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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	For FACS data collection, we used BD FACSDiva (version 8.0.1.1). For microscopy data collection by IN Cell 6000, we used IN Cell Analyzer 6000 Acquisition Software (version 4.0). For immunoblot imaging, we used Image Studio (version 5.2). For qPCR data collection, we used Quant Studio Real Time PCR software (version 1.3)
Data analysis	For flow cytometry analysis, we used FlowJo (version 10.4). For microscopy image analysis, we used ImageJ (version 2.0.0-rc-69/1.52p) and CellProfiler (version 3.1.5). For pooled CRISPR screen analysis, we used MAGeCK-iNC (version 5.2). For CROP-seq analysis, we used Cellranger (version 3.1.0), Scanpy (version 1.4.6) and scikit-learn (version 0.23.0), and custom code, which is available at https://kampmannlab.ucsf.edu/crop-seq. For lipidomics analysis, we used Lipostar (version 1.3.0). For bulk RNA-Seq analysis, we used Salmon (version 0.14.139), tximport (version 1.8.040) and DESeq2 (version 1.20.041). For WGCNA analysis, we used the WGCNA package (version 1.69).

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

All screen datasets are publicly available on the CRISPRbrain website (http://crisprbrain.org/) (associated with Figures 1, 2, and Extended Data Figures 1,2). The accession number for the RNA sequencing datasets reported in this paper is GEO: GSE152988 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE152988),

and mapping of sgRNAs to single cells are available at https://kampmannlab.ucsf.edu/crop-seq (associated with Figures 4, 6, 7 and Extended Data Fig. 4). The DisGeNet database is available at https://www.disgenet.org/ (associated with Figure 6). There are no restrictions on data availability.

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	Sample sizes were determined by existing studies in the field to enable statistical analyses and reproducibility, specifically our previous CRISPRi screens in iPSC-derived neurons (Tian et al. 2019, Neuron 104:239-255).
Data exclusions	No data were exluded.
Replication	Major findings were validated using independent samples and orthogonal approaches. Numbers of replicates are listed in each Figure.
Randomization	Randomization was not relevant to our study since no animals or human subjects were involved, and all experiments were carried out on cell lines.
Blinding	Blinding was not relevant to our study since no subjective rating of data was involved. Instead, we used computational quantification.

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	rabbit anti-PSAP antibody (1:50 dilution; Proteintech; Cat. No. 10801-1-AP), mouse anti-LAMP2 antibody (1:100 dilution; abcam; Cat. No. ab25631), rabbit anti-GM1 antibody (1:20 dilution; abcam; Cat. No. ab23943), rabbit anti-LC3B antibody (1:200 dilution; Cell Signaling Technology; Cat. No. 2775S), chicken anti-TUJ1 antibody (1:500; AVES; Cat. No. TUJ), goat anti-rabbit IgG Alexa Fluor 555 (1:500 dilution; abcam; Cat. No. ab150078), goat anti-mouse IgG Alexa Fluor 488 (1:500 dilution; abcam; Cat. No. ab150113), goat anti-chicken IgG Alexa Fluor 647 (1:500 dilution; abcam; Cat. No. ab150171), mouse anti-β-Actin antibody (1:2000 dilution; Cell Signaling Technology; Cat. No. 3700), IRDye 680RD goat anti-mouse IgG (1:20,000 dilution; LI-COR; Cat. No. 926-68070) and IRDye 800CW goat anti-rabbit IgG(1:20,000 dilution; LI-COR; Cat. No. 926-32211), mouse anti-S100β antibody (1:500; Sigma; Cat. No. S2532), mouse anti-Nestin antibody (1:500; Sigma; Cat. No. MAB5326) and mouse anti-GPR34 antibody (1:50; R&D Systems; Cat. No. MAB4617)
Validation	rabbit anti-PSAP antibody (Proteintech; Cat. No. 10801-1-AP) Validated by us using PSAP KO cells in immunoblotting (Fig 3c), and by manufacturer (https://www.ptglab.com/products/PSAP-Antibody-10801-1-AP.htm) mouse anti-LAMP2 antibody (abcam; Cat. No. ab25631) Validated by manufacturer (https://www.abcam.com/lamp2-antibody-h4b4-lysosome-marker-ab25631.html) rabbit anti-GM1 antibody (abcam; Cat. No. ab23943) Validated by manufacturer (https://www.abcam.com/ganglioside-gm1-antibody-ab23943.html) rabbit anti-LC3B antibody (Cell Signaling Technology; Cat. No. 2775S)

