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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 for data collection
Data analysis	Custom code for RNA-seq and manual patch clamp analysis is deposited at GitHub: https://github.com/recursivesplicing-beep/KCNQ2_2025. The splicing algorithm used in this study is MAJIQ v2.0 (Modeling Alternative Junction Inclusion Quantification) which is available at https://majiq.biociphers.org. Differential gene expression analysis were performed using the DESeq2 package available on Bioconductor. Imaging analysis was done using Nikon Elements (Nikon NIS Elements Software, version 5.41.00) and ImageJ software (ImageJ, version 1.53t) . Patch clamp electrophysiological measurements at UCL were analyzed using Clampfit 10.6 software and custom MATLAB scripts and at NU using Pclamp/Clampfit (Molecular Devices, RRID: SCR_011322) and Easy Electrophysiology software (Easy Electrophysiology Ltd, RRID:SCR_021190). MEA data analysis was done using Axion Integrated Studios (AxIS) software.

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

All RNA sequencing datasets used in this study were previously published and described in the methods section. Briefly, FACS-purified MNs depleted of TDP-43 studies were reported by the Eggan lab: GSE121569, purified TDP-43-high and -low neuronal nuclei datasets were reported by the Lee lab: GSE126543, i3Neurons, SH-SY5Y and SK-N-DZa TDP-43 depletion studies were recently reported by the Fratta and Ward labs: PRJEB42763, mouse TDP-43 depletion studies were reported by the Cleveland group: GSE27394, and TDP-43 iCLIP from SH-SY5Y cells were reported by the Fratta and Ward groups and downloaded from E-MTAB-10297. We obtained access to 1124 ALS/control RNA-seq datasets from the New York Genome Center (NYGC) ALS/ Target ALS Consortium. While these are not publicly available, access to genomics datasets may be sought at https://www.targetals.org/resource/genomic-datasets/. GRCh38.p13 human genome and gene annotation files are available at https://www.gencodegenes.org/human/. Raw experimental data generated here is provided in supplementary tables and source files. Custom code for RNA-seq and manual patch clamp analysis is deposited at GitHub: https://github.com/recursivesplicing-beep/KCNQ2_2025. All other data are available 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	We used commercially available cell lines, including the ESC line W1 (male) and iPSC lines 18a (female) KOLF2.1J (male) and WTC11 (male) . All relevant information on these lines is included in the manuscript. We used predominantly male iPSC lines to avoid random X chromosome inactivation relaxation that occurs in vitro. Details on postmortem samples from ALS/FTD patients are included in Supplementary Table 5
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	Collection of postmortem tissue was performed by VA Biorepository Brain Bank (VABBB, VA Merit BX002466) and Department of Neuropathology, Amsterdam UMC, University of Amsterdam. All procedures involving human participants, including the use of post-mortem tissue samples, were performed by the ethical standards of the institutional and national research committees and the 1964 Helsinki Declaration and its later amendments

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. Our sample sizes were determined based on sample availability, prior experimental experience and are similar to those reported in previous publications.
Data exclusions	No data were excluded from the analyses except for Fig2J-K where 1/7 RNA-Seq datasets was excluded, as it lacked sufficient read coverage for KCNQ2 and cells that did not meet recording criteria for manual patch clamp recordings (see specific methods sections).
Replication	Experiments were replicated n=3 independent times. Key findings from patch clamp recordings and IHC experiments were additionally replicated independently at distinct sites at NU and UCL and Columbia -- see methods and results for details.
Randomization	Samples were not randomized. Randomization was not relevant for experiments described in this study.
Blinding	The Investigators were blinded to allocation during experiments and outcome assessment for most experiments including IHC analysis presented in Fig 7 and Extended Data Fig 8.

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

rabbit polyclonal anti-KCNQ2 (Thermo Fisher: Cat# PA1-929, 1:250)
mouse monoclonal anti-TDP-43 (Proteintech: Cat# 60019-2-Ig, 1:500)
mouse anti-TDP-43 (abcam ab104223)
mouse monoclonal anti-Ubiquitin (Santa Cruz: Cat# sc-8017, 1:100)
chicken polyclonal anti-MAP2 (Abcam: Cat# ab5392, 1:5000)
anti-GFP (Abcam ab6673; 1:10,000)
anti-Calnexin (SantaCruz 46669)
anti-ANK-G (Neuromab 73-146; 1: 200)
anti-pTDP-43 (Cosmo bio, TIPPTD-M01, 1:1000)
anti-Ubiquitin (Millipore, MAB1510-I-100UG, clone Ubi-1, 1:150)
anti-GRP78 (BD Biosciences Cat #:610978, 1:150)
rabbit anti-mCherry (Invitrogen, Cat# PA5-34974, 1:1000)

Secondaries:
anti-rabbit, Alexa Fluor 488 (Invitrogen: Cat# A21206, 1:250)
donkey polyclonal anti-mouse, Alexa Fluor 568 (Invitrogen: Cat# A10037, 1:250)
donkey polyclonal anti-chicken, Alexa Fluor 647 (Jackson Immuno: Cat# 703-606-155, 1:250)

Validation

Commercial antibodies were validated per manufacturer's website. We validated KCNQ2 antibodies using the KCNQ2 engineered cell lines as well as GFP/RFP-tagged transgenes. All other antibodies were assessed for expected sub-cellular localization (TDP-43, MAP2, Calnexin, ANKG, GRP78, Ubiquitin) or validated using reporters (GFP, mCherry).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293-FT (RRID:CVCL_6911)
SH-SY5Y cells (CRL-2266™, ATCC)
WA01 (H1) (RRID:CVCL_9771) is a human embryonic stem cell line derived at the University of Wisconsin Madison and obtained from WiCell. It was engineered in the Eggan lab at Harvard University to derive KCNQ2ΔE5 cells.
CHO-Kv7.3 cell line was generated in the George lab at NU from CHO cells (CRL 9618)
The Halo-tagged TDP-43 iPSC line was generated in the Fratta lab from a WTC11 background (RRID:CVCL_Y803)
iPSC line 18a was generated in the Eggan lab at Harvard university (RRID:CVCL_8993) and KOLF2.1J at Jackson Labs (RRID:CVCL_B5P3)
Primary culture (CD-1 IGS) mouse glia were generated in the Kiskinis Lab from mice purchased from Charles River.

Authentication

The KCNQ2 engineered cell line was characterized by PCR amplification followed by Sanger sequencing, allele-specific quantitative PCR and karyotyping.
The Halo-TDP43 iPSC line was authenticated by genotyping and confirmation of selective TDP-43 depletion upon PROTAC treatment. CHO-Kv7.3 cell line were authenticated by functional analysis and genotyping. 18a, KOLF2.1J and HEK cells were not authenticated.

Mycoplasma contamination

We assessed cultures within the lab for mycoplasma contamination using the MycoAlert kit (Lonza). All cell lines used in this study were tested and none tested positive.

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	Primary culture (CD-1 IGS) mouse glia were derived from P0-2 pups from pregnant mice purchased from Charles River
Wild animals	NA
Reporting on sex	Sex of glial was mixed.
Field-collected samples	NA
Ethics oversight	The Institutional Animal Care and Use Committee (IACUC) at Northwestern approved the protocol for generating primary mice glia

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA