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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	Immunohistochemistry results were visualized using Keyence BZ-X all-in-one fluorescent microscope. Western blots were visualized using a ChemiDoc Imager (Bio-Rad). qPCR were performed on the CFX96 real-time PCR detection system (Bio-Rad). LC-MS/MS was performed on Triple Quad 6500 System (AB SCIEX) in a positive ionization mode. High-throughput sequencing was performed on the NovaSeq platform.
Data analysis	R (version 3.6.3) or Prism (GraphPad Software, version 9.0.2) were used for statistical analysis. For high-throughput sequencing analysis, all raw reads were trimmed with Trimmomatic (version 0.39), aligned by HISAT (version 2.1.0). m6A-seq analysis used MACS2 for peak calling and MeTDiff to identify differentially methylated peaks. Bedtools (v.2.26.0) was used for merging significant peaks in biological replicates. For RNA-seq analysis, HTseq (version 0.12.4) was used for counting and DEseq2 was used to identify differential genes. Enrichment analysis was performed using HOMER (version 4.11) and DAVID (version 6.8).

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

Raw and processed data to support the findings of this study have been deposited in GEO under accession number GSE203581. Proteomic data from Answer ALS and NeuroLINC center can be requested via the websites: http://neurolincs.org/data/, and https://www.answerals.org/. The human reference genome hg38 (https://hgdownload.soe.ucsc.edu/goldenPath/hg38/chromosomes/) was used for alignment. Annotation files (version v29, 2018-08-30, in gtf format) from GENCODE database (https://www.gencodegenes.org/) were used for annotation.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	This study involved data from human lymphoblast samples, human iPSC-derived neuron samples and postmortem human brain samples. The information of biological gender regarding the human samples is listed in Supplementary Tables 1-4 and 6.
Population characteristics	See Supplementary Tables 1-4 and 6 for the demographic information of all the human cell lines, including age and diagnosis.
Recruitment	No recruitment. The study only used de-identified postmortem samples.
Ethics oversight	The study using patient samples/data was approved by the Johns Hopkins University School of Medicine Office of Human Subjects Research Institutional Review Boards.

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 statistical methods were used to pre-determine sample sizes. For analysis using patient data, we used all data available from the respective datasets. For postmortem samples, the samples were provided to us blindly based on availability. For in vitro experiments, our sample sizes are similar to those reported in previous publications (PMID:31587919; PMID: 34389711; PMID: 33789100).
Data exclusions	No data were excluded.
Replication	All experiments were repeated using at least three individual control or patient lines. All replication attempts were successful.
Randomization	We plated the cells in random positions in multi-well plates and randomly assigned them to different experiment groups. We randomly took fluorescence images under microscope.
Blinding	Researchers who performed the ELISA, imaging analysis, and some of the LC/MS/MS were blinded to the genotype and treatment. Blinding was not relevant to high throughput sequencing because libraries were pooled before loading. Other data collection and analysis were not performed blind to the condition, as no group allocation was performed.

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

Methods

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

Antibodies

Antibodies used

METTL14 (Sigma-Aldrich HPA038002, IHC 1:500, western blot 1:1000), METTL3 (Abcam ab195352, IHC 1:500, western blot 1:1000), YTHDC1 (Sigma-Aldrich HPA036462, IHC 1:500, western blot 1:1000), YTHDF2 (Abcam ab246514, IHC 1:2500, western blot 1:1000), ZCCHC8 (Proteintech 23374-1-AP, western blot 1:1000), β-actin (Cell Signaling Technology 3700, western blot 1:1000). mouse or rabbit HRP-linked secondary antibody (Cytiva NA931/NA934, 1:10,000). m6A antibody included in the EpiMark N6-Methyladenosine Enrichment Kit (New England Biolabs, E1610S) and used following the manufacturer's protocol. Rabbit IgG (Sigma Aldrich 12-370) was used as negative control for IP at the same amount as the target protein. 0.35ug/ml biotinylated rabbit anti-GP antibody was used in the ELISA experiment.

Validation

Commercial antibodies were validated by the manufacturer or the human protein atlas (See below link).
METTL14 (https://www.proteinatlas.org/ENSG00000145388-METTL14/antibody)
METTL3 (https://www.abcam.com/mettl3-antibody-epr18810-ab195352.html)
YTHDC1 (https://www.proteinatlas.org/ENSG00000083896-YTHDC1/antibody)
YTHDF2 (https://www.abcam.com/ythdf2-antibody-epr23544-19-ab246514.html)
ZCCHC8 (https://www.ptglab.com/products/ZCCHC8-Antibody-23374-1-AP.htm)
β-actin (https://www.cellsignal.com/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700)
mouse or rabbit HRP-linked secondary antibody (https://www.cytivalifesciences.com/en/us/shop/protein-analysis/blotting-and-detection/blotting-standards-and-reagents/amersham-ecl-hrp-conjugated-antibodies-p-06260#productsupport)
m6A (https://www.neb.com/products/e1610-epimark-n6-methyladenosine-enrichment-kit#Product%20Information)
Rabbit IgG (https://www.sigmaaldrich.com/US/en/product/mm/12370)
Biotinylated rabbit anti-GP antibody was used in previous publications (PMID: 31587919; PMID: 33789100)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Human C9ORF72-ALS/FTD and non-neurological control iPSC lines were obtained from the Answer ALS repository at Cedars-Sinai iPSC core.
Patient lymphoblast cells were obtained from Coriell Institute.
HeLa Flp-In (Invitrogen) dual luciferase reporter were used in the previous publication (PMID: 31587919).
HEK293T were used for lentivirus packaging (American Type Culture Collection CRL-1573)
Human i3Neuron iPSC line was obtained from Dr. Michael E Ward (PMID:31422865)
The isogentic pair of C9-ALS iPSC was obtained from Dr. Kevin Talbot (PMID:32504093)

Authentication

We have done repeat prime PCR to examine the (GGGGCC)n expansion and routine ELISA check for poly(GP) expression in the control and patient iPSC. The patient lymphoblast cells were authenticated by FISH against C9 repeat intron in a previous publication (PMID: 34389711). The expression of the luciferase reporter was authenticated by luciferase assays. All the lines have been previously published.

Mycoplasma contamination

All cell lines were routinely tested using the PlasmoTest™ - Mycoplasma Detection Kit (InvivoGen). No mycoplasma contamination was detected in the samples tested.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines were used