nature portfolio

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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	PClamp software v10.7 (Molecular Devices)
Data analysis	Neurolucida 360 (MBF Biosciences), Neurolucida Explorer (MBF Biosciences), AxIS Navigator v3.10.1.9 (Axion Biosystems Inc), Axion Data Export Tool v3.5.0 (Axion Biosystems Inc), Offline Sorter v4.5.0 (Plexon Inc), Neural Metric Tool v3.1.7 (Axion Biosystems Inc), Custom code for transcriptomic analysis, RStudio (RStudio: Integrated Development for R), R (The R Project), Harmony 4.9 (PerkinElmer), ImageJ (NIH), Python v3.12.2 (Anaconda), Prism v10.1.1 (GraphPad Software Inc.), Cell Ranger 7.0.0 or 9.0.0 (10X Genomics), Seurat v5 (Satija Lab), qPCRanalysis tool v1.2 (STEMCELL Technologies).

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

The raw single-nuclei RNA sequencing data generated in this study is protected and not available for public access due to patient privacy. The processed data is

available in the supplementary information. All other raw data files generated in this study can be made available upon reasonable request by contacting the corresponding author, otherwise data generated in this study is provided in the Source Data file.

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	Patient sex was considered in the study design, skin biopsies were collected from five children with Sanfilippo syndrome type A (MPS IIIA; 3x Female, 2x Male) and five age and sex-matched control cases (2x Female, 3x Male).
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	MPS IIIA patients (n=5) were aged between 4 and 11 years and age-matched to healthy controls (n=5) that were aged between 4 and 12 years of age. MPS IIIA patients were clinically confirmed to have SGSH mutations and symptoms. Controls were defined as clinically and genotypically neurotypical at the time of biopsy.
Recruitment	Participants were recruited from the Women's and Children's Hospital in Adelaide, South Australia. All participants consented to involvement in the study prior to tissue extraction under ethics application HREC/18/WCHN/147.
Ethics oversight	Women's and Children's Health Network Human Research Ethics Committee

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 formal statistical sample-size calculation was performed prior to this study. Instead, sample size was determined by the availability of clinically confirmed MPS IIIA patient samples and matched healthy controls. Skin biopsies were collected from five children with MPS IIIA (3 female, 2 male, aged 4–11 years) and five age- and sex-matched neurotypical controls (2 female, 3 male, aged 4–12 years). Each donor contributed two independent isogenic iPSC clones (except BIAD05 who contributed one), providing biological replication at both the donor and clone levels. These sample sizes are consistent with other published iPSC studies using patient-derived lines, and were considered sufficient to detect robust genotype-associated phenotypes in downstream analyses while balancing the practical limitations of patient sample availability and the intensive nature of iPSC culture and differentiation experiments.
Data exclusions	No data were excluded on the basis of experimental outcome, and no statistical outliers were removed. Exclusions were limited to predefined instances of technical failure, such as failed antibody staining, focus failure, or cell loss/detachment during immunocytochemistry. For synaptic imaging data, additional quality control was applied at the field-of-view (FOV) level. Each well contained 25 FOVs, and FOVs were excluded if they showed: (i) a clumping area ≥104,000 µm² (≥25% of the total FOV), (ii) ≥4 clumping events (each >5,000 µm²), or (iii) neuronal density (MAP2+DAPI⁺ cells) <1. These criteria were pre-established to avoid artefacts from cellular detachment or clumping that compromise neuronal morphology analyses. For multielectrode array (MEA) data, wells with a network event frequency of 0 Hz were excluded only from the Network event frequency (Hz) metric, as their inclusion would artificially lower group averages. This exclusion was applied consistently and transparently reported, and did not differ between MPSIIIA and control groups. All exclusion criteria was pre-established, applied consistently, and is detailed throughout the manuscript.
Replication	All key findings were successfully replicated across independent experiments. Data was reproduced in at least three independent “playground” experiments with a minimum of six technical replicates per experiment. Results were consistent across multiple iPSC lines and clones derived from five healthy donors and five MPSIIIA patients (two clones per individual), as well as in additional isogenic knockout controls. While variability was observed between patient-derived lines, results within the same donor were reproducible, indicating that the variation reflects true biological diversity rather than technical artefacts.
Randomization	Samples were allocated to groups based on clinical status (MPSIIIA vs. healthy control) and age/sex matching at the time of biopsy. Within each donor, two independent iPSC clones were generated and analysed. Experimental allocation was therefore not randomized, but key covariates (age and sex) were controlled through matching of cases and controls, and technical variation was minimised by processing lines in parallel under identical conditions.
Blinding	Investigators were not blinded to group allocation during data collection or analysis for most assays, as this was not feasible during tissue culture and cell line characterisation. The exception was whole-cell patch-clamp recordings, where experimenters were blinded to genotype

and treatment during acquisition and analysis to minimise selection bias.

