

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

Data analysis

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD035196. [<https://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PX035196>]
Previously published HITS-CLIP dataset for Ptbp2 are available at Gene Expression Omnibus (GEO) under accession code GSE47564 [<https://www.ncbi.nlm.nih.gov/>]

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	<input type="text" value="N/A"/>
Population characteristics	<input type="text" value="N/A"/>
Recruitment	<input type="text" value="N/A"/>
Ethics oversight	<input type="text" value="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](https://www.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	<input type="text" value="Most experiments were carried out independently at least in triplicates. No calculations were done to predetermine sample size. Sample size was chosen based on author's experiences and established standards for cell lines and primary neurons and tissues from animal models."/>
Data exclusions	<input type="text" value="No data were excluded from the analysis."/>
Replication	<input type="text" value="To ensure reproducibility, all experiments except sucrose gradient fractionation were performed at least in triplicates using similar conditions and reagents. For sucrose gradient fractionation of motoneurons, two replicates (control and knockdown condition) and single replicate (EDTA treatment) were done due to the large number of cells required for the procedure. Replication was successful in all experiments."/>
Randomization	<input type="text" value="All experiments were carried out with primary neurons and tissues from mice with defined genetic backgrounds, and NSC-34 cells. For all experiments, samples were allocated randomly, and control and treatment conditions were derived from the same batch of cells."/>
Blinding	<input type="text" value="Samples for different experimental conditions were processed simultaneously such that blinding was not necessary."/>

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	Included in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Included in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	<input type="text" value="Primary antibodies:"/>
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Antibodies used

1. Rabbit Polyclonal anti-Ptbp2, Proteintech, Cat# 55186-1-AP, PRID: AB_10837230, For IP (1µg), IF (1:250), WB (1:2000).
2. Mouse monoclonal anti-Ptbp1, Invitrogen, Cat# 32-4800, PRID: AB_2533082, For WB (1:2000).
3. Rabbit Polyclonal anti-hnRNP R, Sigma-Aldrich, Cat# HPA026092, RRID: AB_1850885, IF (1:200).
4. Rabbit Polyclonal anti-hnRNP R, Abcam, Cat# ab30930, PRID: AB_2295539, WB (1:2000), IF (1:100).
5. Rabbit Polyclonal anti-Histone-H3, Abcam, Cat# ab1791, PRID: AB_302613, WB (1:10000).
6. Rabbit Polyclonal anti-EIF5A1/EIF5A2, Proteintech, Cat# 17069-1-AP, RRID: AB_2262009, IP (3µg).
7. Mouse Polyclonal anti-EIF5A1/EIF5A2, BD Biosciences, Cat# 611976, RRID: AB_399397, WB (1:5000), IF (1:100).
8. Goat Polyclonal anti-Calnexin, Sicgen, Cat# AB0037, RRID: AB_2333118, WB (1:10000).
9. Chicken polyclonal anti-GFP, Abcam, Cat# ab13970, RRID: AB_300798, IF (1:500), WB (1:1000).
10. Rabbit monoclonal anti-Eif2α, Cell Signaling Technology, Cat# 5324, RRID: AB_10692650, WB (1:5000).
11. Mouse monoclonal anti-α-Tubulin, Sigma-Aldrich, Cat# T5168, RRID: AB_477579, IF (1:500).
12. Mouse monoclonal anti-GAPDH, EMD Millipore, Cat# CB1001, RRID: AB_2107426, WB (1:4000).
13. Mouse monoclonal anti-Puromycin, Sigma-Aldrich, Cat# MABE343, RRID: AB_2566826, IF (1:200).
14. Mouse monoclonal anti-p75NTR, Biosensis, Cat# M-009-100, RRID: AB_2492396.
15. Rabbit polyclonal anti-Tau, Sigma-Aldrich, Cat# T6402, RRID: AB_261728, IF (1:500).
16. Mouse monoclonal anti-Synapsin1, Synaptic Systems, Cat# 106 011, RRID: AB_2271476, IF (1:500).
17. Guinea pig polyclonal anti-Piccolo, Synaptic Systems, Cat# 142104, RRID: AB_2619831, IF (1:500).
18. Mouse monoclonal anti-Ribosomal Protein S5, Santa Cruz Biotechnology, Cat# sc-390935, RRID: AB_2713966, WB (1:1000).
19. Mouse monoclonal anti-Ribosomal RNA (Y10b), Santa Cruz Biotechnology, Cat# sc-33678, RRID: AB_628226, IP (1µg).
20. Goat Polyclonal anti-Choline Acetyltransferase (ChAT), EMD Millipore, Cat# AB144P, RRID: AB_90661, IF (1:500).

