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Supplemental information

Mechanism-free repurposing of drugs for *C9orf72*-related ALS/FTD using large-scale genomic data

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SUPPLEMENTARY METHODS

Replication cohort

The DNA samples for the replication cohort were obtained from a different study (principal investigator: Christopher Shaw, King's College London). These samples were collected at (A) King's College London and (B) Project MinE Sequencing Consortium (Utrecht University) as described below:

(A) The King's College London

The DNA samples of 464 novel *C9orf72* repeat expansion carriers were collected at King's College London. The participants were recruited through the SLAGEN Consortium, Boğaziçi University, and Scotland University. Details of the cohorts are as follows:

(1) **Italy (SLAGEN Consortium).** DNA samples of the novel Italian carriers were collected by the SLAGEN Consortium through the contribution of several Tertiary Centers and Clinical Laboratories across Italy. Patients were diagnosed with ALS according to the El Escorial revised criteria at the ALS tertiary referral center of Istituto Auxologico Italiano IRCCS [S1]. All patients had probable or definite familial ALS according to the Byrne criteria for FALS. Cognitive assessment in the care of ALS patients adopted standard neuropsychological assessment suitable for patients with verbal and motor impairment, such as the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) [S2]. All individuals gave written informed consent, and the Ethics Committee of the Istituto Auxologico Italiano IRCCS, Milan, approved this protocol. Screening for the expanded repeats in the *C9orf72* gene was performed by a two-step protocol, including genotyping PCR followed by a repeat-primed PCR, as previously described [S3, S4].

(2) **Turkey (Boğaziçi University).** DNA samples of the Turkish carriers were collected at the Boğaziçi University and recruited across Turkey between 2002 and 2019. All individuals gave written informed consent, and the Ethics Committee on Research with Human Participants (INAREK) and Boğaziçi University, Istanbul, approved this protocol. Genomic DNA was isolated from whole blood using the MagNa Pure Compact System (Roche, Switzerland). The *C9orf72* GGGGCC repeat expansion was screened by Repeat-primed touchdown PCR using FastStart Universal Master Mix (Roche, Switzerland). FAM-labeled PCR products were subjected to fragment length analysis (Macrogen, Korea), and a saw-tooth pattern in expansion-positive cases was visualized in PeakScanner Software (ThermoFisher Scientific, USA) [S5, S6].

(3) **Scotland (Edinburgh University).** DNA samples were obtained from patients with ALS who donated blood for research to the Scottish Regenerative Neurology Tissue Bank as part of the Scottish Motor Neurone Disease (MND) Register. ALS patients were diagnosed following the 'El Escorial' criteria [S7]. Ethical approval for research analysis of the Scottish Regenerative Neurology Tissue Bank samples affiliated with the Scottish MND register was obtained from the East of Scotland Research Ethics Service.

(B) Project MinE Sequencing Consortium cohort (Utrecht University)

A total of 456 *C9orf72* carriers were obtained from the Project MinE data, which consisted of a collection of ALS patients recruited worldwide through the collaboration of tertiary referral clinics for motor neuron disease. Neurologists from the European participating Tertiary Centers who are members of the EU Joint Program – Neurodegenerative Diseases Research (JPND) project STRENGTH and the Project MinE Sequencing Consortium have agreed to follow shared standard parameters in the collection of clinical information of ALS patients. Neurologic examination and diagnostic tests were used to determine whether participants met the revised El Escorial criteria for possible, probable, laboratory-supported, or definite ALS as fully described elsewhere [S1, S8]. Details of the cohorts are as follows:

(1) **The Netherlands.** ALS patients were diagnosed with ALS at the tertiary referral clinic for motor neuron disease at the University Medical Center Utrecht (Dutch ALS Center) or were included in the Prospective ALS Study in The Netherlands. Patients were not pre-screened for any mutations related to ALS. All individuals gave written informed consent, and the University Medical Center Utrecht Medical Ethics Committee, Utrecht, approved this protocol.

(2) **UK MNDA Biobank.** Neurologists diagnosed cases with ALS in one of twenty UK hospitals specialized in motor neuron diseases, and patients had no family history of ALS. All participated in the UK National Biobank for Motor Neuron Disease Research. All individuals gave written informed consent, and the Trent University Medical Ethics Committee approved this protocol.

(3) **Turkey.** ALS patients were recruited from hospitals across Turkey between 2002 and 2019. DNA samples were collected at Boğaziçi University. A full description of individuals gave written informed consent, and the Ethics Committee on Research with Human Participants (INAREK) at Boğaziçi University, Istanbul, approved this protocol.

(4) **Belgium.** Patients were diagnosed with ALS at the tertiary referral clinic for motor neuron diseases at the University Hospitals in Leuven. All individuals gave written informed consent, and the Ethical Committee of the University Hospitals in Leuven approved this protocol.

(5) **Ireland.** Cases were diagnosed with probable or definite ALS according to the El Escorial Criteria by neurologists specialized in motor neuron diseases at Beaumont Hospital in Dublin [S7]. Patients were part of an ongoing population-based prospective ALS registry. Patients were selected for sequencing so that all areas of Ireland were adequately represented. All individuals reported Irish ancestry for at least three generations. All individuals gave written informed consent, and the Beaumont Hospital Research & Ethics Committee, Dublin, approved this protocol.

(6) **Spain.** According to the El Escorial criteria, ALS patients were diagnosed with definite or probable ALS [S7]. Neurologists and neurophysiologists saw patients at the tertiary referral centers: the Bellvitge Hospital and Carlos III Hospital for Catalonia and Madrid, respectively. All individuals gave written informed consent, and the Bellvitge University Hospital Ethics Committee, Barcelona, and "Comité de Ética de la Investigación del Hospital Carlos III," Madrid, approved this protocol.

(7) **The United States.** All samples were taken from patients seen at the Emory ALS Center in Atlanta, Georgia, USA. The Emory Center is a tertiary care ALS clinic that cares for many patients in Georgia and the surrounding states. Diagnoses were made by neurologists specializing in neuromuscular diseases and motor neuron diseases. After informed consent, complete demographic and clinical information was stored in the clinic database. DNA was collected and stored. All individuals gave written informed consent, and the Committee for the Protection of Human Subjects in Research of the University of Massachusetts Medical School, Worcester, approved this protocol.

(8) **France.** ALS patients were diagnosed with probable or definite ALS according to the El Escorial criteria by neurologists specialized in motor neuron diseases at the Reference centers for ALS of the University Hospitals of Limoges and Tours (LITORALS federation), members of the French FILSLAN networks [S7]. All individuals gave written informed consent, and the ethics committee of Tours Hospital and Limoges University Hospital approved this protocol.

(9) **Sweden.** Cases were diagnosed with probable or definite ALS according to the revised El Escorial Criteria by neurologists specialized in motor neuron diseases [S1]. All participants were of Swedish descent and had reported Northern Swedish citizenship for at least three generations. All individuals gave written informed consent, and the Regional Ethical Review Board in Umeå approved this protocol.

(10) **Israel.** ALS patients were diagnosed with probable or definite ALS according to the El Escorial criteria [S7] and in follow-up at the tertiary referral ALS clinic at the Hadassah University Hospital, Jerusalem, or Tel-Aviv Sourasky Medical Center in Tel-Aviv. Patients were not pre-screened for any

mutations related to ALS. Patients were referred from all regions in Israel and participated in a prospective ALS database and sample repository. All individuals gave written informed consent, and the Hadassah University Hospital Institutional Review Board, Hadassah, and The Institutional Review Board of Tel Aviv Sourasky Medical Center, Tel Aviv, approved this protocol.

(11) **Portugal.** According to the revised El-Escorial criteria [S1], neurologists specialized in motor neuron diseases diagnosed patients with possible, probable, or definite ALS. All individuals gave written informed consent, and the Local Research Ethics Committee at the Faculty of Medicine, University of Lisbon, approved this protocol.

(12) **Italy.** Patients were diagnosed with ALS according to the El Escorial revised criteria at the ALS tertiary referral center of Istituto Auxologico Italiano IRCCS [S1]. All patients had probable or definite familial ALS according to the Byrne criteria for FALS in the *SOD1*, *TARDBP*, *FUS*, and *C9orf72* genes. All individuals gave written informed consent, and the Ethics Committee of the IRCCS Istituto Auxologico Italiano, Milan, approved this protocol.

(13) **Switzerland.** ALS patients were diagnosed at the Muskelzentrum/ALS clinic at the Kantonsspital St. Gallen, a tertiary referral center in Northern Switzerland. Patients fulfilled the El-Escorial Criteria for probable lab supported, probable or definite, or ALS [S7]. All individuals gave written informed consent, and the Kantonale Ethikkomission des Kantons St. Gallen approved this protocol.

Overall, of the 836 ALS/FTD *C9orf72* repeat expansion carriers who passed the quality control (QC) thresholds, complete clinical information was available for 713 (n = 385 males and n = 328 females) individuals. Of those, only 699 did not overlap with the training dataset.

SNP array-based genotyping in the replication dataset

C9orf72 carriers (n=464) from the King's College London cohort were genotyped on the Illumina InfiniumOmni2-5-8v1-4_A1 platform in the Illumina certified laboratory of the Department of Social Genetic & Developmental Psychiatry, King's College London. Genotype raw data were first annotated to the dbSNP150 and merged after alignment to the same genomic coordinate (coordinates GRCh37). All multi-allelic and A/T or C/G SNPs were excluded. Pre-phasing quality control steps were performed according to PLINK's standard protocols (version 1.9) [S9]. SNPs were excluded by low call rate < 99% (--geno 0.01), minor allele frequency (MAF) > 0.01, and Hardy-Weinberg disequilibrium (HWE) < 1 x 10^{-6}. Individuals were removed by missingness genotype value of 3%, (--mind 0.03), by the +/-3SD to the mean of the inbreeding distribution F (+/- 0.25), if with mismatches between genetic and reported gender. Related and duplicated individuals were identified by calculating each pair of individuals' identity by state (IBS) status. Those who passed the threshold of PI_HAT > 0.175 were excluded from further analyses. Ancestry differences were estimated by principal components analysis (PCA) using EIGENSTRAT software, and the outliers identified by the first ten principal components (PCs) were removed.

After pre-imputation quality control, 836 individuals (457 males, 379 females) with coverage of 1,349,769 SNPs passed the filter thresholds. Filtered data were phased according to the Haplotype Reference Consortium Release 1.1 (HRC.r1-1) through the Wellcome Sanger Institute Imputation Service (https://www.sanger.ac.uk/tool/sanger-imputation-service/) adopting the Eagle method (version 2.4.1). Imputation analysis generated ~ 39,000,000 variants. Post-imputation QC was performed using QCTOOL (version 2.0.8) and SNPTEST (version 2.5.6) software. The quality of the inferred variants was estimated according to the following thresholds: INFO score > 0.6, average posterior probability (APP) > 0.9, MAF > 0.01, and Hardy-Weinberg disequilibrium > 1 x 10^{-6}. After post-imputation quality control, 7,635,605 SNPs remained for further analysis.

Whole-genome sequencing in the replication dataset

C9orf72 carriers (n=456) from Project MinE were whole-genome sequenced, and standard quality control criteria were applied. At the variant level, sites with a genotype quality (GQ) < 10 or missing and SNVs and indels with quality (QUAL) scores < 20 and < 30 were removed. Kinship coefficients (i.e., relatedness) were calculated using the KING method, as implemented in the *SNPRelate* package in R. All pairs of related individuals (kinship > 0.0625) were identified. The transition-transversion ratio in each sample was calculated using SnpSift27 (version 4.3p). The expected transition-transversion (Ti/Tv) ratio in whole-genome sequence data is ~2.0. Samples with a Ti/Tv ratio ± 6 SD from the entire distribution of samples were removed. The number of single nucleotide variants (SNV) and singletons was calculated per sample. Samples with total SNVs or singletons > 6 SD from the mean were removed.

The transition in sequencing platforms from HiSeq 2000 to HiSeq X caused an increase in observed indels per sample. Accordingly, samples were filtered by platform (HiSeq 2000 or HiSeq X) and were excluded if the number of indels was ± 6 SD from the mean of their respective group. After this step, the average sample depth was calculated again. It was higher for samples sequenced on the HiSeq 2000 (35X, on average) than for samples sequenced on the HiSeq X (25X, on average). However, no samples were removed at this step.