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

Chicken polyclonal anti-MAP2, Abcam 1:2000 (ab5392)
Rabbit polyclonal anti-Synapsin I, Millipore 1:1000 (AB1543P)
Rabbit polyclonal anti-Ki67, Abcam 1:500 (ab15580)
Mouse monoclonal anti-PSD-95 (Clone 6G6-1C9), Thermo Fisher Scientific 1:500 (MA1-90314)
Mouse monoclonal anti-Gephyrin (Clone mAb7a), Synaptic Systems 1:250 (147021)
Mouse monoclonal anti-GFAP (Clone 2A5), Abcam 1:300 (ab4648)
Mouse monoclonal anti-Nestin (Clone 10C2), Abcam 1:200 (ab22035)
Mouse monoclonal anti-SOX2 (Clone 9-9-3), Abcam 1:200 (ab79351)
Mouse monoclonal anti-human TRA-1-60 (Clone TRA-1-60R) Alexa 488 Conjugated , STEMCELL Technologies 1:200 (60064AD)
Mouse monoclonal anti-human SSEA-4 (Clone MC-813-70) PE conjugated, STEMCELL Technologies 1:100 (60062PE)
Rabbit monoclonal recombinant anti-KLF4, (Clone EPR19590) Abcam 1:100 (ab215036)
Rabbit polyclonal anti-FOXG1, Abcam 1:400 (ab18259)
Goat monoclonal anti-Oct4, Abcam 1:100 (ab27985)
Goat polyclonal anti-SOX2, Abcam 1:100 (ab110145)
Donkey anti Chicken AlexaFluor 488-conjugated, Jackson ImmunoResearch 1:250 (703-545-155)
Donkey anti Rabbit AlexaFluor 568-conjugated, Abcam 1:250 (AB175692)
Donkey anti Mouse AlexaFluor 647-conjugated, Abcam 1:250 (AB150111)
Donkey anti Mouse AlexaFluor 568-conjugated, Abcam 1:250 (ab175472)
Donkey anti Rabbit AlexaFluor 647-conjugated, Abcam 1:250 (AB150063)
Donkey anti Mouse AlexaFluor 488-conjugated, Abcam 1:250 (AB150109)
Donkey anti Goat AlexaFluor 568-conjugated, Abcam 1:250 (AB175704)
Donkey anti Goat AlexaFluor 647-conjugated, Abcam 1:250 (AB150131)

Validation

All primary antibodies used in this study were selected based on manufacturer validation for species reactivity and application (immunocytochemistry/immunofluorescence), with reference to validation statements and supporting literature available on the suppliers' websites, and profiles in antibody databases where applicable. Dilutions were initially guided by manufacturer recommendations and published studies, then optimised on our own iPSC-derived cell lines. Specificity was confirmed by expected staining patterns consistent with known cellular localization or marker expression in our system (e.g. MAP2 in neurons, Synapsin I along neurites, OCT4/SSEA-4/TRA-1-60 in pluripotent cells). This combination of manufacturer information, published citations, and in-house optimisation served as the validation of each antibody for the species and application in this study.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Cell lines were derived from skin biopsies collected from five children with MPS IIIA (3 female, 2 male, aged 4–11 years) and five age- and sex-matched neurotypical controls (2 female, 3 male, aged 4–12 years). H9 embryonic stem cells were sourced WiCell.

Authentication

Human pluripotent stem cell lines used in this study were authenticated prior to and during this study. Cell line genotype was confirmed by patient genotyping. Pluripotency of iPSC lines was extensively characterized and confirmed by expression of pluripotency markers and tri-lineage differentiation potential. Genomic integrity was assessed by karyotype analysis to exclude major chromosomal abnormalities.

Neural progenitor cells (NPCs) and differentiated neural cultures derived from these iPSC lines were authenticated by lineage-specific marker expression using immunocytochemistry and qPCR, confirming appropriate neural identity and

	developmental stage. Cells were used at low passage numbers and maintained under standardized culture conditions to minimize genetic drift.
Mycoplasma contamination	IPSC and H9 lines tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	N/A

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.*

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	For surface marker analysis, human pluripotent stem cell (hPSC) cultures were dissociated into single cells with Accutase (5 min, 37 °C). Cells were collected, washed, and resuspended in sorting medium (mTESR Plus with 10% CloneR). Cells were stained with conjugated antibodies against SSEA-4 and TRA-1-60 for 45 min at 4 °C. DRAQ5 (1:100) was added near the end of incubation to identify nucleated cells. After centrifugation (100 × g, 5 min, 4 °C), cells were washed in PBS, filtered through a 40 µm strainer, and stained with DAPI immediately prior to analysis.
Instrument	BD LSRFortessa X-20 Cell Analyzer (BD Biosciences)
Software	All flow cytometry data was analyzed using FloJoTM v10.8.2 software
Cell population abundance	Following FACS, the abundance and purity of the target cell populations were confirmed by post-sort re-analysis. Purity was determined using the same gating strategy applied during sorting. Appropriate controls (e.g., unstained, single-stained, were used to establish gates.
Gating strategy	For hPSC surface marker analysis, preliminary gates were applied on forward scatter area (FSC-A) versus side scatter area (SSC-A) to exclude debris and select the main cell population. Doublets were removed by plotting FSC-A against FSC-H. Nucleated cells were identified using DRAQ5 staining, and viable cells were selected as DAPI-negative. SSEA-4 and TRA-1-60 positivity was defined using single-stained and unstained controls prepared in parallel, ensuring clear separation between positive and negative populations.

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