Validated by manufacturer (https://www.cellsignal.com/products/primary-antibodies/lc3b-antibody/2775)

chicken anti-TUJ1 antibody (AVES; Cat. No. TUJ)
Validated by manufacturer (https://www.aveslabs.com/products/beta-tubulin-3-tuj)
mouse anti-β-Actin antibody (Cell Signaling Technology; Cat. No. 3700)
Validated by manufacturer (https://www.cellsignal.com/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700)

mouse anti-S100β antibody (Sigma; Cat. No. S2532)
Validated by manufacturer (https://www.sigmaaldrich.com/catalog/product/sigma/s2532?lang=en&region=US)

mouse anti-Nestin antibody (Sigma; Cat. No. MAB5326)
Validated by manufacturer (https://www.sigmaaldrich.com/catalog/product/mm/mab5326?lang=en&region=US)

mouse anti-GPR34 antibody (R&D Systems; Cat. No. MAB4617)
Validated by manufacturer (https://www.rndsystems.com/cn/products/human-gpr34-antibody-419859_mab4617)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The human iPSC line WTc11 was obtained from Coriell (GM25256). The human iPSC line NCRM was obtained from Dr. Michael Ward, NIH and is available from tne NINDS Human Cell and Data Depository (NDS00262). HEK293Ts were obtained from ATCC (CRL-3216).
Authentication	iPSCs were characterized for normal karyotypes and for maintaining capability to differentiate into glutamatergic neurons. We did not authenticate the HEK293T cells.
Mycoplasma contamination	All lines were tested negative for mycoplasma using the Universal Mycoplasma Detection Kit (ATCC 30-1012KTM).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

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	Cells were dissociated using Papain (for neurons), Accutase (for iPSCs) or Trypsin (for HEK293s) and analyzed or sorted by flow cytometry based on staining signals. All stains were performed according to manufacturing protocols. For Filipin staining, cells were washed with PBS three times and fixed with 3% paraformaldehyde for 1 hr at room temperature. Cells were then washed 3 times with PBS and incubated with 0.05 mg/ml Filipin III from Streptomyces filipinensis (Sigma; Cat. No. F4767-1MG) in PBS for 2 h at room temperature. Cells were washed with PBS 3 times before analysis. For staining using other live cell probes, cells were washed with PBS and incubated in DMEM containing appropriate concentrations of the probes at 37 °C as detailed below. Cells were washed with PBS before analysis. Concentrations and staining conditions for different probes were as follows: CellRox Green, 2.5 µM for 30 minutes; Lysotracker Green, 50 nM for 5 minutes; FeRhoNox-1, 5 µM for 60 minutes; Liperfluo, 5 µM for 30 minutes; C11-BODIPY, 2.5 µM for 30 minutes; FerroOrange (Dojindo Molecular Technologies, Inc.; Cat. No. F374-10), 1 µM for 30 minutes; Hoechst 33342 (Thermo Fisher Scientific; Cat. No. H3570), 1 µg/ml for 10 minutes; Propidium Iodide (PI)(Thermo Fisher Scientific; Cat. No. P1304MP), 1 µg/ml for 10 minutes.
Instrument	BD LSRFortessa X14
Software	BD FACSDiva (version 8.0.1.1) and FlowJo (version 10.4)
Cell population abundance	In the experiments to analyze staining signals, cells were analyzed and not sorted. The starting populations were pure (either iPSCs, glutamatergic neurons or HEK293s). For pooled screens, these cells were sorted into high and low signal populations corresponding to the top 40% and the bottom 40% of the staining signal distribution.
Gating strategy	Preliminary FSC/SSC gates encompassed all live, single cells in the population. For pooled screens, these cells were sorted into high and low signal populations corresponding to the top 40% and the bottom 40% of the staining signal distribution.

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