Samples with mismatched sex information or missing phenotypic information were excluded. The remaining sample quality control was performed on high-quality variants: multi-allelic SNVs, variants with missingness > 2%, variants with Hardy-Weinberg equilibrium p-value < 1×10^{-5}, variants with differential missingness between cases, and controls with p-value < 1×10 were removed. The final steps of sample quality control were performed on a set of variants with an MAF > 10%, SNP missingness < 0.1%, variants residing outside four complex regions (the major histocompatibility complex (MHC) on chromosome 6; the lactase locus (LCT) on chromosome 2; and inversions on chromosomes 8 and 17). A/T and C/G variants were also excluded. We used the SNVs to calculate observed and expected autosomal homozygous genotype counts for each sample, and they were removed if |F| > 0.1. Samples with a PI_HAT > 0.8 were excluded to avoid duplicated samples.

Principal component analysis, as implemented in EIGENSOFT, was used to visualize potential structure in the data induced by population stratification or other variables. Projections onto the HapMap3 and the 1000 Genomes (phase 3, version 5) populations indicated that the samples were primarily of European ancestry. However, some were of African or East Asian ancestries, while others appeared admixed. Outliers from the European population (HapMap3: > 10 SD on principal components (PC) 1-4, 1000 Genomes: > 4 SD on PCs 1-4) were excluded from further analyses.

All samples were sent in batches to Illumina for sequencing. Thus, all variants were regressed against batch using PLINK (version 1.9) [S9]. Finally, all variants with an association p-value < 1.0×10^{-10} in at least one batch were excluded.

New York Genome Center RNA sequencing

Patient and control samples were acquired from the New York Genome Center (NYGC) Consortium Database and can be accessed by contacting the NYGC at https://www.nygenome.org/contact/. *Trimmomatic* software was used to trim the original sequencing files obtained from NYGC to 80 base pairs to remove barcodes and improve sample quality [S10]. To account for sample sequencing depth differences, the reads from all samples were downsampled to 25 million reads. These reads were aligned to the hg38 reference genome (https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.26/) with the *Spliced Transcripts Alignment to a Reference* (STAR) software [S11].

These raw mRNA transcript counts were collated into a single count matrix file for differential expression analysis (DEA). First, samples in the count matrix file collected from the motor cortex were selected. Next, genes with a low number of transcript counts (5 or less) were removed, as well as those that were

significantly associated with biological sex (DESeq2 FDR adjusted p-value < 0.05). The NYGC provided information regarding which patients had a pathogenic C9orf72 expansion. The number of patients with a pathogenic *C9orf72* expansion only (i.e., no other known genetic predisposition to ALS) was 36. The DEA was performed on the resulting non-normalized count data matrix using the *DESeq2* package in R to compare the expression profiles of *C9orf72* ALS patients and controls [S12]. Differentially expressed genes (DEGs) were counted with a Bonferroni adjusted p-value < 0.05.

Individual-level variant analysis

We evaluated associations of the rs113247976 (*KIF5A*) variant with age at onset using linear regression models adjusted for sex and principal components one to ten. The variant was studied under an additive genotypic and a dominant genotypic model.

Induced pluripotent stem cells (iPSCs) maintenance and differentiation into motor neurons

Differentiation of iPSCs (**Table S10**) into motor neurons was performed as previously described with modifications [S13]. For the differentiation, induced pluripotent stem cells (iPSCs) were seeded in growth factor-reduced Matrigel-coated plates (0.1 µg/ml). On day zero, iPSCs at 100% confluence were washed once with PBS. Neuralization was initiated by switching to iPSC-NPC day 1-6 differentiation media (containing 50% KnockOut DMEM/F-12, 50% neurobasal medium, 0.5× N2 supplement, 0.5× B27 supplement, 1× GlutaMAX, 1% penicillin/streptomycin; this will be referred as a basal medium) supplemented with 2 µM dorsomorphin homolog 1 (DMH1), 2 µM SB431542, 3 µM CHIR (a GSK3 inhibitor), which was replaced every 24 hours. On day 7 of the differentiation, cells were switched to day 7-12 iPSC-NPC differentiation media (which contains basal medium, supplemented with 1 µM CHIR, 2 µM DMH1, 2 µM SB431542, 0.1 µM all-*trans* retinoic acid, and 0.5 µM purmorphamine (PMN)). For the passage, cells were washed with HBSS without calcium and magnesium and incubated for 7 minutes with Accutase at 37°C. Accutase was neutralized with double the medium quantity, and the cell suspension was centrifuged at 200 g for 4 minutes. The supernatant was discarded, and the cell pellet was resuspended in 7-12 iPSC-NPC differentiation media supplemented with 10µM Y27632 ROCK inhibitor. Cells were re-plated onto new matrigel-coated 6-well plates at a ratio of 1:1, and differentiation was continued. By day 12 of the differentiation, neural rosettes should have formed, and the cells should express classical neural progenitor cell (NPC) markers (Pax6, Nestin).

For the motor neuron differentiation, NPCs were plated in Matrigel-coated 6-well plates at a density of $7x10^5$ cells per well. After 24 hours of incubation, the medium was changed to basal medium supplemented with 0.5 µM all-*trans* retinoic acid and 0.1 µM PMN, and the medium was changed every day for six days. On day 19, the motor neuron progenitors were passaged with accutase onto matrigel-coated plates. The medium was replaced with basal medium supplemented with 0.5 µM all-*trans* retinoic acid, 0.1 µM PMN, 0.1 µM compound E (Cpd E), 10 ng/mL BDNF, 10 ng/mL CNTF, and 10 ng/mL IGF-1 (19-28 days medium) and seeded into an optic 96-well plate (Perkin Elmer) for staining, at a density of $2x10^4$ cells per well. On day 29 of the differentiation, the cells were switched to day 29-40 neuronal differentiation medium (which contains Neurobasal basal medium, supplemented with 1x of B27, 10 ng/mL BDNF, 10 ng/mL CNTF, and 10 ng/mL IGF-1). The cells were fed on alternate days with the neuronal medium until day 40. Cells were previously characterized at day 40 of differentiation and were found to express classical mature motor neuron markers (ChAT, SMI32, Islet 1/2, MAP2, NeuN) (**Figure S5**).

Drug Treatments

Acamprosate was obtained from Sigma-Aldrich (acamprosate calcium A6981), stored at 10 mM in dH_2O, and kept out of light at -20ºC until use. To evaluate the effect of acamprosate on neuronal survival, on day 40, motor neurons were treated with acamprosate (0.01-30 µM) diluted in 29-40 neuronal differentiation medium for 72 hours. As a positive control for cell death, motor neurons were treated with 2 µM

camptothecin (CPT) made up in day 29-40 neuronal differentiation medium for 1 hour at 37ºC. Control cultures were treated with dH_2O, the vehicle of dilution of acamprosate.

Apoptosis assessment

On day 40, motor neurons were treated with the selected drugs. After three days of treatment, motor neurons were fixed and stained for (i) active Caspase-3 to identify cells undergoing apoptosis and (ii) MAP2, a neuron-specific cytoskeletal protein, to define the cytoplasmic boundaries of cells. 4,6-diamidino-2-phenylindole (DAPI) was used for nuclear counterstain. Quantitative imaging analysis was conducted through the Opera Phenix high content Screening System at 40x magnification, using the Harmony software for analysis. The percentage of Caspase-3 positive cells and the number of fragmented nuclei were assessed per every condition. At least 25 fields were randomly selected and scanned per well of a 96-well plate in triplicate. To identify and remove any false readings generated by the system, three random treated and untreated wells were selected and counted manually (blind to the group).

Immunocytochemistry

Cells were washed with PBS and incubated with 4% paraformaldehyde warmed to approximately 37°C for 10 minutes at room temperature, then washed with PBS. Fixed motor neurons were permeabilized with 0.3% Triton X-100 in PBS for 5 minutes and incubated in 5% donkey serum blocking solution for 1 hour to block non-specific staining. Antibodies were diluted in 5% donkey serum. Cells were incubated with primary antibodies (**Table S11**) overnight at 4°C and washed three times with PBS (5 minutes per wash). Cells were incubated with AlexaFluor secondary antibodies (1:400 dilution) (**Table S11**) for 1 hour at room temperature in the dark. They were washed once with PBS before incubating in 1 µg/mL of DAPI for 5 minutes at room temperature in the dark. Cells were washed three times with PBS and stored in the dark at 4ºC until imaging.

Imaging

All the imaging was performed using the Opera Phenix™ High Content Screening System (Perkin Elmer) at × 40 magnification to allow high throughput analysis without experimental bias. Z-stacks of at least eight or more planes separated by 0.7 µm were obtained from a minimum of 25 fields per well from three technical replicate wells per experiment, thus assessing > 6000 cells per experiment. 405, 488, 594, and 647nm lasers, and the appropriate excitation and emission filters were used. Settings were kept consistent while taking images from all cultures. For active caspase-3 analysis, the total number of caspase-3 positive cells was counted using the automated image analysis software Harmony (Perkin Elmer) and divided by the total number of cells.

MTT assay

The effect of acamprosate on cell viability was assessed via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to purple formazan crystals by metabolically active cells. For this assay, motor neurons were seeded in clear 96-well plates, treated with different acamprosate concentrations, and incubated for 72 hours at 37°C. Motor neurons were treated with 2 µM camptothecin (CPT) as a positive control for cell death. After incubation, the media was removed, and the cells were washed with PBS (100µl per well). MTT solution was then added to a final concentration of 0.5 mg/mL and incubated at 37°C for 2 hours to allow the formation of formazan crystals. The cells were washed with PBS, and DMSO was added (100µl per well) to dissolve the formazan crystals. The plates were shaken for 10 minutes to lyse the cells. Plate absorbance was read

at 570nm in a PherAstar plate reader. The percentage of cell viability was normalized to the vehicle group.

SUPPLEMENTARY FIGURES

Figure S1. The sporadic ALS genetic risk influenced the age at onset among ALS *C9orf72* and FTD *C9orf72* carriers, related to Figure 3.

(A) The regression line shows the association between ALS genetic risk score and age at onset among 817 ALS/FTD patients carrying *C9orf72* repeat expansions (p-value = 0.024, beta = -0.765, 95% CI = -1.429 – -0.101) (ALS & FTD group), 666 ALS patients carrying *C9orf72* repeat expansions (p-value = 0.028, beta = -0.815, 95% CI = -1.54 – -0.09) (ALS group), and 151 FTD patients carrying *C9orf72* repeat expansions (p-value = 0.705, beta = -0.333, 95% CI = -2.06 – 1.39) (FTD group). The shadow areas represent the 90% confidence interval of the regression model. (B) The forest plot shows the meta-analysis results of the ALS and FTD groups (p-value 0.029, beta = -0.742, 95% CI = -1.412– -0.072).

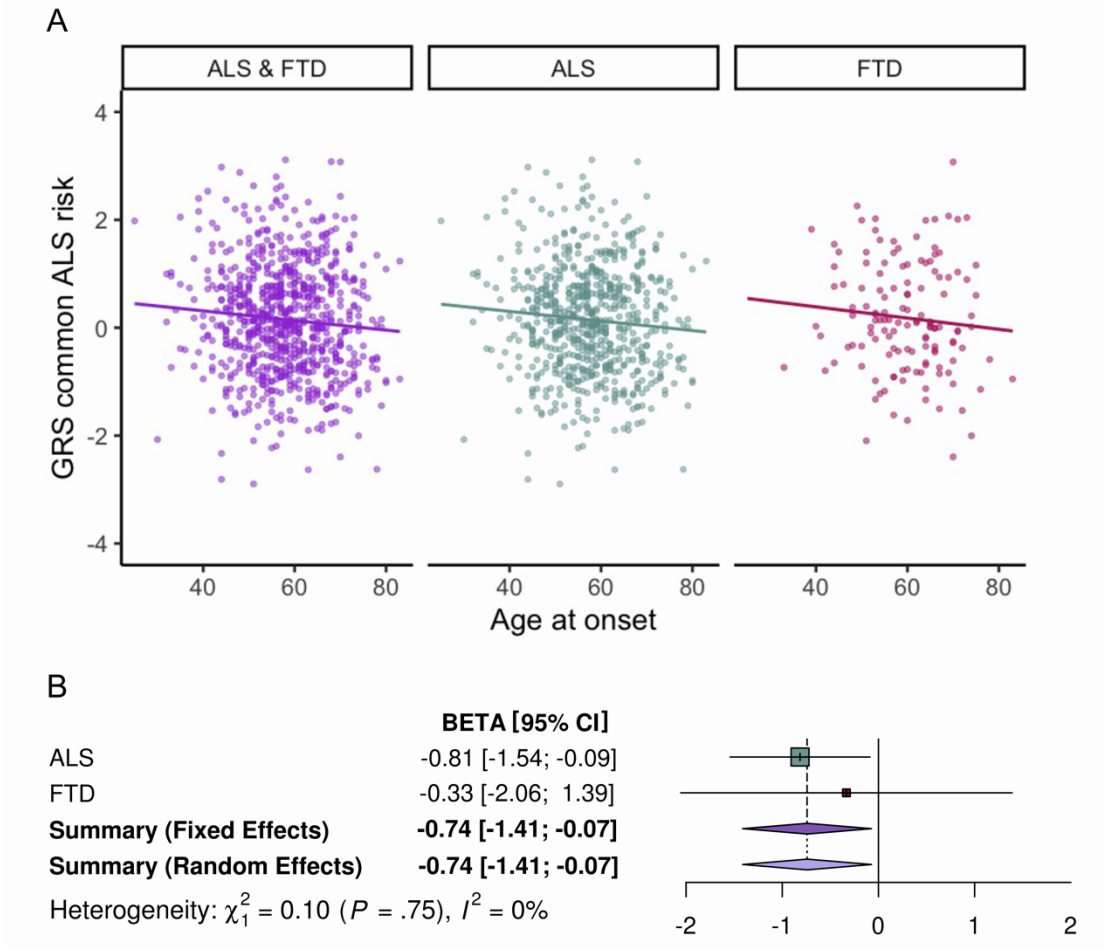


Figure S2. The sporadic ALS genetic risk score did not influence age at onset among patients without the *C9orf72* repeat expansion, related to Figure 3.

