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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	No software was used.
Data analysis	Standard software for behavior and histologic analysis: Prism version 10; imaging analyzed using LAS X software and quantified using ImageJ software, except for iPSC imaging quantification which was done using FIJI 2. Standard software for transcriptomic analysis: R version 3.6.1 and R studio for visualization. R packages include : DESeq2 (version 1.20.1); clusterProfiler (version 4.1.4); EnhancedVolcano (version 1.9.11); Tidyverse (version 1.3.1); Dplyr (version 2.1.1);ggplot2 (version 3.3.5); pheatmap (version 1.0.12); RColorBrewer (version 1.1.2). All code for data pre-processing and analysis is available on GitHub in the Davidson Lab repository: https://github.com/DavidsonLabCHOP/Amado_2025_NatComm. DOI: 10.5281/zenodo.15427428.

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

In addition to the data available in the main text and supplementary materials, source data are provided with this paper. Additionally, the raw and processed next-generation sequencing data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database under accession code GSE283631 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE283631).

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	A human male C9orf72-ALS patient iPSC line (CS7VCZiALS-nxx) available through the Answer-ALS Project was obtained via the Cedars-Sinai iPSC repository (https://biomanufacturing.cedars-sinai.org). The cell line is reported to contain >800 repeats in the C9orf72 gene; presence of a large repeat expansion was confirmed using the AmplideX-C9 assay. A dox-inducible transcription factor cassette for lower motor neuron differentiation was inserted by Dr. Esteban Mazzoni at NYU. Karyotyping and CNV analysis were conducted prior to initiation of experiments and cells tested negative for mycoplasma. This information is also in the manuscript.
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 for these studies were determined based on predicted effect size and resultant power calculations from a previously published study using the same model and target transcript (Becker et al. Nature 2017). Treatment endpoints and a detailed analysis plan for each study were determined prior to initiation of the overall study. Samples or mice were added to the study if technical errors (e.g., tissue damage during embedding) prevented inclusion of a sample. In some studies, if the magnitude of effect exceeded what was predicted (e.g., in cortical inflammation studies) and statistical significance was achieved early, collection was stopped at that point.
Data exclusions	Histology data were excluded only in circumstances where outliers were identified by Tukey's fences (1.5 IQR, ~0.7%); behavior data were never excluded.
Replication	All dots on graphs in our mouse studies represent individual mice (i.e. biological replicates), with the exception of upper motor neuron counts for which dots represent tissue sections from a total of at least 3 mice per treatment group and which were analyzed with general linear mixed modeling following the approach outlined by Becker et al. 2017. All attempts at replication were successful with the rare exception of histologic outliers as described above.
Randomization	Mice were randomized by litter to receive PM-AAV9.miAtxn2, PM-AAV9.miCtrl, or virus buffer. Treatments were prepared by an independent investigator not involved in the execution of the studies, and the investigator performing the injections was blinded as to treatment. For bulk RNA-sequencing samples were randomized and blinded for library preparation. Samples were unblinded for data analysis but were processed identically.
Blinding	Treatments were prepared by an independent investigator not involved in the execution of the studies. Injections, all experiments, and all analyses were conducted in a blinded fashion, including the investigators performing the injections or experiments, the investigators analyzing them, and all animal caretakers. Unblinding occurred at the end of the study. RNA-sequencing samples were blinded for library preparation