Secondary antibodies:

1. Monoclonal Mouse Anti-Rabbit (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 211-032-171, RRID: AB_2339149, For WB (1: 10000).
2. Donkey polyclonal anti-Rabbit (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 711-035-152, RRID: AB_10015282, For WB (1: 10000).
3. Donkey polyclonal anti-Goat (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 705-035-003, RRID: AB_2340390, For WB (1: 10000).
4. Donkey polyclonal anti-Chicken (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 703-035-155, RRID: AB_10015283, For WB (1: 10000).
5. Goat anti-mouse IgG (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 115-035-174, RRID: AB_2338512, For WB (1: 10000).
6. Donkey polyclonal anti-Chicken (Alexa Fluor® 488 Conjugated), Jackson ImmunoResearch, Cat# 703-545-155, RRID: AB_2340375, For IF (1:500).
7. Donkey polyclonal anti-Rabbit (Alexa Fluor® 647 Conjugated), Thermo Fisher Scientific, Cat# A31573, RRID: AB_2536183, For IF (1:500).
8. Donkey polyclonal anti-Mouse (DyLight 488 Conjugated), Thermo Fisher Scientific, Cat# SA5-10166, RRID: AB_2556746, For IF (1:500).
9. Donkey polyclonal anti-Mouse (CyTM3 Conjugated), Jackson ImmunoResearch, Cat# 715-165-150, RRID: AB_2340813, For IF (1:500).
10. Donkey polyclonal anti-Guinea Pig (Cy5), Jackson ImmunoResearch, Cat# 706-175-148, RRID: AB_2340462, IF (1:500).
11. Donkey polyclonal anti-Rabbit (Cy3), Jackson ImmunoResearch, Cat# 711-166-152, RRID: AB_2307443, IF (1:500).

Validation

Primary antibodies:

1. Rabbit Polyclonal anti-Ptbp2, Proteintech, Cat# 55186-1-AP, PRID: AB_10837230, For IP (1µg), IF (1:250), WB (1:2000). Validation data provided by the supplier (<https://www.ptglab.com/products/PTBP2-Antibody-55186-1-AP.htm>), validated in our lab by shRNA knock down.
2. Mouse monoclonal anti-Ptbp1, Invitrogen, Cat# 32-4800, PRID: AB_2533082, For WB (1:2000). Validation data provided by the supplier (<https://www.thermofisher.com/antibody/product/PTBP1-Antibody-clone-1-Monoclonal/32-4800>), validated in our lab by shRNA knock down.
3. Rabbit Polyclonal anti-hnRNP R, Sigma-Aldrich, Cat# HPA026092, RRID: AB_1850885, IF (1:200). (<https://www.sigmaaldrich.com/DE/de/product/sigma/hpa026092>), validated in our lab by shRNA knock down and knock out.
4. Rabbit Polyclonal anti-hnRNP R, Abcam, Cat# ab30930, PRID: AB_2295539, WB (1:2000), IF (1:100). Validation data provided by the supplier (<https://www.abcam.com/hnRNP-R-antibody-ab30930.html>), validated in our lab by shRNA knock down and knock out.
5. Rabbit Polyclonal anti-Histone-H3, Abcam, Cat# ab1791, PRID: AB_302613, WB (1:10000). Validation data provided by the supplier (<https://www.abcam.com/histone-h3-antibody-nuclear-marker-and-chip-grade-ab1791.html>)
6. Rabbit Polyclonal anti-EIF5A1/EIF5A2, Proteintech, Cat# 17069-1-AP, RRID: AB_2262009, IP (3µg). Validation data provided by the supplier (<https://www.ptglab.com/Products/EIF5A2-Antibody-17069-1-AP.htm>), validated in our lab by shRNA knock down.
7. Mouse Polyclonal anti-EIF5A1/EIF5A2, BD Biosciences, Cat# 611976, RRID: AB_399397, WB (1:5000), IF (1:100). Validation data provided by the supplier (<https://www.bdbiosciences.com/en-de/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-eif-5a.611976>), validated in our lab by shRNA knock down.
8. Goat Polyclonal anti-Calnexin, Sicgen, Cat# AB0037, RRID: AB_2333118, WB (1:10000). Validation data provided by the supplier (<https://www.citeab.com/antibodies/1620098-ab0037-200-anti-canx>).
9. Chicken polyclonal anti-GFP, Abcam, Cat# ab13970, RRID: AB_300798, IF (1:500), WB (1:1000). Validation data provided by the supplier (<https://www.abcam.com/GFP-antibody-ab13970.html>).
10. Rabbit monoclonal anti-Eif2α, Cell Signaling Technology, Cat# 5324, RRID: AB_10692650, WB (1:5000). Validation data provided by the supplier (<https://www.cellsignal.com/products/primary-antibodies/eif2a-d7d3-xp-rabbit-mab/5324>).
11. Mouse monoclonal anti-α-Tubulin, Sigma-Aldrich, Cat# T5168, RRID: AB_477579, IF (1:500). Validation data provided by the supplier (<https://www.sigmaaldrich.com/DE/de/product/sigma/t5168>).
12. Mouse monoclonal anti-GAPDH, EMD Millipore, Cat# CB1001, RRID: AB_2107426, WB (1:4000). Validation data provided by the supplier (https://www.merckmillipore.com/DE/de/product/Anti-GAPDH-Mouse-mAb-6C5,EMD_BIO-CB1001).
13. Mouse monoclonal anti-Puromycin, Sigma-Aldrich, Cat# MABE343, RRID: AB_2566826, IF (1:200). Validation data provided by the supplier (<https://www.sigmaaldrich.com/DE/de/product/mm/mabe343>).
14. Mouse monoclonal anti-p75NTR, Biosensis, Cat# M-009-100, RRID: AB_2492396. Validated in our lab (Wiese et al. 2009 Nature Protocols).