The regression line shows the lack of association between ALS genetic risk score and age at onset in 7,037 ALS patients without *C9orf72* repeat expansions (p-value = 0.437, beta = 0.115, 95% CI = -0.175-0.404). The shadow areas represent the 99% confidence interval of the regression model. The forest plot shows the regression result and the 95% confidence interval.

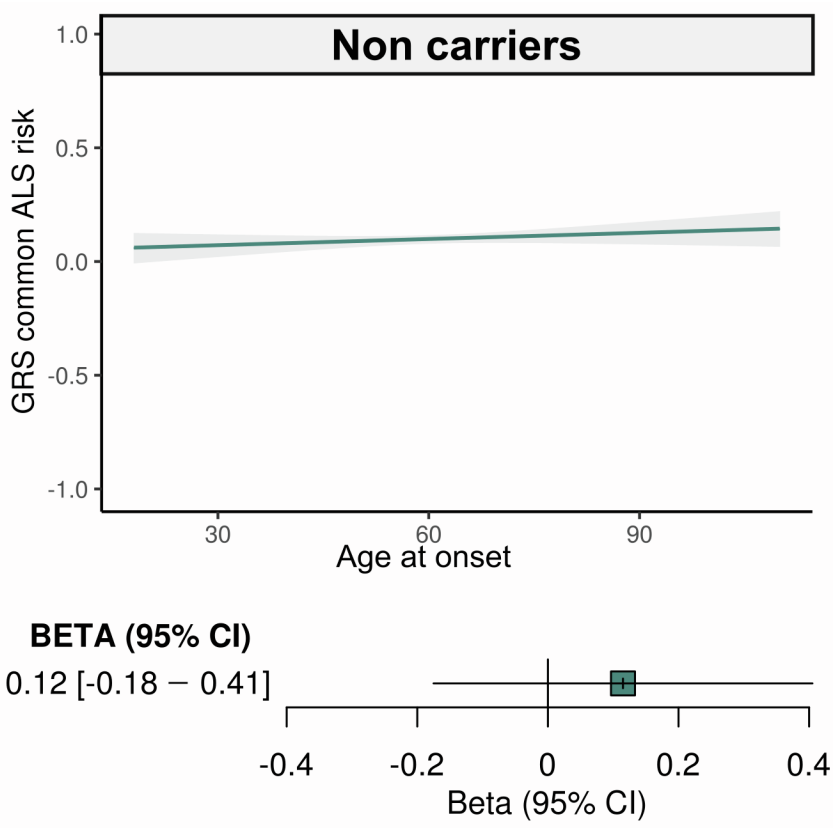


Figure S3. A schematic illustration of the leave-one-out analysis and decile calculation, related to Figure 4.

Leave-one-out analyses were performed by excluding one variant from the ALS genetic risk score analysis (based on 161 predictors) and re-estimating the causal effect on age at the onset of the remaining 160 variants. The variants were ordered based on their impact on age at onset, and ten ranked deciles, each containing 16 SNPs, were generated. Scores were recalculated using the deciles, and regression analysis evaluated the contribution of the 16 variants within each decile to age at onset.

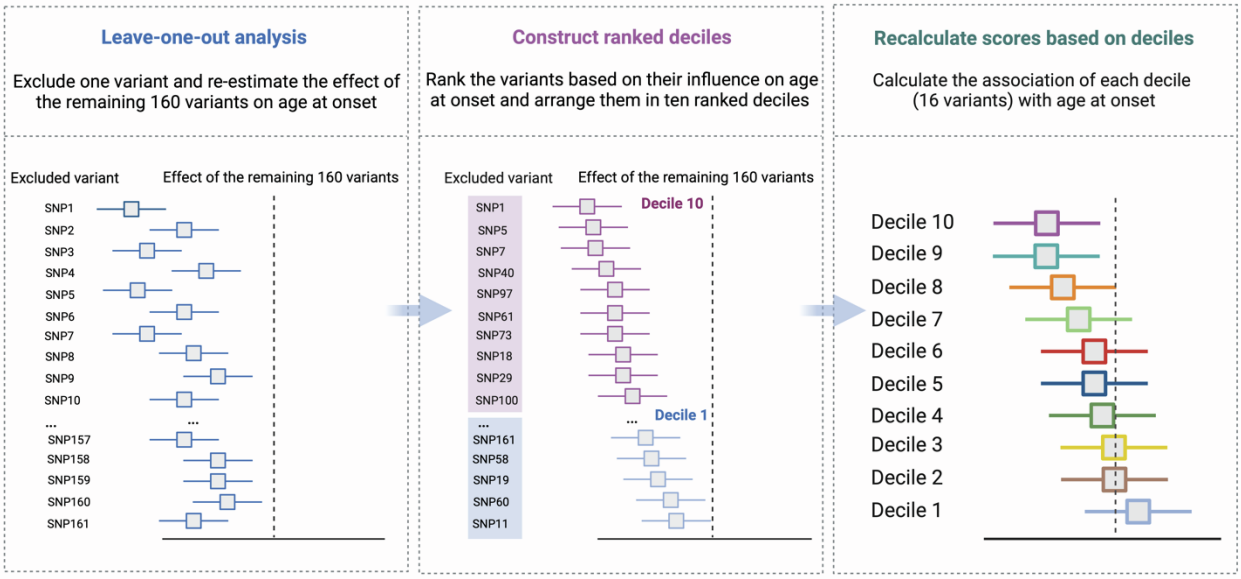


Figure S4. The genetic risk score of each decile showed no relationship to age of symptom onset among the ALS cases who did not carry the *C9orf72* expansion, related to Figure 4.

The Forest plot shows the effect estimates of the genetic risk score on age at onset in ALS non-*C9orf72* cases based on deciles obtained from the leave-one-out analysis. It also shows the regression results and the 95% confidence interval per decile. The analysis was performed in 7,037 ALS patients without *C9orf72* repeat expansions.

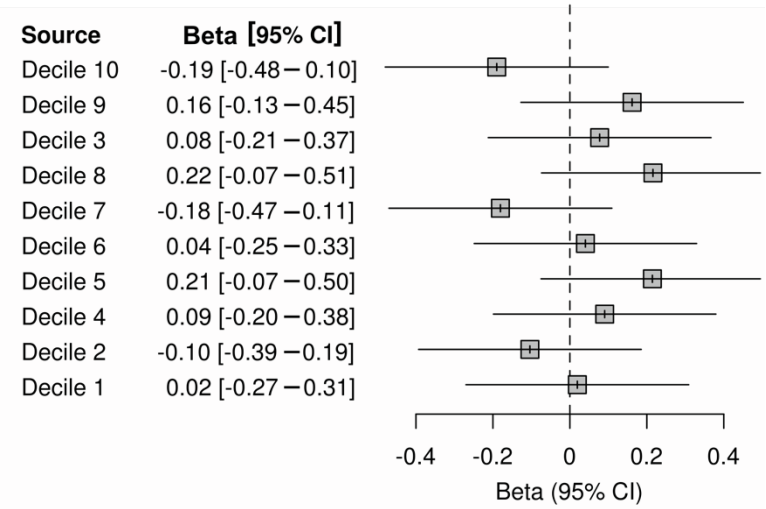


Figure S5. Enrichment analysis of the decile ten genes identified pathways influencing the age of onset among *C9orf72* carriers, related to Figure 4.

Twelve out of sixteen variants within decile ten are mapped to genes based on genomic coordinates. These genes were used for enrichment analysis based on Gene Ontology (GO) terms, including biological processes, molecular functions, and KEGG pathways.

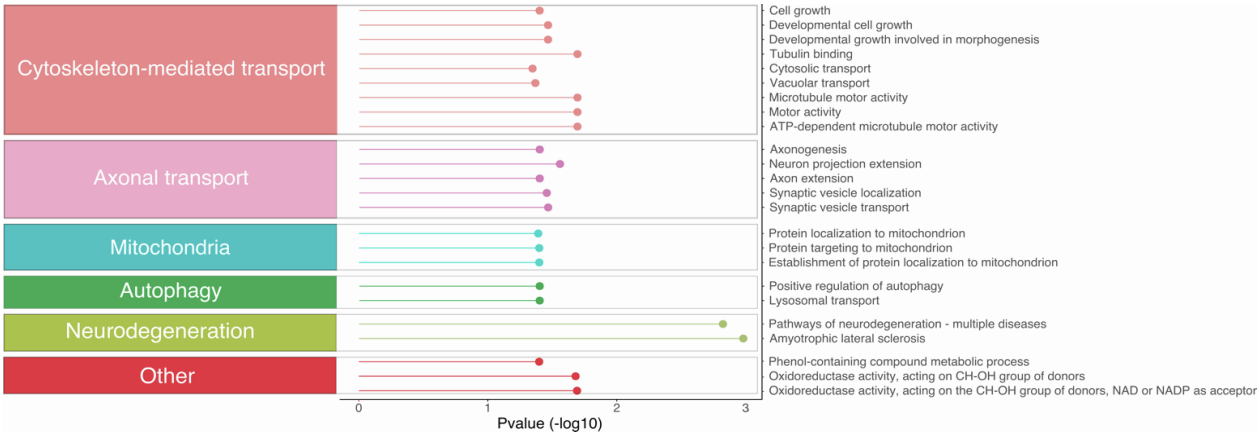


Figure S6. The cell lines displayed characteristics of motor neurons, related to Figure 6.

The picture shows motor neurons derived from unaffected controls (control), *C9orf72* ALS patients (ALS-C9orf72), and an isogenic control line (ISO-C9orf72). Chat, Choline acetyltransferase; DAPI, 4',6-diamidino-2-phenylindole; Islet 1/2, ISL LIM Homeobox 1/2; MAP2, Microtubule-associated protein 2; NeuN, neuronal nuclei antigen; SMI, neurofilament H; and TUJ, beta-tubulin III. The nuclei were counterstained with DAPI (Blue). Scale bar, 50 µm.

