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	Detection of smFISH signals and accompanied immunofluorecence spot was done using FISHquant (PMID: 23538861)
Data analysis	https://github.com/LR-MVU/neuron RNA-seq analysis was performed on usegalaxy.org. Adaptors and reads with a quality below 20 within 4-base sliding windows were removed using Trimmomatic (galaxy version 0.38.0; https://doi.org/10.1093/bioinformatics/btu170). Trimmed single-end reads were aligned to the mouse mm10 genome using STAR (galaxy version 2.7.8a+galaxy0; https://doi.org/10.1093/bioinformatics/bts635) with default parameters, and the number of reads per transcript was determined using featureCounts (galaxy version 2.0.1+galaxy2; https://doi.org/10.1093/bioinformatics/btt656) using default parameters. Differential gene expression was determined using DESeq2 (galaxy version 2.11.40.7 +galaxy1; https://doi.org/10.1186/s13059-014-0550-8) using default parameters. Gene ontology analysis to identify biological processes enriched in differentially expressed genes was performed using geneontology.org.

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

RNA-seq data: GEO accession number: GSE202202, Reviewer token: uviluycclvsbpuz
Proteomics data: ProteomeXchange Project accession: PXD046036. Username: reviewer_pxd046036@ebi.ac.uk Password: icaFMkF9

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	Primary neurons were a mix of male and female pups because the time for genotype postnatal day 0 and embryonic E13.5 pups before preparing viable cultures decreased the viability of the cultures. The human iPSC derived into motor neurons were previously established and published as state in references 126 and 128
Reporting on race, ethnicity, or other socially relevant groupings	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

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	We did not predetermined the sample size but we count a higher number of neurons and dendrites than in previous reports using the similar single-neuron and single-molecule systems (Das et al., PMID: 37100055, Das et al PMID: 29938222, and Eliscovich et al PMID: 28223507)
Data exclusions	No data were excluded
Replication	Data were obtained from 2 (iPSC derived to motor neurons in total 6 cultures) to 5 (murine primary neurons) biological replicates.
Randomization	not applicable
Blinding	not applicable

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

GFP AVES GFP1010
FUS Proteintech 11570-I-AP
MAP2 EMD Millipore AB5622
PSD95 Antibodies Incorporated 75-028
TAU R&D Systems/Bio-Techne AF3494
GCN4 NOVUS NBP2-81273S
β-Actin Sigma A2228
Goat Anti- chicken Alexa Fluor 488 Invitrogen A32931
Goat Anti-mouse Alexa Fluor 488 Invitrogen A32723
Goat Anti-rabbit Alexa Fluor 488 Invitrogen A32731
Goat Anti-rabbit Alexa Fluor 750 Invitrogen A-21039
Goat Anti-mouse Alexa Fluor 750 Invitrogen A-21037
Goat Anti-mouse Alexa Fluor 647 Invitrogen A-21236
HSP70 R&D Systems/Bio-Techne AF1663
B-Actin R&D Systems/Bio-Techne MAB8929
STAU2 Generated in Michael Kiebler's lab
GFP R&D Systems MAB42401
HSP70 (Rb)R&D Systems/Bio-Techne AF1663
Hsc70 Proteintech 10654-1-AP
Puromycin Sigma MABE343
STAU2 generated in Michael Kiebler's lab
HNRNP Proteintech 67445-1-Ig

Validation

GCN4 and GFP antibodies validated by stained non-injected neurons. Specific signal belong to injected cells
FUS antibody validated by KD/KO
MAP2 control in human brain tissue or human glioblastoma T98G cells
PSD95 Produced by in vitro bioreactor culture of hybridoma line followed by Protein A affinity chromatography. Purified mAbs are >90% specific antibody
TAU . Tau was detected in immersion fixed paraffinembedded sections of human Alzheimer's disease brain (cortex)
STAU2 Generated and validated as indicated in https://doi.org/10.1016/j.celrep.2013.11.039
B-actin Peptide containing a sequence at the N-terminus of human beta Actin Accession # P60709
HSP70 (Rb) Only detectable upon stress. Detects the induced form of recombinant endogenous human, mouse and rat HSP70/ HSPA1A (HSP72) in Western blots. In Western blots, no cross-reactivity with the constitutively expressed HSC70 (HSP73) is detected

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

primary hippocampal neurons - both sexes
primary motor neurons - both sexes
human motor neurons differentiated from iPSC - (reported in references 126 and 128 where they were generated)
Neuro-2a - Male
293T - Female
MEFs - both sexes

Authentication

None of the cell lines used were authenticated

Mycoplasma contamination

Neuro-2a, MEF and 293T are stained with DAPI and observed under the microscope. No DAPI signal is observed in the cytoplasm over time.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines per ICLAC register were used

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	CD1, C57BL/6, and FVB mice purchsed from Charles River and keep at McGill Animal Facility following Canadian Council of Animal Health. Neurons were obtained from P0 pups from females ages between 8-36 weeks.
Wild animals	not applicable
Reporting on sex	Findings apply to male and females. Primary neuronal cultures were obtained from female and male pups and mixed in the culture.
Field-collected samples	not applicable
Ethics oversight	Housing and euthanasia were performed in compliance with the Canadian Council on Animal Care. Ethical approval was provided by McGill University

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

Plants

Seed stocks	not applicable
Novel plant genotypes	not applicable
Authentication	not applicable