and unblinded for data analysis but were processed identically.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies used for tissue immunostaining included the following: anti-Atxn2 rabbit polyclonal antibody (1:500; 21776-1-AP; Proteintech); anti-TDP-43 phospho-Ser409/410 rabbit polyclonal antibodies (1:200; 22309-1-AP; Proteintech | 1:2,000; 80007-1-RR; Proteintech); anti-ChAT goat polyclonal antibody (1:400; AB144P; Millipore, Burlington, MA); anti-NeuN rabbit monoclonal antibody (1:1,000; EPR 12763; Abcam); anti-Gfap rabbit polyclonal antibody (1:2,000 [brain] or 1:4,000 [spinal cord]; Z0334; Dako/Agilent, Santa Clara, CA); anti-Iba1 rabbit polyclonal antibody (1:2,000 [brain] or 1:4,000 [spinal cord]; 019-19741; Wako, Richmond, VA); and anti-Cleaved Caspase-3 polyclonal antibody (1:400; 9661; Cell Signaling, Danvers, MA). All primary antibodies were diluted in 2% serum (of the secondary antibody species) in 0.1% Triton-X100/PBS. Secondary antibodies used for immunofluorescence included Donkey anti-rabbit AlexaFluor 488 (A-21206), Donkey anti-mouse Alexafluor 488 (A-21202), and Donkey anti-goat AlexaFluor 568 (A-11057) polyclonal IgG antibodies at a concentration of 1:1,000 (ThermoScientific, Waltham, MA). Secondary antibodies used for immunohistochemistry included biotinylated goat anti-rabbit (BA-1000) and biotinylated horse anti-goat (BA-9500) at a concentration of 1:200 (Vector laboratories, Burlingame, CA).

Validation

Antibodies against Atxn2 and TDP-43 phospho-Ser409/410 were validated by Western blot. Anti-ChAT, NeuN, Gfap, Iba1 and CC3 antibodies were selected based on extensive prior use in the Davidson and Amado laboratories as well as on the basis of extensive citation in the literature (anti-ChAT, >2000 citations, https://www.citeab.com/antibodies/222823-ab144p-anti-choline-acetyltransferase-antibody; anti-NeuN: 868 publications, https://www.abcam.com/en-us/products/primary-antibodies/neun-antibody-epr12763-neuronal-marker-ab177487?srsltid=AfmBOoprHoue4KDd6U2jZ2L7NKCVIjDAHKhBys2XTX0yN819iTUc6elr#; anti-Gfap, 410 citations, https://www.labome.com/product/Dako/Z0334.html; anti-Iba1, 491 citations, https://www.labome.com/product/Wako-Chemicals-USA/019-19741.html; anti-CC3, 686 citations, https://www.labome.com/product/Cell-Signaling-Technology/9661.html).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The patient-derived iPSC line used in our study was from a male patient harboring a repeat expansion in the C9orf72 gene and the line was originally obtained from Answer-ALS and modified by Dr. Esteban Mazzoni as described in the manuscript to differentiate into lower motor neurons after induction with doxycycline. N2A cells, which are a mouse neuroblast cell line of unknown sex, were used in the screening of miRNAs early in the study.

Authentication

The patient-derived iPSC line underwent karyotyping and CNV analysis in our Stem Cell Core, and genotyped to confirm presence of the repeat expansion using an AmplideX-C9 assay.

Mycoplasma contamination

The C9orf72-iPSC line used was tested (and was negative) for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this 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

TDP-43 transgenic mice generated by Samir Kumar-Singh (TAR4 strain) were purchased from JAX (strain 012836, B6;SJL-Tg(Thy1-TARDBP)4Singh/J). Mice were maintained on a B6/SJL background by crossing hemizygous mice with F1 hybrid mice from JAX (strain 100012, B6SJLF1/J). Hemizygous mice were crossed with one another to generate the TAR4/4, TAR4, and WT offspring used in our studies. Regarding age, survival analyses allowed animals to reach humane endpoint, which occurred in untreated TAR4/4 mice at an

	average of 22 days of age. Histologic studies were conducted at 21-23 days of age for TAR4/4 mice and wildtype littermates, and transcriptomic studies at 19 days of age. Longitudinal studies in hemizygous TAR4 mice and wildtype littermates were conducted at various timepoints through 21 months of age.
Wild animals	The study did not involve wild animals.
Reporting on sex	Male and female mice were used and data pooled for all studies based on published reports of a lack of sex-based phenotypic differences in this model (Wils et al. 2010, Becker et al. 2017). For the longitudinal studies in TAR4 hemizygous mice, weight analyses were conducted by sex without meaningful differences found, so were reported in the manuscript with both sexes pooled. Sex was recorded for all studies and this information is available if needed.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal protocols were approved by the Children's Hospital of Philadelphia Animal Care and Use Committee.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A