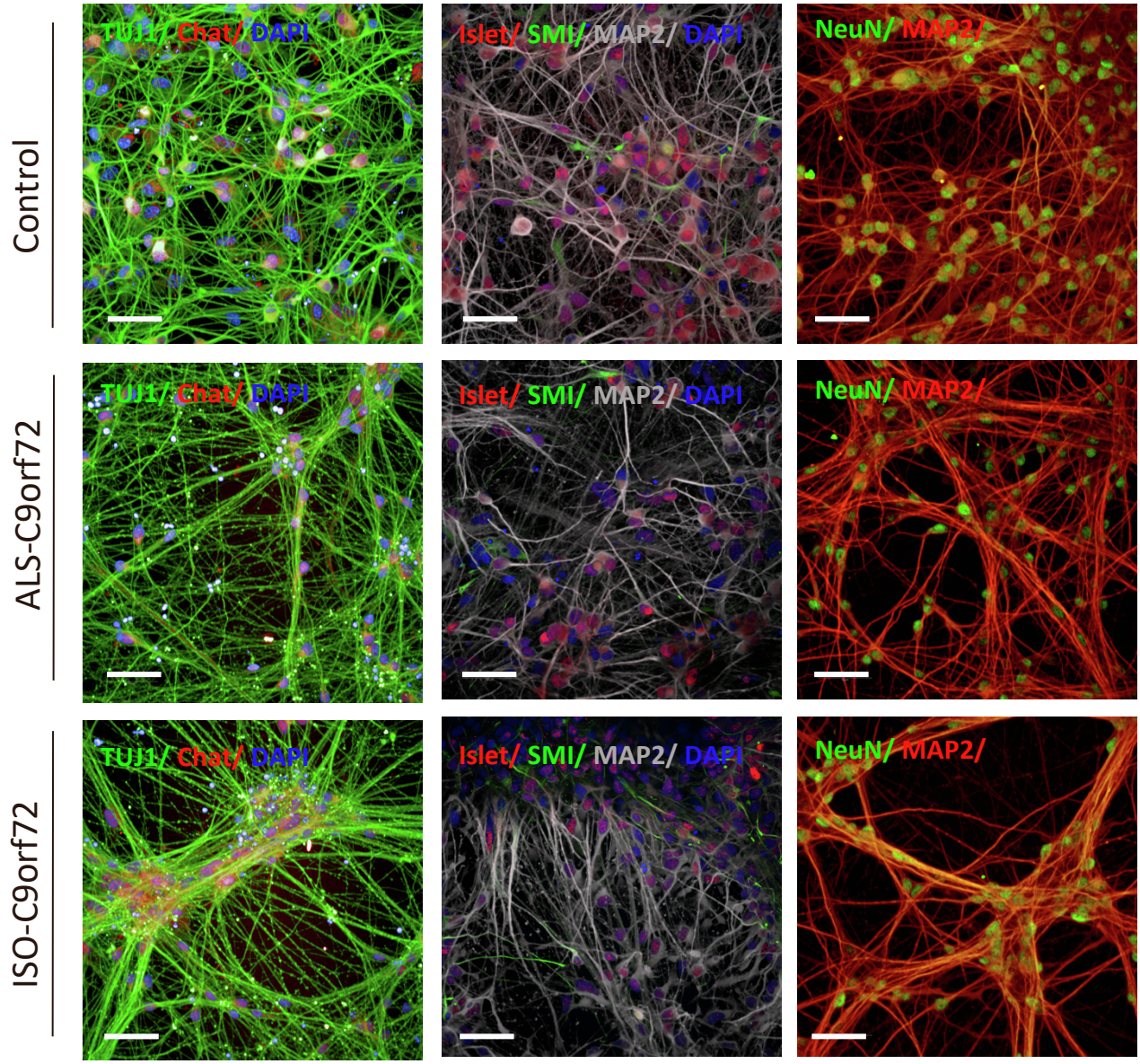


Figure S7. Acamprosate showed no evidence of toxicity in healthy-derived motor neurons, related to Figure 6.

Data are shown as the percentage of viable cells normalized to the vehicle (water). After 72 hours of treatment, acamprosate was not toxic for iPSC-derived motor neurons from two healthy donors. Camptothecin (CPT), an apoptosis inductor, was used as a positive control of cell death. The comparison was performed using one-way ANOVA. Each dot represents a technical replicate, and the data were pooled from two motor neuron lines to calculate the means and standard deviations.

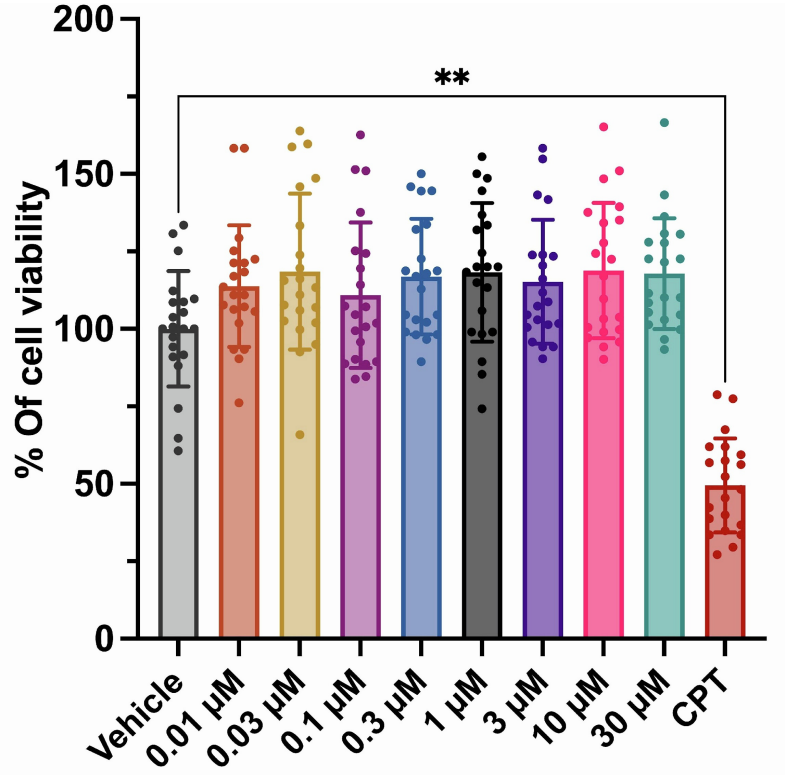


Figure S8. Acamprosate showed no evidence of toxicity in ALS-derived motor neurons, related to Figure 6.

Data are shown as the percentage of viable cells normalized to the vehicle (water). Acamprosate was not toxic in two iPSC-derived motor neuron cell lines derived from ALS patients carrying *C9orf72* expansion after 72 hours of treatment. The lines used were CS52iALS-C9nxx and CS28iALS-C9nxx; more information is listed in Table S10. Camptothecin (CPT), an apoptosis inductor, was used as a positive control of cell death. The comparison was performed using one-way ANOVA. Each dot represents a technical replicate, and the data were pooled to calculate the means and standard deviations.

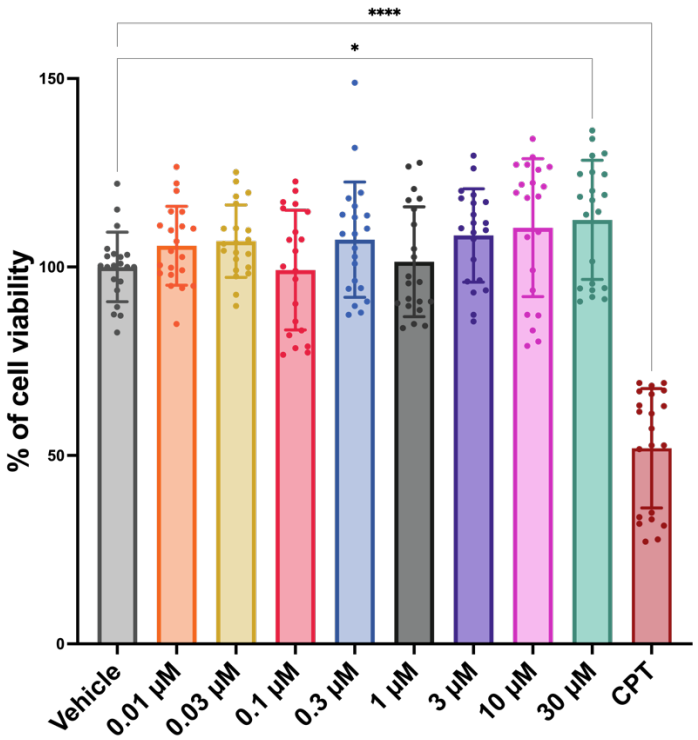
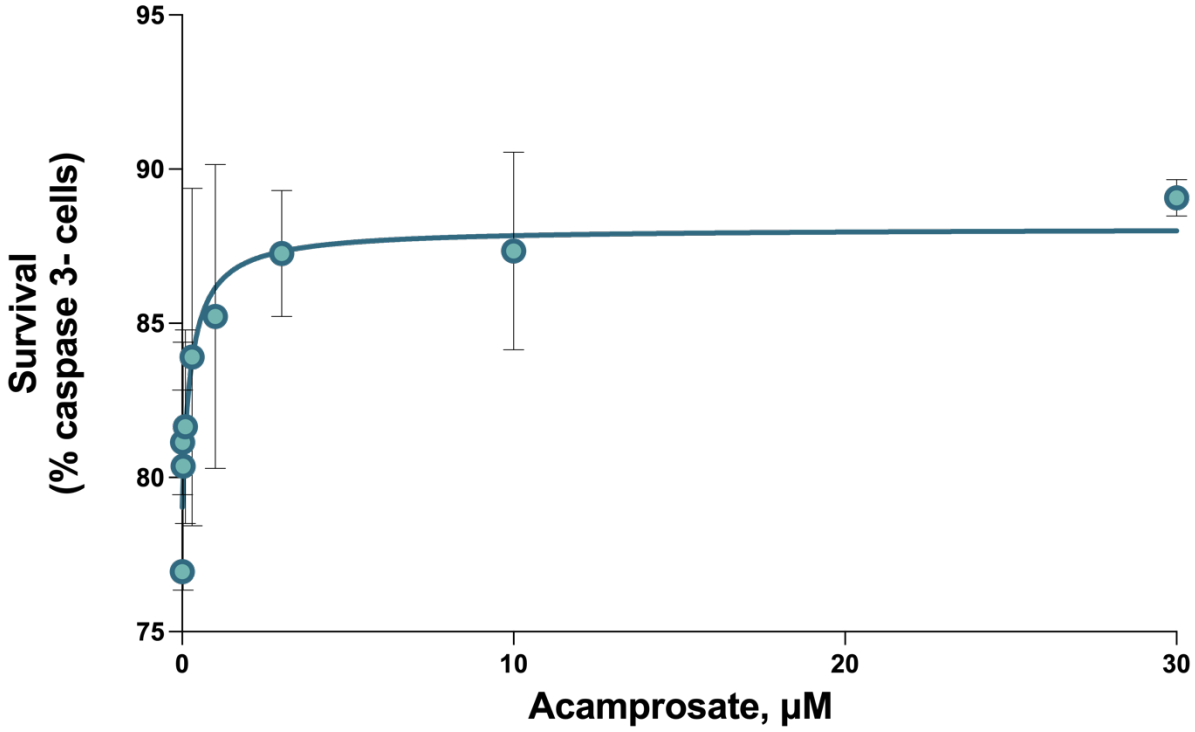


Figure S9. The dose-response curve of acamprosate in ALS-derived motor neurons showed a neuroprotective effect, related to Figure 6.

Dose-response curve and half maximal effective concentration (EC50 = 0.271 µM, 95% CI = 0.0218 – 2.95) of acamprosate in *C9orf72* ALS-derived motor neurons. The dose-response curve illustrates the effect of increasing concentrations of acamprosate (x-axis) on the survival of two different lines of *C9orf72*-carrying motor neurons (y-axis). For each line, data were averaged across three biological replicates, with three technical replicates for each one. Data are means ± standard deviations.



SUPPLEMENTARY TABLES

Table S1. The ALS genetic risk profile was based on 161 SNPs, related to Figure 2.

The polygenic risk score was generated using genetic data from Van Rheenen et al., 2016 [S14]. EA, effect allele; Chr, chromosome; Pos, position in build hg 38.

rsID	Chr	Pos (hg38)	EA	Gene names	Beta	P-value
rs10938692	4	8116834	T	*ABLIM2*	-0.079	$2.74x10^{-5}$
rs12369156	12	120729872	A	*ACADS*	0.261	$7.17x10^{-6}$
rs116488199	10	1675116	A	*ADARB2*	0.132	$8.83x10^{-5}$
rs1159918	4	99321852	A	*ADH1B*	-0.080	$2.03x10^{-5}$
rs320019	1	48610454	A	*AGBL4*	-0.078	$6.75x10^{-5}$
rs73103977	12	53513226	T	*ATF7*	-0.251	$1.78x10^{-5}$
rs11065961	12	111585263	A	*ATXN2*	-0.083	$5.81x10^{-5}$
rs6737916	2	32372917	A	*BIRC6*	0.121	$2.59x10^{-5}$
rs75087725	21	44333234	A	*CFAP410*	0.479	$8.65x10^{-11}$
rs10067826	5	10282407	A	*CMBL*	-0.143	$4.21x10^{-5}$
rs10443173	1	86068071	A	*COL24A1*	-0.106	$7.95x10^{-6}$
rs2271689	10	17046273	A	*CUBN*	-0.090	$9.43x10^{-5}$
rs6947666	7	137708989	A	*DGKI*	0.333	$4.92x10^{-5}$
rs10876069	12	50599395	T	*DIP2B*	0.072	$8.51x10^{-5}$
rs62073477	17	78448064	T	*DNAH17*	0.102	$4.65x10^{-5}$
rs77238283	17	11797907	T	*DNAH9*	0.167	$6.95x10^{-5}$
rs35059420	5	169995487	A	*DOCK2*	-0.159	$6.05x10^{-5}$
rs1442671	18	69528820	A	*DOK6*	0.078	$4.60x10^{-5}$
rs7764458	6	83116819	T	*DOP1A*	0.121	$5.74x10^{-5}$
rs11608027	11	34492865	T	*ELF5*	0.123	$3.05x10^{-5}$
rs17171046	7	37438260	T	*ELMO1*	0.116	$3.70x10^{-5}$
rs9901522	17	14770617	T	*ENSG00000205325*	0.146	$4.61x10^{-5}$
rs11185388	1	104198732	T	*ENSG00000215869*	-0.070	$9.25x10^{-5}$
rs2893656	7	106534655	A	*ENSG00000243797*	0.070	$8.87x10^{-5}$
rs3798105	5	133194937	T	*ENSG00000248245*	0.075	$9.76x10^{-5}$
rs4273590	5	159335610	A	*ENSG00000249738*	-0.191	$9.59x10^{-5}$
rs118072482	8	138017250	T	*ENSG00000253288*	0.297	$8.11x10^{-5}$
rs72973932	11	74400615	A	*ENSG00000254631*	0.182	$9.56x10^{-5}$
rs117219925	12	23142104	A	*ENSG00000256995*	-0.318	$7.15x10^{-5}$
rs111704832	15	93374070	T	*ENSG00000257060*	-0.155	$1.80x10^{-5}$
rs11171999	12	56846925	A	*ENSG00000258679*	0.077	$8.21x10^{-5}$
rs6603044	15	83015059	T	*ENSG00000259805*	-0.079	$1.08x10^{-5}$
rs56024498	16	76893238	A	*ENSG00000259995*	-0.070	$8.18x10^{-5}$
rs12991146	2	59884087	A	*ENSG00000271955*	-0.170	$6.05x10^{-5}$
rs144476584	9	23029711	T	*ENSG00000284418*	0.270	$7.46x10^{-5}$
rs116876275	13	65954214	T	*ENSG00000286395*	-0.287	$8.80x10^{-5}$

