statistical modeling & inference

Model type and settings: n/a

Effect(s) tested: n/a

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)

n/a

Correction

n/a

Models & analysis

n/a	Involved in the study
x	☐ Functional and/or effective connectivity
x	☐ Graph analysis
x	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

n/a

Graph analysis

n/a

Multivariate modeling and predictive analysis

n/a