15. Rabbit polyclonal anti-Tau, Sigma-Aldrich, Cat# T6402, RRID: AB_261728, IF (1:500). Validation data provided by the supplier (<https://www.sigmaaldrich.com/DE/de/product/sigma/t6402>).
16. Mouse monoclonal anti-Synapsin1, Synaptic Systems, Cat# 106 011, RRID: AB_2271476, IF (1:500). Validation data provided by the supplier (<https://sysy.com/product/106011>).
17. Guinea pig polyclonal anti-Piccolo, Synaptic Systems, Cat# 142104, RRID: AB_2619831, IF (1:500). Validation data provided by the supplier (<https://sysy.com/product/142104>).
18. Mouse monoclonal anti-Ribosomal Protein S5, Santa Cruz Biotechnology, Cat# sc-390935, RRID: AB_2713966, WB (1:1000). Validation data provided by the supplier (<https://www.scbt.com/de/p/ribosomal-protein-s5-antibody-a-8>).
19. Mouse monoclonal anti-Ribosomal RNA (Y10b), Santa Cruz Biotechnology, Cat# sc-33678, RRID: AB_628226, IP (1µg). Validation data provided by the supplier (<https://www.scbt.com/p/rrna-antibody-y10b/>).
20. Goat Polyclonal anti-Choline Acetyltransferase (ChAT), EMD Millipore, Cat# AB144P, RRID: AB_90661, IF (1:500). Validation data provided by the supplier (https://www.merckmillipore.com/DE/de/product/Anti-Choline-Acetyltransferase-Antibody,MM_NF-AB144P?ReferrerURL=https%3A%2F%2Fwww.bing.com%2F&bd=1).

Secondary antibodies:

1. Monoclonal Mouse Anti-Rabbit (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 211-032-171, RRID: AB_2339149, For WB (1: 10000). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
2. Donkey polyclonal anti-Rabbit (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 711-035-152, RRID: AB_10015282, For WB (1: 10000). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
3. Donkey polyclonal anti-Goat (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 705-035-003, RRID: AB_2340390, For WB (1: 10000). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
4. Donkey polyclonal anti-Chicken (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 703-035-155, AB_10015283, For WB (1: 10000). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
5. Goat anti-mouse IgG (Peroxidase Conjugated), Jackson ImmunoResearch, Cat# 115-035-174, RRID: AB_2338512, For WB (1: 10000). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
6. Donkey polyclonal anti-Chicken (Alexa Fluor® 488 Conjugated), Jackson ImmunoResearch, Cat# 703-545-155, RRID: AB_2340375, For IF (1:500). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
7. Donkey polyclonal anti-Rabbit (Alexa Fluor® 647 Conjugated), Thermo Fisher Scientific, Cat# A31573, RRID: AB_2536183, For IF (1:500). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
8. Donkey polyclonal anti-Mouse (DyLight 488 Conjugated), Thermo Fisher Scientific, Cat# SA5-10166, RRID: AB_2556746, For IF (1:500). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
9. Donkey polyclonal anti-Mouse (Cy3 Conjugated), Jackson ImmunoResearch, Cat# 715-165-150, RRID: AB_2340813, For IF (1:500). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
10. Donkey polyclonal anti-Guinea Pig (Cy5), Jackson ImmunoResearch, Cat# 706-175-148, RRID: AB_2340462, IF (1:500). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.
11. Donkey polyclonal anti-Rabbit (Cy3), Jackson ImmunoResearch, Cat# 711-166-152, RRID: AB_2307443, IF (1:500). Validated in our lab by omission of primary antibody, absence of target antigen and used under similar condition for other project.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	NSC-34 cells (Cedarlane, cat. no. CLU140) and HEK293TN cells (System Biosciences, cat. no. LV900A-1)
Authentication	NSC-34 and HEK293TN cells were obtained commercially from Cedarlane and System Biosciences, respectively, and were not authenticated.
Mycoplasma contamination	NSC-34 and HEK293TN cells tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

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-mice and Hnrnp1+/+ and -/- mice of both genders were housed in the animal facility of the Institute of Clinical Neurobiology at the University Hospital of Wuerzburg. Mice were maintained on a 12 h/12 h day/night cycle under controlled conditions at 20-22°C and 55-65% humidity with food and water in abundant supply. Breeding animals were between 6 and 20 weeks of age. Pregnancy in female mice was detected by daily plug control, and mouse embryos were isolated at E13 for generation of culture of primary motoneurons. Tissues were obtained from embryonic and postnatal mice at defined ages as indicated in the manuscript.
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Wild animals	The study did not involve wild animals.
Reporting on sex	Sex was not considered in the study design and information on sex was not collected because embryonic mice were used for motoneuron cultures.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experiments were performed strictly according to the regulations on animal protection of the German federal law and the Association for Assessment and Accreditation of Laboratory Animal Care, in agreement with and under the control of the local veterinary authority.

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