rs538622	5	172920676	A	*ERGIC1*	0.079	$1.33x10^{-5}$
rs2985994	13	45539849	T	*ERICH6B*	-0.081	$6.17x10^{-5}$
rs7930973	11	44159478	A	*EXT2*	0.076	$2.27x10^{-5}$
rs72792226	10	48204534	T	*FRMPD2*	0.285	$1.36x10^{-5}$
rs9903355	17	36580791	T	*GGNBP2*	-0.081	$5.29x10^{-6}$
rs7258235	19	2612120	A	*GNG7*	-0.156	$1.48x10^{-5}$
rs112820958	5	79465868	T	*HOMER1*	0.148	$4.75x10^{-5}$
rs11718653	3	122759011	T	*HSPBAP1*	0.221	$8.75x10^{-6}$
rs144049425	2	162505368	T	*KCNH7*	-0.251	$7.80x10^{-5}$
rs113247976	12	57581917	T	*KIF5A*	0.288	$1.13x10^{-5}$
rs61954176	13	40198035	T	*LINC00598*	-0.096	$5.32x10^{-6}$
rs11695294	2	176608890	A	*LINC01117*	0.108	$5.27x10^{-5}$
rs28407220	2	33816106	T	*LINC01320*	0.103	$7.11x10^{-6}$
rs150278778	1	209379025	A	*LINC01698*	-0.254	$4.82x10^{-5}$
rs72733862	5	8446181	A	*LINC02226*	0.148	$6.08x10^{-6}$
rs35318094	5	180245984	T	*MAPK9*	0.184	$1.15x10^{-5}$
rs17326496	5	113340882	T	*MCC*	-0.091	$5.54x10^{-5}$
rs79502718	18	50919413	A	*ME2*	0.169	$9.23x10^{-5}$
rs12972250	19	329746	A	*MIER2*	0.073	$6.10x10^{-5}$
rs9653747	21	18669100	A	*MIR548XHG*	-0.088	$4.64x10^{-6}$
rs12079484	1	181048307	A	*MR1*	0.075	$7.81x10^{-5}$
rs2240601	17	57673751	A	*MSI2*	-0.090	$5.07x10^{-5}$
rs4292737	8	10401605	A	*MSRA*	0.073	$5.11x10^{-5}$
rs4945276	11	78453938	T	*NARS2*	0.076	$5.90x10^{-5}$
rs150949995	5	150518937	T	*NDST1*	0.373	$4.28x10^{-6}$
rs642811	11	78053929	T	*NDUFC2-KCTD14*	0.085	$7.08x10^{-5}$
rs34432311	2	177346958	T	*NFE2L2*	-0.207	$3.20x10^{-5}$
rs68072647	17	9224590	A	*NTN1*	-0.077	$2.65x10^{-5}$
rs12886280	14	31829453	T	*NUBPL*	-0.083	$3.15x10^{-6}$
rs118036547	15	27863948	T	*OCA2*	0.251	$4.49x10^{-5}$
rs35346557	3	190120229	T	*P3H2*	-0.090	$4.92x10^{-5}$
rs3109207	4	168675360	A	*PALLD*	0.071	$9.49x10^{-5}$
rs36037136	1	164710739	A	*PBX1*	-0.291	$1.72x10^{-5}$
rs10492593	13	66919985	A	*PCDH9*	0.123	$2.89x10^{-5}$
rs2477866	1	233152025	A	*PCNX2*	-0.140	$3.02x10^{-5}$
rs16865645	2	177696567	T	*PDE11A*	0.096	$9.42x10^{-5}$
rs5766195	22	44921429	T	*PHF21B*	-0.072	$4.36x10^{-5}$
rs11652752	17	67379776	A	*PITPNC1*	-0.119	$6.49x10^{-5}$
rs8053191	16	81117558	T	*PKD1L2*	-0.152	$6.50x10^{-5}$
rs10430614	10	131935136	T	*PPP2R2D*	-0.079	$6.04x10^{-5}$
rs9355960	6	161901879	T	*PRKN*	-0.084	$1.37x10^{-5}$
rs2253050	16	74040378	T	*PSMD7-DT*	0.173	$7.43x10^{-5}$
rs28660489	12	64560784	A	*RASSF3*	-0.081	$6.13x10^{-5}$

rs12229321	12	64518074	T	*RASSF3*	0.071	$8.42x10^{-5}$
rs9813285	3	29329178	T	*RBMS3*	-0.077	$2.42x10^{-5}$
rs6683585	1	240967882	T	*RGS7*	-0.068	$9.16x10^{-5}$
rs143747467	16	11367549	T	*RMI2*	-0.293	$1.37x10^{-5}$
rs115348904	4	158390602	T	*RXFP1*	-0.208	$5.48x10^{-5}$
rs2294928	22	43986973	A	*SAMM50*	0.104	$4.27x10^{-5}$
rs35714695	17	28392769	A	*SARM1*	-0.134	$1.29x10^{-8}$
rs10139154	14	30678292	T	*SCFD1*	0.081	$1.92x10^{-5}$
rs118082508	12	56925035	T	*SDR9C7*	0.288	$3.76x10^{-5}$
rs111970477	3	47064091	A	*SETD2*	-0.262	$6.96x10^{-5}$
rs430979	4	2812971	T	*SH3BP2*	-0.085	$2.63x10^{-6}$
rs9995307	4	146488500	A	*SLC10A7*	0.080	$8.43x10^{-5}$
rs118038177	11	121459793	T	*SORL1*	-0.216	$4.79x10^{-5}$
rs60318796	17	32991387	T	*SPACA3*	0.109	$4.87x10^{-5}$
rs13387347	2	168898336	T	*SPC25*	0.075	$3.15x10^{-5}$
rs12967284	18	12532099	T	*SPIRE1*	0.079	$1.74x10^{-5}$
rs76805704	12	64138597	A	*SRGAP1*	0.188	$5.95x10^{-6}$
rs79612353	20	59884601	A	*SYCP2*	0.190	$3.84x10^{-5}$
rs112348322	4	118882809	A	*SYNPO2*	0.276	$2.60x10^{-5}$
rs74654358	12	64488187	A	*TBK1*	0.206	$7.72x10^{-7}$
rs11067262	12	114724621	T	*TBX3-AS1*	0.078	$8.56x10^{-5}$
rs79496463	8	132904843	T	*TG*	0.194	$7.17x10^{-5}$
rs13410191	2	137643025	A	*THSD7B*	-0.069	$9.98x10^{-5}$
rs651001	6	11569169	A	*TMEM170B*	-0.074	$3.81x10^{-5}$
rs115980385	7	141463227	T	*TMEM178B*	0.292	$9.81x10^{-5}$
rs10463311	5	151031274	T	*TNIP1*	-0.100	$8.51x10^{-7}$
rs4958888	5	151093281	A	*TNIP1*	0.089	$1.53x10^{-5}$
rs78549703	19	17638733	A	*UNC13A*	0.110	$1.31x10^{-8}$
rs8180839	7	5200339	A	*WIPI2*	0.151	$6.46x10^{-5}$
rs138116283	4	4318540	A	*ZBTB49*	0.337	$7.77x10^{-6}$
rs4974650	4	2309992	A	*ZFYVE28*	0.090	$1.63x10^{-6}$
rs8101883	19	56681170	A	*ZIM2-AS1*	0.084	$1.97x10^{-5}$
rs6997565	8	2560505	T	Intergenic	-0.146	$1.54x10^{-6}$
rs7118388	11	34432600	A	Intergenic	-0.084	$2.34x10^{-6}$
rs144387708	12	119264395	A	Intergenic	0.348	$4.95x10^{-6}$
rs116900480	12	58262322	T	Intergenic	0.294	$7.07x10^{-6}$
rs12900374	15	82741261	T	Intergenic	0.107	$7.27x10^{-6}$
rs10050775	5	38007940	A	Intergenic	-0.109	$1.08x10^{-5}$
rs117860708	11	1537217	A	Intergenic	0.237	$1.30x10^{-5}$
rs116946806	7	131997812	T	Intergenic	0.224	$1.43x10^{-5}$
rs4676496	3	39456514	A	Intergenic	0.077	$1.44x10^{-5}$
rs71472777	11	24121389	T	Intergenic	0.241	$1.45x10^{-5}$
rs112913348	5	108554187	T	Intergenic	-0.156	$1.51x10^{-5}$

rs34384833	5	91942338	A	Intergenic	0.265	$1.75x10^{-5}$
rs12472309	2	7266695	T	Intergenic	0.229	$2.45x10^{-5}$
rs10488631	7	128954129	T	Intergenic	-0.120	$2.52x10^{-5}$
rs7041171	9	111939350	T	Intergenic	-0.094	$2.71x10^{-5}$
rs12138742	1	119591406	T	Intergenic	-0.123	$2.74x10^{-5}$
rs62290425	4	4963737	A	Intergenic	0.126	$2.93x10^{-5}$
rs3098553	15	27631110	T	Intergenic	0.267	$3.04x10^{-5}$
rs79676202	12	49786775	T	Intergenic	0.204	$3.08x10^{-5}$
rs970258	2	5138399	T	Intergenic	-0.100	$3.11x10^{-5}$
rs72716562	5	7957484	A	Intergenic	-0.209	$3.12x10^{-5}$
rs141347161	7	42377714	T	Intergenic	0.149	$3.30x10^{-5}$
rs10008582	4	146026232	A	Intergenic	-0.106	$3.53x10^{-5}$
rs77058105	20	17070347	T	Intergenic	-0.198	$3.85x10^{-5}$
rs144129573	12	114485139	T	Intergenic	-0.209	$4.61x10^{-5}$
rs141730255	7	138253804	A	Intergenic	-0.180	$5.11x10^{-5}$
rs79446108	7	137863942	T	Intergenic	0.186	$5.33x10^{-5}$
rs6020200	20	50017227	A	Intergenic	-0.101	$5.36x10^{-5}$
rs6420358	13	84715333	A	Intergenic	-0.080	$6.14x10^{-5}$
rs12220832	10	80806689	T	Intergenic	0.123	$6.27x10^{-5}$
rs117452182	13	27293529	A	Intergenic	0.284	$6.31x10^{-5}$
rs7602576	2	112942040	T	Intergenic	-0.087	$6.39x10^{-5}$
rs11702120	21	23786163	A	Intergenic	0.202	$6.40x10^{-5}$
rs118071175	14	28515686	T	Intergenic	0.123	$6.78x10^{-5}$
rs7209200	17	5066645	T	Intergenic	-0.075	$6.82x10^{-5}$
rs9567838	13	47359837	T	Intergenic	0.095	$6.87x10^{-5}$
rs112288580	11	98729819	A	Intergenic	-0.290	$7.00x10^{-5}$
rs9819308	3	1691812	A	Intergenic	-0.086	$7.06x10^{-5}$
rs6037557	20	405220	T	Intergenic	-0.097	$7.56x10^{-5}$
rs9956309	18	38136697	A	Intergenic	-0.091	$8.42x10^{-5}$
rs76323495	16	16859460	A	Intergenic	-0.200	$8.54x10^{-5}$
rs16905848	11	20223160	T	Intergenic	-0.097	$8.72x10^{-5}$
rs35851984	17	28239061	A	Intergenic	-0.070	$8.98x10^{-5}$
rs76427181	6	86684739	A	Intergenic	-0.224	$9.38x10^{-5}$
rs2176039	22	45189151	A	Intergenic	0.069	$9.44x10^{-5}$
rs1570281	6	146517546	A	Intergenic	-0.077	$9.66x10^{-5}$
rs72838433	2	127913724	A	Intergenic	0.073	$9.87x10^{-5}$
rs193044924	17	15767328	A	Intergenic	-0.077	$9.89x10^{-5}$
rs73152707	3	86619077	T	Intergenic	0.170	$9.94x10^{-5}$
rs1146342	1	118440554	T	Intergenic	0.269	$9.98x10^{-5}$

Table S2. The genetic risk score influenced the age of symptom onset among *C9orf72* carriers, related to Figure 3.

