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Corresponding author(s): Rudolf Jaenisch

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Reporting Summary

Nature Research 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 Research 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	We downloaded human hg38 and mouse mm10 genome assemblies and their gene annotation files (version 97) from ftp.ensembl.org.
Data analysis	Cell Ranger (v3.0.2) was used for mapping and assigning sequenced reads to genes. Seurat v3 was used for dimentional reduction and cell clustering. Differentially expressed genes were identified by Muscat (https://github.com/HelenaLC/muscat). The trajectory analysis was done with Slingshot (v.1.4.0)

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.
The figures that have associated raw data are: Figure 1b,c,d,e; Figures 2-5 : Suppl. Figures 2-7.
Raw data for single cell RNA seq analysis can be accessed here by reviewers: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi
Record: GSE155661
Token: sbodgygovxepdir

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	For immunofluorescence analyses, sample size was chosen according to numerous previous studies assessing neuronal phenotypes in disease.
Data exclusions	For single cell RNA seq analysis, one pair of control/FXS samples was excluded, as the FXS NPC quality was poor and the had not adequately differentiated into neural cells as assessed by immunofluorescence analyses performed on a mouse brain from the same injected litter. For arborization analyses, neurons obviously cut (less than three branches visible) were excluded from the analysis. For dendritic protrusion density and morphology analyses, very poorly labeled dendrites were excluded from the analysis, when the experimenter was not able to clearly distinguish the dendritic protrusions from the background.
Replication	NA
Randomization	NA
Blinding	Confocal microscopy data acquisition was not blinded. For analysis of confocal images, images were converted to grayscale so that the experimenter could not determine if the neuron analyzed was control or FXS. Files were assigned numbers and analyzed by the experimenter or other authors.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Provided in Table S4.
Validation	All antibodies used were validated by the supplier for use to detect the human species. We tested each antibody and determined the

optimal antibody dilution for our stainings. We compared the staining observed to stainings available in the literature to validated each antibody used.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	C_1_2 and FXS_SW were obtained from Prof. Stephen Warren's group. dCas9-Tet1 and dCas9-dTet1 were obtained from Dr. Shawn Liu and are available in the Jaenisch lab.
Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	All the cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	NA

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	We used male and female Nod scid gamma mice. We injected P0-P3 pups and analyzed the animals at 1, 3 or 6 months post-injection.
Wild animals	NA
Field-collected samples	NA
Ethics oversight	Committee on Animal Care (CAC)

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