nature portfolio

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Reporting Summary

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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	Nikon microscope system. ImageJ 1.54f, NeuronJ 1.4.3
Data analysis	Microsoft excel, R 4.2.2, ImageJ 1.54f, AME v5.5.5, ClusterProfiler v4.6.2, Guitar v2.14.0

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 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

Source data are provided with this paper. The raw RNA-seq data generated in this study have been deposited in the Gene Expression Omnibus (GEO) under accession codes GSE277229, GSE277230, and GSE277231. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD080605. Due to the large size of the raw imaging datasets, these data are available from the corresponding authors upon request.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	Sample sizes were not pre-determined based on statistical methods but were similar to those reported in previous publications (DOI: https://doi.org/10.1038/s41586-018-0847-y, https://doi.org/10.1093/nar/gkac251, https://doi.org/10.1016/j.cell.2017.09.003, https://doi.org/10.1038/s41467-019-11123-x). Sample sizes are provided in the figure legends.
Data exclusions	Tissue samples were excluded if the sample is poorly dissected. Primary neuron samples were excluded depend on cell's condition and culture density.
Replication	All image analyses and RNA sequencing replicated at least three independent samples. Except the SACseq and stability assay which replicated with two independent samples.
Randomization	Order of *in utero* experiment, behavior test, and sample selection was randomized per mouse before the genotyping. Primary neuron samples were randomly selected for growth analysis, live imaging, smFISH, and PLA experiments.
Blinding	All tissue histochemistry, primary neuron growth analysis, live imaging, smFISH, and PLA experiments were performed and data collected by different individual researchers in blinded status.

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

Primary antibodies: L1CAM, Merck MAB5272, AB_2133200; NTNG1, R&D Systems AF1166, AB_2154822; YTHDF2, Aviva systems biology ARP67917_P050, AB_2861185; FMRP Thermo Fisher PA5-34584, AB_2551936; ACTIN, Cytoskeleton AAN01, AB_10708070; RFP, Chromotek 5f8-100, AB_2336064; Chicken GFP, Aves Labs GFP-1020, AB_10000240; TUJ1, Sigma-Aldrich T3952, AB_1841226; SATB2, Abcam ab92446, AB_10563678; TBR1, Abcam ab31940, AB_2200219; CTIP2, Abcam ab18465, AB_2064130; METTL14, ABclonal AB8530; KIF5C, ABclonal ab5630; Anti-HA Roche, 3F10; Myc-Tag Cell signaling, 9B11; Rabbit GFP Abcam, ab290; Normal rabbit IgG Merck, 12-370; Normal mouse IgG Merck, 12-371;

Secondary antibodies: anti-rabbit IgG-HRP, Santa Cruz sc-2004, AB_631746; anti-mouse IgG-HRP, Santa Cruz sc-2005, AB_631736; (Jackson ImmunoResearch) Alexa Fluor® 488 AffiniPure™ Donkey Anti-Rat IgG (H+L), 712-545-150, AB_2340683; Alexa Fluor® 488 AffiniPure™ Donkey Anti-Chicken IgG (H+L), 703-545-155, AB_2340375; Alexa Fluor® 488 AffiniPure™ Donkey Anti-Mouse IgG (H+L), 715-545-150, AB_2340846; Alexa Fluor® 488 AffiniPure™ Donkey Anti-Rabbit IgG (H+L), 711-545-152, AB_2313584; Alexa Fluor® 594 AffiniPure™ Donkey Anti-Rat IgG (H+L), 712-585-153, AB_2340689; Alexa Fluor® 594 AffiniPure™ Donkey Anti-Goat IgG (H+L), 705-585-003, AB_2340432; Alexa Fluor® 594 AffiniPure™ Donkey Anti-Rabbit IgG (H+L), 711-585-152, AB_2340621; Alexa Fluor® 647 AffiniPure™ Donkey Anti-Rat IgG (H+L), 712-605-153, AB_2340694

Validation

Antibodies used in this study were purchased from commercially available sources and were validated by the manufacturer.

L1CAM: https://www.sigmaaldrich.com/KR/ko/product/mm/mab5272

NTNG1: https://www.rndsystems.com/products/mouse-netrin-g1a-antibody_af1166

YTHDF2: https://www.avivasysbio.com/ythdf2-antibody-c-terminal-region-arp67917-p050.html

FMRP: https://www.thermofisher.com/antibody/product/FMRP-Antibody-Polyclonal/PA5-34584

ACTIN: https://www.cytoskeleton.com/antibodies/actin/aan01

RFP: https://www.ptglab.com/products/RFP-antibody-5F8.htm

Chicken GFP: https://www.antibodiesinc.com/products/anti-green-fluorescent-protein-antibody-gfp

TUJ1: https://www.sigmaaldrich.com/KR/ko/product/sigma/t3952

SATB2: https://www.abcam.com/en-us/products/primary-antibodies/satb2-antibody-epncir130a-ab92446

TBR1: https://www.abcam.com/en-us/products/primary-antibodies/tbr1-antibody-ab31940

CTIP2: https://www.abcam.com/en-us/products/primary-antibodies/ctip2-antibody-25b6-ab18465

METTL14: https://www.abclonal.com/catalog-antibodies/KDValidatedMETTL14RabbitmAb/A24396

KIF5C: https://www.abcam.com/en-us/products/primary-antibodies/kif5c-antibody-ab5630

Anti-HA: https://www.sigmaaldrich.com/KR/ko/product/roche/roahaha

Myc-Tag: https://www.cellsignal.com/products/primary-antibodies/myc-tag-9b11-mouse-monoclonal-antibody/2276

Validation	Rabbit GFP: https://www.abcam.com/en-us/products/primary-antibodies/gfp-antibody-ab290 Normal IgG rb: https://www.sigmaaldrich.com/KR/ko/product/mm/12370 Normal IgG ms: https://www.sigmaaldrich.com/KR/ko/product/mm/12371

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research Cell line

source(s)	N2A cell line: Mouse neuroblasts Neuro-2a cell line. HEK 293 cell line: Human embryonic kidney cell line.
Authentication	NA
Mycoplasma contamination	Mycoplasma contamination was routinely tested on every cell line throughout the study. We used Mycoplasma contamination test service from CosmoGenetech Inc.
Commonly misidentified lines (See ICLAC register)	We did not use any ICLAC lines in this study.

Animals and other research organisms

Laboratory animals	This study used embryonic mouse, early postnatal mouse (P0-P14), and pregnant mouse. Strains: C57BL/6J. Mettl14 allele. (B6.Cg-Tg(Nes-cre)1Kln/J; Jackson Laboratory stock: 003771) Ythdf2 allele were received as a gift from Dr. Chuan He and Dr. Xiaoxi Zhuang at the university of Chicago. Fmr1 allele (B6.129P2-Fmr1tm1Cgr/J; Jackson Laboratory stock:003025) were purchased from Jackson Laboratory. For in utero and in vitro electroporation studies, timed pregnant C57BL/6J (wild type) mice were commercially obtained from KRIBB (Taconic), South Korea. Mice were housed in standard conditions on a 12-hour light/dark cycle in a temperature- and humidity-controlled barrier facility and allowed ad libitum access to standard chow and water.
Wild animals	This study did not used wild animals.
Reporting on sex	This study used both sexes in every experiment, except for Supplementary Figure 6i; weight difference between DF2 WT, het-cre, and cKO mouse at P14
Field-collected samples	This study did not used field collected samples.
Ethics oversight	All procedures were in accordance with the guidelines of and approved by the Institutional Animal Care and Use Committee at KAIST