The *bottom 3%* group is composed of individuals whose Z-score is in the bottom 3% of the genetic risk score distribution among *C9orf72* carriers and non-carriers, respectively. The *medium* group is comprised of individuals whose z-score is between 20-80% of the genetic risk score distribution. The *top 3%* group is comprised of individuals whose z-score is between 97-100% of the genetic risk score distribution.

	Age at onset mean	Age at onset standard deviation	Z-score mean	Z-score standard deviation	Count
Carriers					
Bottom 3%	58.11	10.23	-2.21	0.4	27
Medium	57.51	9.25	0.04	0.49	488
Top 3%	55.12	9.92	2.35	0.36	32
Non-Carriers					
Bottom 3%	60.25	12.18	-2.2	0.35	213
Medium	60.07	12.48	-0.03	0.47	4288
Top 3%	60.07	12.63	2.21	0.4	280

Table S3. The leave-one-out analysis stratified the 161 SNPs of the ALS genetic risk into ten deciles, related to Figure 4.

Decile ten contains the variants with a more significant contribution to age at onset. LOO, leave-one-out (indicating the removed SNP); Chr, chromosome; Pos (hg38), genomic position, GRCh38 assembly; SE, standard error.

LOO	Chr	Pos (hg38)	Gene name	Beta	SE	P-value	Decile	Rank
rs9901522	17	14770617	*ENSG00000205325*	-0.678	0.336	0.044	10	1st
rs118036547	15	27863948	*OCA2*	-0.705	0.337	0.037	10	2nd
rs2294928	22	43986973	*SAMM50*	-0.708	0.336	0.036	10	3rd
rs61954176	13	40198035	*LINC00598*	-0.711	0.337	0.035	10	4th
rs62073477	17	78448064	*DNAH17*	-0.715	0.337	0.034	10	6th
rs79502718	18	50919413	*ME2*	-0.715	0.336	0.034	10	5th
rs113247976	12	57581917	*KIF5A*	-0.716	0.336	0.034	10	7th
rs118082508	12	56925035	*SDR9C7*	-0.716	0.337	0.034	10	8th
rs1146342	1	118440554	Intergenic	-0.718	0.336	0.033	10	9th
rs117219925	12	23142104	*ENSG00000256995*	-0.72	0.337	0.033	10	10th
rs116900480	12	58262322	*Intergenic*	-0.721	0.336	0.032	10	11th
rs10876069	12	50599395	*DIP2B*	-0.725	0.336	0.031	10	12th
rs6420358	13	84715333	Intergenic	-0.727	0.336	0.031	10	13th
rs7118388	11	34432600	Intergenic	-0.728	0.336	0.031	10	14th
rs9355960	6	161901879	*PRKN*	-0.728	0.336	0.031	10	15th
rs10938692	4	8116834	*ABLIM2*	-0.73	0.336	0.03	10	16th
rs10488631	7	128954129	Intergenic	-0.731	0.337	0.03	9	17th
rs34384833	5	91942338	Intergenic	-0.732	0.336	0.03	9	18th
rs116876275	13	65954214	*ENSG00000286395*	-0.733	0.336	0.03	9	19th
rs12972250	19	329746	*MIER2*	-0.738	0.336	0.029	9	20th
rs11608027	11	34492865	*ELF5*	-0.739	0.336	0.028	9	22nd
rs17171046	7	37438260	*ELMO1*	-0.739	0.336	0.028	9	24th
rs62290425	4	4963737	Intergenic	-0.739	0.336	0.028	9	21st
rs6683585	1	240967882	*RGS7*	-0.739	0.337	0.028	9	23rd
rs6737916	2	32372917	*BIRC6*	-0.739	0.336	0.028	9	25th
rs10008582	4	146026232	Intergenic	-0.74	0.336	0.028	9	26th
rs7041171	9	111939350	Intergenic	-0.74	0.336	0.028	9	27th
rs9995307	4	146488500	*SLC10A7*	-0.74	0.336	0.028	9	28th
rs4958888	5	151093281	*TNIP1*	-0.741	0.337	0.028	9	29th
rs12991146	2	59884087	*ENSG00000271955*	-0.742	0.337	0.028	9	32nd
rs4945276	11	78453938	*NARS2*	-0.742	0.336	0.028	9	31st
rs56024498	16	76893238	*ENSG00000259995*	-0.742	0.336	0.028	9	30th
rs144476584	9	23029711	*ENSG00000284418*	-0.743	0.336	0.027	8	33rd
rs642811	11	78053929	*NDUFC2-KCTD14*	-0.743	0.336	0.027	8	34th
rs35346557	3	190120229	*P3H2*	-0.744	0.336	0.027	8	35th
rs71472777	11	24121389	Intergenic	-0.744	0.336	0.027	8	36th

rs118072482	8	138017250	*ENSG00000253288*	-0.745	0.336	0.027	8	37th
rs13410191	2	137643025	*THSD7B*	-0.745	0.336	0.027	8	38th
rs35714695	17	28392769	*SARM1*	-0.745	0.336	0.027	8	39th
rs11718653	3	122759011	*HSPBAP1*	-0.746	0.336	0.027	8	40th
rs2240601	17	57673751	*MSI2*	-0.746	0.336	0.027	8	41st
rs1570281	6	146517546	Intergenic	-0.747	0.336	0.027	8	43rd
rs74654358	12	64488187	*TBK1*	-0.747	0.337	0.027	8	42nd
rs2253050	16	74040378	*PSMD7-DT*	-0.748	0.336	0.027	8	46th
rs34432311	2	177346958	*NFE2L2*	-0.748	0.336	0.026	8	47th
rs76805704	12	64138597	*SRGAP1*	-0.748	0.337	0.027	8	45th
rs77058105	20	17070347	Intergenic	-0.748	0.336	0.027	8	44th
rs10463311	5	151031274	*TNIP1*	-0.749	0.336	0.026	8	48th
rs16905848	11	20223160	Intergenic	-0.749	0.336	0.026	7	50th
rs2477866	1	233152025	*PCNX2*	-0.749	0.337	0.026	7	49th
rs12138742	1	119591406	Intergenic	-0.75	0.336	0.026	7	52nd
rs138116283	4	4318540	*ZBTB49*	-0.75	0.336	0.026	7	53rd
rs6020200	20	50017227	Intergenic	-0.75	0.336	0.026	7	51st
rs11695294	2	176608890	*LINC01117*	-0.751	0.336	0.026	7	56th
rs60318796	17	32991387	*SPACA3*	-0.751	0.336	0.026	7	54th
rs73103977	12	53513226	*ATF7*	-0.751	0.336	0.026	7	55th
rs10430614	10	131935136	*PPP2R2D*	-0.752	0.336	0.026	7	58th
rs117452182	13	27293529	Intergenic	-0.752	0.336	0.026	7	57th
rs9956309	18	38136697	Intergenic	-0.752	0.336	0.026	7	59th
rs1159918	4	99321852	*ADH1B*	-0.753	0.336	0.025	7	60th
rs143747467	16	11367549	*RMI2*	-0.753	0.337	0.026	7	61st
rs2893656	7	106534655	*ENSG00000243797*	-0.754	0.336	0.025	7	62nd
rs2176039	22	45189151	Intergenic	-0.755	0.336	0.025	6	65th
rs28660489	12	64560784	*RASSF3*	-0.755	0.336	0.025	7	63rd
rs7602576	2	112942040	Intergenic	-0.755	0.336	0.025	7	64th
rs118038177	11	121459793	*SORL1*	-0.756	0.336	0.025	6	68th
rs6947666	7	137708989	*DGKI*	-0.756	0.336	0.025	6	66th
rs9567838	13	47359837	Intergenic	-0.756	0.336	0.025	6	67th
rs112288580	11	98729819	Intergenic	-0.757	0.336	0.025	6	70th
rs115980385	7	141463227	*TMEM178B*	-0.757	0.336	0.025	6	69th
rs6997565	8	2560505	Intergenic	-0.757	0.337	0.025	6	71st
rs72973932	11	74400615	*ENSG00000254631*	-0.758	0.337	0.025	6	72nd
rs11171999	12	56846925	*ENSG00000258679*	-0.759	0.336	0.024	6	73rd
rs141730255	7	138253804	Intergenic	-0.761	0.336	0.024	6	74th
rs111704832	15	93374070	*ENSG00000257060*	-0.762	0.336	0.024	6	77th
rs141347161	7	42377714	Intergenic	-0.762	0.336	0.024	6	76th
rs538622	5	172920676	*ERGIC1*	-0.762	0.336	0.024	6	75th
rs72792226	10	48204534	*FRMPD2*	-0.762	0.336	0.024	6	78th
rs10492593	13	66919985	*PCDH9*	-0.763	0.336	0.024	5	83rd

rs12900374	15	82741261	Intergenic	-0.763	0.336	0.024	6	80th
rs17326496	5	113340882	*MCC*	-0.763	0.336	0.023	5	81st
rs28407220	2	33816106	*LINC01320*	-0.763	0.336	0.023	5	86th
rs35059420	5	169995487	*DOCK2*	-0.763	0.336	0.023	5	84th
rs36037136	1	164710739	*PBX1*	-0.763	0.336	0.023	5	85th
rs4273590	5	159335610	*ENSG00000249738*	-0.763	0.336	0.024	5	82nd
rs9653747	21	18669100	*MIR548XHG*	-0.763	0.336	0.024	6	79th
rs12967284	18	12532099	*SPIRE1*	-0.764	0.336	0.023	5	89th
rs144049425	2	162505368	*KCNH7*	-0.764	0.336	0.023	5	87th
rs68072647	17	9224590	*NTN1*	-0.764	0.336	0.023	5	88th
rs112820958	5	79465868	*HOMER1*	-0.765	0.336	0.023	5	91st
rs12079484	1	181048307	*MR1*	-0.765	0.336	0.023	5	90th
rs144129573	12	114485139	Intergenic	-0.765	0.336	0.023	5	93rd
rs35851984	17	28239061	Intergenic	-0.765	0.336	0.023	5	92nd
rs11652752	17	67379776	*PITPNC1*	-0.766	0.336	0.023	5	94th
rs12220832	10	80806689	Intergenic	-0.766	0.336	0.023	4	99th
rs150278778	1	209379025	*LINC01698*	-0.766	0.336	0.023	4	100th
rs5766195	22	44921429	*PHF21B*	-0.766	0.336	0.023	5	95th
rs77238283	17	11797907	*DNAH9*	-0.766	0.336	0.023	4	97th
rs7764458	6	83116819	*DOP1A*	-0.766	0.336	0.023	4	101st
rs79612353	20	59884601	*SYCP2*	-0.766	0.336	0.023	5	96th
rs8101883	19	56681170	*ZIM2-AS1*	-0.766	0.336	0.023	4	98th
rs12229321	12	64518074	*RASSF3*	-0.767	0.336	0.023	4	103rd
rs1442671	18	69528820	*DOK6*	-0.767	0.336	0.023	4	102nd
rs72838433	2	127913724	Intergenic	-0.768	0.336	0.023	4	104th
rs430979	4	2812971	*SH3BP2*	-0.769	0.336	0.022	4	106th
rs6037557	20	405220	Intergenic	-0.769	0.336	0.022	4	107th
rs79446108	7	137863942	Intergenic	-0.769	0.336	0.022	4	105th
rs116488199	10	1675116	*ADARB2*	-0.77	0.336	0.022	4	108th
rs13387347	2	168898336	*SPC25*	-0.77	0.336	0.022	4	111th
rs2985994	13	45539849	*ERICH6B*	-0.77	0.336	0.022	4	112th
rs76427181	6	86684739	Intergenic	-0.77	0.336	0.022	4	109th
rs78549703	19	17638733	*UNC13A*	-0.77	0.336	0.022	4	110th
rs193044924	17	15767328	Intergenic	-0.771	0.336	0.022	3	114th
rs6603044	15	83015059	*ENSG00000259805*	-0.771	0.336	0.022	3	113th
rs10139154	14	30678292	*SCFD1*	-0.772	0.336	0.022	3	116th
rs150949995	5	150518937	*NDST1*	-0.772	0.336	0.022	3	117th
rs8053191	16	81117558	*PKD1L2*	-0.772	0.337	0.022	3	115th
rs72733862	5	8446181	*LINC02226*	-0.773	0.336	0.022	3	118th
rs76323495	16	16859460	Intergenic	-0.773	0.336	0.022	3	119th
rs4974650	4	2309992	*ZFYVE28*	-0.774	0.336	0.022	3	120th
rs79676202	12	49786775	Intergenic	-0.774	0.336	0.022	3	121st
rs16865645	2	177696567	*PDE11A*	-0.775	0.336	0.021	3	122nd

rs116946806	7	131997812	Intergenic	-0.776	0.336	0.021	3	123rd
rs320019	1	48610454	*AGBL4*	-0.776	0.336	0.021	3	124th
rs11185388	1	104198732	*ENSG00000215869*	-0.777	0.336	0.021	3	125th
rs3109207	4	168675360	*PALLD*	-0.777	0.336	0.021	3	126th
rs11065961	12	111585263	*ATXN2*	-0.778	0.336	0.021	3	127th
rs111970477	3	47064091	*SETD2*	-0.779	0.336	0.021	3	128th
rs115348904	4	158390602	*RXFP1*	-0.779	0.337	0.021	2	129th
rs112913348	5	108554187	Intergenic	-0.78	0.336	0.021	2	131st
rs72716562	5	7957484	Intergenic	-0.78	0.336	0.021	2	130th
rs8180839	7	5200339	*WIPI2*	-0.782	0.336	0.02	2	132nd
rs12472309	2	7266695	Intergenic	-0.783	0.336	0.02	2	134th
rs2271689	10	17046273	*CUBN*	-0.783	0.336	0.02	2	133rd
rs12369156	12	120729872	*ACADS*	-0.785	0.336	0.02	2	135th
rs75087725	21	44333234	*CFAP410*	-0.785	0.336	0.02	2	136th
rs10067826	5	10282407	*CMBL*	-0.786	0.336	0.02	2	138th
rs35318094	5	180245984	*MAPK9*	-0.786	0.336	0.02	2	139th
rs73152707	3	86619077	Intergenic	-0.786	0.336	0.02	2	137th
rs970258	2	5138399	Intergenic	-0.786	0.336	0.02	2	140th
rs117860708	11	1537217	Intergenic	-0.788	0.336	0.019	2	141st
rs4676496	3	39456514	Intergenic	-0.788	0.336	0.019	2	142nd
rs11067262	12	114724621	*TBX3-AS1*	-0.789	0.336	0.019	2	144th
rs3098553	15	27631110	Intergenic	-0.789	0.336	0.019	2	143rd
rs9819308	3	1691812	Intergenic	-0.79	0.336	0.019	1	145th
rs10050775	5	38007940	Intergenic	-0.791	0.336	0.019	1	146th
rs10443173	1	86068071	*COL24A1*	-0.796	0.336	0.018	1	148th
rs144387708	12	119264395	Intergenic	-0.796	0.336	0.018	1	150th
rs3798105	5	133194937	*ENSG00000248245*	-0.796	0.336	0.018	1	149th
rs9903355	17	36580791	*GGNBP2*	-0.796	0.336	0.018	1	147th
rs7209200	17	5066645	Intergenic	-0.797	0.336	0.018	1	151st
rs651001	6	11569169	*TMEM170B*	-0.798	0.336	0.018	1	153rd
rs79496463	8	132904843	*TG*	-0.798	0.336	0.018	1	152nd
rs9813285	3	29329178	*RBMS3*	-0.804	0.336	0.017	1	154th
rs118071175	14	28515686	Intergenic	-0.805	0.336	0.017	1	155th
rs7930973	11	44159478	*EXT2*	-0.806	0.336	0.017	1	156th
rs11702120	21	23786163	Intergenic	-0.808	0.336	0.016	1	157th
rs7258235	19	2612120	*GNG7*	-0.811	0.336	0.016	1	158th
rs4292737	8	10401605	*MSRA*	-0.814	0.336	0.016	1	159th
rs112348322	4	118882809	*SYNPO2*	-0.833	0.336	0.013	1	160th
rs12886280	14	31829453	*NUBPL*	-0.834	0.336	0.013	1	161st

Table S4. Enrichment analysis identified pathways influencing the age of symptom onset among *C9orf72* carriers, related to Figure 4.

Enrichment analysis for decile ten was based on the variants that make up decile ten plus the *C9orf72* gene. g:Profiler maps intronic variants to their corresponding gene. Decile refers to the decile that yielded the pathways on enrichment analysis. No significant pathways were identified for deciles 1–4 and 9. GO:MF, Gene Ontology, molecular function; GO:BP, Gene Ontology, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Source	Term name	Category	Term ID	Adjusted p-value	Decile
GO:MF	ATP-dependent microtubule motor activity	Transport	GO:1990939	0.020	10
GO:MF	Motor activity	Transport	GO:0003774	0.020	10
GO:MF	Microtubule motor activity	Transport	GO:0003777	0.020	10
GO:BP	Vacuolar transport	Transport	GO:0007034	0.043	10
GO:BP	Cytosolic transport	Transport	GO:0016482	0.045	10
GO:MF	Tubulin binding	Transport	GO:0015631	0.020	10
GO:BP	Developmental growth involved in morphogenesis	Transport	GO:0060560	0.034	10
GO:BP	Developmental cell growth	Transport	GO:0048588	0.034	10
GO:BP	Cell growth	Transport	GO:0016049	0.040	10
GO:BP	Synaptic vesicle transport	Axonal transport	GO:0048489	0.034	10
GO:BP	Synaptic vesicle localization	Axonal transport	GO:0097479	0.035	10
GO:BP	Axon extension	Axonal transport	GO:0048675	0.040	10
GO:BP	Neuron projection extension	Axonal transport	GO:1990138	0.028	10
GO:BP	Axonogenesis	Axonal transport	GO:0007409	0.040	10
GO:BP	Lysosomal transport	Autophagy	GO:0007041	0.040	10
GO:BP	Positive regulation of autophagy	Autophagy	GO:0010508	0.040	10
GO:BP	Establishment of protein localization to mitochondrion	Mitochondria	GO:0072655	0.040	10
GO:BP	Protein targeting to mitochondrion	Mitochondria	GO:0006626	0.040	10
GO:BP	Protein localization to mitochondrion	Mitochondria	GO:0070585	0.041	10
KEGG	Amyotrophic lateral sclerosis	Neurodegeneration	KEGG:05014	0.001	10
KEGG	Pathways of neurodegeneration - multiple diseases	Neurodegeneration	KEGG:05022	0.002	10
GO:MF	Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	Other	GO:0016616	0.020	10
GO:MF	Oxidoreductase activity, acting on CH-OH group of donors	Other	GO:0016614	0.021	10
GO:BP	Phenol-containing compound metabolic process	Other	GO:0018958	0.040	10
GO:BP	Regulation of innate immune response	Defense response	GO:0045088	0.011	8
GO:BP	Positive regulation of response to biotic stimulus	Response to biotic stimulus	GO:0002833	0.017	8
GO:BP	Innate immune response-activating signaling pathway	Defense response	GO:0002758	0.017	8
GO:BP	Positive regulation of innate immune response	Defense response	GO:0045089	0.017	8
GO:BP	Pattern recognition receptor signaling pathway	Defense response	GO:0002221	0.017	8

GO:BP	Activation of innate immune response	Defense response	GO:0002218	0.017	8
GO:BP	Toll-like receptor signaling pathway	Receptor signaling pathway	GO:0002224	0.017	8
GO:BP	Immune response-activating signaling pathway	Signal transduction	GO:0002757	0.023	8
GO:BP	Positive regulation of defense response	Defense response	GO:0031349	0.023	8
GO:BP	Immune response-regulating signaling pathway	Signal transduction	GO:0002764	0.025	8
GO:MF	2-oxoglutarate-dependent dioxygenase activity	Oxidoreductase activity	GO:0016706	0.031	8
GO:MF	Dioxygenase activity	Oxidoreductase activity	GO:0051213	0.037	8
GO:BP	Stem cell differentiation	Cellular developmental process	GO:0048863	0.049	8
GO:MF	Transcription coactivator binding	Transcription factor binding	GO:0001223	0.006	7
GO:MF	Transcription coregulator binding	Transcription factor binding	GO:0001221	0.018	7
GO:BP	Neutral lipid metabolic process	Cellular lipid metabolic process	GO:0006638	0.016	6
GO:BP	Acylglycerol metabolic process	Cellular lipid metabolic process	GO:0006639	0.016	6
GO:BP	Endoplasmic reticulum to Golgi vesicle-mediated transport	Intracellular transport	GO:0006888	0.016	6
GO:MF	Small GTPase binding	Enzyme binding	GO:0031267	0.019	6
GO:MF	GTPase binding	Enzyme binding	GO:0051020	0.019	6
GO:MF	Molecular function inhibitor activity	Molecular function regulatory activity	GO:0140678	0.019	6
GO:MF	Enzyme inhibitor activity	Enzyme regulator activity	GO:0004857	0.019	6
GO:BP	Golgi vesicle transport	Vesicle-mediated transport	GO:0048193	0.026	6
GO:BP	Glycerolipid metabolic process	Cellular lipid metabolic process	GO:0046486	0.030	6
GO:MF	T cell receptor binding	Signaling receptor binding	GO:0042608	0.002	5

Table S5. The list of genes used in the gene-gene similarity network *GREP* analysis, related to Figure 5.

Seed genes refer to the genetically identified genes in decile ten.

Rank	Gene
Seed gene	*ABLIM2*
Seed gene	*C9orf72*
Seed gene	*DIP2B*
Seed gene	*DNAH17*
Seed gene	*KIF5A*
Seed gene	*LINC00598*
Seed gene	*ME2*
Seed gene	*OCA2*
Seed gene	*PRKN*
Seed gene	*SAMM50*
Seed gene	*SDR9C7*
1	*RIMS3*
2	*CAMKV*
3	*CLASP2*
4	*TUBB4A*
5	*KCNQ2*
6	*BSN*
7	*KCNC1*
8	*MAPK4*
9	*SNCB*
10	*CLVS2*
11	*CNTN2*
12	*SEPTIN3*
13	*NCAN*
14	*KIF1A*
15	*LGI3*
16	*CDH22*
17	*CDHR1*
18	*ADCYAP1R1*
19	*OTUD7A*
20	*KIF1B*
21	*SLC8A2*
22	*TMEM151B*
23	*TRIM9*
24	*PHF24*
25	*VSTM2B*
26	*CACNA1B*
27	*DOCK3*

28	*NDRG4*
29	*ATP1A3*
30	*GPM6B*
31	*CRHR1*
32	*RBFOX1*
33	*TPPP*
34	*GRIA2*
35	*NEFL*
36	*SLC35F1*
37	*SNAP91*
38	*KCNJ9*
39	*HMP19*
40	*ACTL6B*
41	*CARMIL3*
42	*PLP1*
43	*SYP*
44	*CELSR2*
45	*SULT4A1*
46	*NEFM*
47	*RIMBP2*
48	*CPLX2*
49	*ASTN1*
50	*CNTN1*
51	*SHISA7*
52	*STMN4*
53	*SYN2*
54	*GNAL*
55	*DLG2*
56	*KCNA2*
57	*ARHGEF4*
58	*KIF5C*
59	*IGSF11*
60	*NSG1*
61	*CELF3*
62	*ZDHHC22*
63	*HRH3*
64	*GDAP1L1*
65	*SCRT1*
66	*INA*
67	*NRXN1*
68	*WNK2*
69	*PTPN5*
70	*MMD2*

71	*HPCAL4*
72	*CHRNB2*
73	*JPH3*
74	*ATP2B3*
75	*MAPK8IP2*
76	*HAPLN2*
77	*MYT1*
78	*PAFAH1B1*
79	*CDK5R1*
80	*NOL4*
81	*SYNPR*
82	*ZNF536*
83	*GALNT8*
84	*CTNNA2*
85	*ST8SIA3*
86	*ABCG4*
87	*LINGO1*
88	*ADAM22*
89	*AMER2*
90	*PAK5*
91	*PPFIA3*
92	*SH3GL2*
93	*PREPL*
94	*ELMOD1*
95	*ATP2B2*
96	*LRRC4B*
97	*SORCS1*
98	*DUSP26*
99	*JPH4*
100	*MAST1*
101	*BRSK2*
102	*CA11*
103	*ELAVL3*
104	*GNG3*
105	*STXBP5L*
106	*PTPRZ1*
107	*MAPT*
108	*ZCCHC12*
109	*RIMS4*
110	*SYT4*
111	*TMEM179*
112	*CLASP1*
113	*ELAVL4*

114	*SOCS7*
115	*ADGRB3*
116	*RUNDC3A*
117	*SCN3B*
118	*PSD2*
119	*EFR3B*
120	*GARNL3*
121	*GRID1*
122	*ZDHHC11B*
123	*TMEM63C*
124	*RAB3C*
125	*CHD5*
126	*ADGRL3*
127	*DPYSL5*
128	*GRIK3*
129	*PHYHIPL*
130	*CCDC177*
131	*GRIA4*
132	*FBXL16*
133	*IGSF21*
134	*SEZ6L*
135	*TTBK1*
136	*CNTFR*
137	*NRXN2*
138	*LANCL1*
139	*OLFM3*
140	*SCG3*
141	*ABCC8*
142	*UNC13A*
143	*SH3GL3*
144	*GRIK5*
145	*NELL1*
146	*ATP1B2*
147	*SCN4B*
148	*CADM2*
149	*RIPPLY2*
150	*B3GAT1*
151	*IGSF9B*
152	*ANK2*
153	*CADM4*
154	*UBE2QL1*
155	*PCDH8*
156	*SYN1*

157	*ADGRA1*
158	*ADAM11*
159	*KCNB2*
160	*SMIM10L2A*
161	*NXPH1*
162	*CHRNA4*
163	*NAPB*
164	*GAP43*
165	*MAP3K9*
166	*PGBD5*
167	*LRRTM3*
168	*PIP4K2B*
169	*RAB3A*
170	*SLIT1*
171	*TCEAL5*
172	*GABRG2*
173	*GNAO1*
174	*GRID2*
175	*PPP2R2C*
176	*RUFY3*
177	*CASKIN1*
178	*ADGRL1*
179	*SORCS3*
180	*SPTBN4*
181	*CNTNAP4*
182	*PDZD4*
183	*PEX5L*
184	*SOX8*
185	*CSPG5*
186	*ATCAY*
187	*RPRD1A*
188	*RGS8*
189	*PHF21B*
190	*ACTN2*
191	*GAD2*
192	*SLC6A11*
193	*SLC32A1*
194	*KCNJ4*
195	*MEGF11*
196	*POU3F3*
197	*SV2B*
198	*ELAVL2*
199	*IGLON5*

200	*LRRC3B*

Table S6. The list of repurposable, approved drugs identified by the GREP analysis, related to Figure 5.

Drug indications and mechanisms of action were curated from the Drugbank database [S15]. GABA, gamma-aminobutyric acid; GABA(A), gamma-aminobutyric acid Type A; VDCC, Voltage-dependent calcium channel; KCNK3, Potassium Two Pore Domain Channel Subfamily K Member 3; CHRNA4, Cholinergic Receptor Nicotinic Alpha 4 Subunit; CaV2.2, Neuronal voltage-gated N-type Calcium Channel.

Drug	Indication	Mechanism of action
Acamprosate	Withdrawal symptoms of alcoholism	Analogue of GABA
Adinazolam	Seizures	GABA positive allosteric modulator
Alprazolam	Anxiety and panic disorders	GABA(A) receptor positive allosteric modulator
Betahistine	Vertigo	H1-receptor agonist
Brivaracetam	Seizures	Unknown, synaptic GABA release
Bromazepam	Anxiety and panic disorders	GABA(A) receptor positive allosteric modulator
Chlordiazepoxide	Withdrawal symptoms of alcoholism	GABA(A) receptor positive allosteric modulator
Cinolazepam	Sleep disorders	GABA positive allosteric modulator
Clobazam	Seizures	GABA positive allosteric modulator
Clonazepam	Anxiety and panic disorders	GABA(A) receptor positive allosteric modulator
Clotiazepam	Anxiety	GABA(A) receptor positive allosteric modulator
Desflurane	Anesthetic	GABA(A) receptor positive allosteric modulator
Diazepam	Anxiety and alcohol withdrawal	GABA(A) receptor positive allosteric modulator
Enflurane	Anesthetic	GABA(A) receptor potentiator
Estazolam	Insomnia	GABA(A) receptor positive allosteric modulator
Eszopiclone	Insomnia	GABA(A) receptor potentiator
Ethchlorvynol	Insomnia	GABA(A) receptor positive allosteric modulator
Etizolam	Anxiety and insomnia	GABA(A) receptor positive allosteric modulator
Etomidate	Anesthetic	GABA receptor subunit alpha-1 potentiator
Fludiazepam	Convulsion	GABA receptor subunit alpha-1 agonist
Flurazepam	Anxiety and convulsion	GABA(A) receptor positive allosteric modulator
Gabapentin	Convulsion	VDCC subunit alpha-2/delta-1 inhibitor
Glutethimide	Sedative	GABA receptor subunit alpha-1 agonist
Halazepam	Seizures and anxiety	GABA(A) receptor positive allosteric modulator
Halothane	Anesthetic	KCNK3 binder
Isoflurane	Anesthetic	GABA receptor subunit alpha-1 agonist
Levetiracetam	Seizures	CaV2.2 subunit alpha-1B inhibitor
Lorazepam	Seizures, anxiety, and panic disorders	GABA(A) receptor positive allosteric modulator
Lormetazepam	Anxiety	GABA(A) receptor positive allosteric modulator
Meprobamate	Anxiety	GABA agonist
Metharbital	Convulsion	GABA receptor subunit alpha-2 potentiator
Methoxyflurane	Anesthetic	GABA receptor subunit alpha-1 agonist
Methylphenobarbital	Seizures	Depressant of the central nervous system
Midazolam	Anxiety and convulsion	GABA(A) receptor positive allosteric modulator
Nicotine	Smoking cessation.	Neuronal CHRNA4 agonist

Nitrazepam	Anxiety and insomnia	GABA positive allosteric modulator
Oxazepam	Withdrawal symptoms of alcoholism	GABA(A) receptor positive allosteric modulator
Pentobarbital	Seizures and sedation	GABA(A) receptor potentiator
Pitolisant	Narcolepsy	Antagonist at the histamine H3 receptor
Prazepam	Anxiety	GABA(A) receptor positive allosteric modulator
Primidone	Seizures	GABA receptor subunit beta-2 potentiator
Propofol	Sedative	GABA receptor subunit beta-2 potentiator
Quazepam	Insomnia	GABA positive allosteric modulator
Sevoflurane	Anesthetic	GABA(A) receptor agonist
Stiripentol	Seizures	GABA(A) receptor agonist allosteric modulator
Talbutal	Sedative	GABA receptor subunit alpha-2 potentiator
Temazepam	Anxiety and panic disorders	GABA(A) receptor positive allosteric modulator
Topiramate	Seizures	GABRA1 agonist
Triazolam	Insomnia	GABA(A) receptor positive allosteric modulator
Varenicline	Smoking cessation	CHRNA4 partial agonist
Ziconotide	Chronic pain	CaV2.2 subunit alpha-1B inhibitor

Table S7. *KIF5A* genotypes influenced the age of symptom onset among *C9orf72* carriers, related to Figure 4.

The table summarizes the age at onset of *C9orf72* individuals carrying the rs113247976 (chr12:57581917) variant in the *KIF5A* gene. N, number of cases.

Variant	Status	Genotype	Age at onset mean	Age at onset standard deviation	Cases (n)
rs113247976 (*KIF5A*)	*C9orf72* carrier	CC	57.81	9.49	780
		CT	54.19	11.09	37
		TT	NA	NA	0
	Non-carrier	CC	60.08	12.54	6,827
		CT	60.06	12.14	206
		TT	61.75	11.76	4

Table S8. The sources of the cohorts used in this study, related to Star Methods.

Dataset	Sample size	Reference
Reference	12,577 ALS cases & 23,475 controls	16
Training	7,030 ALS cases & 34,235 controls	16,17
Test (*C9orf72*)	817 ALS/FTD	16,18
Replication (*C9orf72*)	699 ALS/FTD	14

Table S9. The clinical descriptions of the test cohort, related to Star Methods.

The test cohort is composed of 817 *C9orf72* carriers. FTLD, frontotemporal lobar degeneration; FTLD-Tau, frontotemporal lobar degeneration with tau pathology; PSP, progressive supranuclear palsy; FTLD TDP, frontotemporal lobar degeneration with TDP-43 inclusions; FTLD-U, frontotemporal lobar degeneration with ubiquitin-positive inclusions; NOS, not otherwise specified. *One FTLD NOS patient was initially clinically misdiagnosed as PSP.

Type	Subtype	Size
Asymptomatic		13
Clinical	ALS	666
Clinical	FTD Motor neuron disease	81
Clinical	FTD Behavioral variant	23
Clinical	FTD Language variant NOS	1
Clinical	FTD Nonfluent variant	3
Clinical	FTD NOS	2
Clinical	FTD Semantic variant	3
Pathological	FTLD NOS*	9
Pathological	FTLD Tau (PSP)	2
Pathological	FTLD TDP Type A	4
Pathological	FTLD TDP Type B	1
Pathological	FTLD TDP Type Unknown	6
Pathological	FTLD U	3

Table S10. The cell lines used for the drug validation experiments, related to Figure 6 and Figure S7.

F, female; M, male.

iPSC cell line	Source	Clinical remarks	Mutation	Race	Sex	Age at collection	Supplier
CS14iCTR-nxx	Fibroblast	Clinically normal and healthy volunteer	Unknown	Caucasian	F	52	Cedars-Sinai
GM23338	Fibroblast	Clinically normal and healthy volunteer	Unknown	Caucasian	M	55	Coriell Biorepository
MIFF1	Fibroblast	Clinically normal and healthy volunteer	Unknown	Caucasian	M	<1 month old	University of Sheffield
CS02iCTR-NTn1	PBMC	Clinically normal and healthy volunteer	Unknown	Caucasian	M	51	Cedars-Sinai
ALS-183-C9	Fibroblast	ALS, age of onset = 48; disease duration = 27 months.	*C9orf72* repeat expansion	Caucasian	M	50	University of Sheffield
ALS-78	Fibroblast	ALS, age of onset = unknown; disease duration = 31.7 months	*C9orf72* repeat expansion	Caucasian	M	66	University of Sheffield
CS28iALS-C9nxx	Fibroblast	ALS, age of onset = 46; site of onset = left upper extremity	*C9orf72* repeat expansion	Caucasian	M	47	Cedars-Sinai
CS29iALS-C9nxx	Fibroblast	ALS, age of onset = unknown; disease duration = unknown	*C9orf72* repeat expansion	Caucasian	M	47	Cedars-Sinai
CS52iALS-C9nxx	Fibroblast	ALS, age of onset = 57; disease duration = 48 months	*C9orf72* repeat expansion	Unknown	M	49	Cedars-Sinai
CS29iALS-C9n1.ISOxx	Fibroblast	ALS, Age of onset: Unknown; disease duration, Unknown	Isogenic control line of CS29iALS-C9nxx	Caucasian	M	47	Cedars-Sinai
CS52iALS-C9n6.ISOxx	Fibroblast	ALS, age of onset = 57; disease duration = 48 months	Isogenic control line of CS52iALS-nxx	Unknown	M	49	Cedars-Sinai

Table S11. The antibodies used in motor neuron staining, related to Star Methods.

ChAT, Choline acetyltransferase; MAP2, Microtubule-associated protein 2; Islet 1/2, ISL LIM Homeobox 1/2.

	Host	Dilution	Wavelength (nm)	Supplier	Catalogue Number
Primary Antibodies					
Beta III tubulin	Mouse	1:1000	-	Biolegend	801201
Caspase-3	Rabbit	1:200	-	Merck Millipore	AB3623
ChAT	Goat	1:100	-	Merck Millipore	AB144P
MAP-2	Guinea pig	1:1000	-	Synaptic systems	188004
Islet 1/2	Rabbit	1:500	-	Abcam	ab109517
NeuN	Mouse	1:1000	-	Millipore	MAB377
Secondary Antibodies					
Anti-rabbit	Donkey	-	488	Thermofisher	A21206
Anti-rabbit	Donkey	-	568	Thermofisher	A10042
Anti-mouse	Donkey	-	568	Thermofisher	A10037
Anti-mouse	Donkey	-	488	Thermofisher	A21202
Anti-goat	Donkey	-	555	Thermofisher	A21432
Anti-guinea pig	Goat	-	647	Thermofisher	A21450

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