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Supplementary Materials for

Human genetics and neuropathology suggest a link between miR-218 and amyotrophic lateral sclerosis pathophysiology

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/11/523/eaav5264/DC1)

Data file S1 (Microsoft Excel format). Individual-level data for miR-218 expression.

Data file S2 (Microsoft Excel format). NanoString nCounter data for miRNAs measured in lumbar ventral horns.
Data file S3 (Microsoft Excel format). Source data for Kv10.1 (KCNH1) Western blot studies.
Data file S4 (Microsoft Excel format). Source data for Kv4.2 (KCND2) Western blot studies.

Materials and Methods

Ethical approval of studies

All subjects contributed DNA for genetic studies approved by local IRBs at the submitting sites. Human materials were studied under approval of Institutional review boards at U.C. San Diego and Weizmann Institute of Science Institutional Review Board. miRNA analysis was performed as previously described (*3*). All animal experiments were approved by Weizmann Institute of Science IACUC (protocols 33230117-2; 24390116-2; 32271216-1; 23690116-1; 23660116-2; 07201113-1; 02170413-2; 07811212-2).

Human genetics

Project MinE ALS sequencing consortium (*31*), comprises of seven independent cohorts: the Netherlands, Belgium, Ireland, Spain, United Kingdom, United States and Turkey, served as a discovery cohort. Whole-genome sequencing (WGS) of 6,579 samples was performed on Illumina HiSeq2000 or HiSeqX platforms. Reads were aligned to human genome build hg19 and sequence variants called with Isaac Genome Alignment Software and variant caller (*61*). Individual genomic variant call format files (GVCFs) were merged with 'agg' tool: a utility for aggregating Illumina-style GVCFs. Multi-allelic variants were excluded from analyses. Following completion of the raw data merge, multiple quality control (QC) filtering steps were performed: (i) setting genotypes with GQ<10 to missing; (ii) removing low-quality sites (QUAL< 30 and QUAL< 20 for SNPs and indels, respectively); (iii) removing sites with missingness > 10%. (iv) Samples excluded if deviated from mean by more than 6SD for total numbers of SNPs, singletons and indels, Ti/Tv ratio, het/hom-non-ref ratio and inbreeding (by cohort). (v) missingness > 5%, (vi) genotyping-sequence concordance (made possible by genotyping data

generated on the Illumina Omni 2.5M SNP array for all samples; 96% concordance), (vii) depth of coverage, (viii) a gender check (to identify mismatches), (ix) relatedness (drop samples with >100 relatedness pairs). Related individuals were further excluded until no pair of samples had a kinship coefficient > 0.05). (x) missing phenotype information. A total of 6119 samples (4281 ALS cases and 1838 healthy controls) passed all QC and were included in downstream analysis. Per-nucleotide site QC was performed on QC-passing samples only, for biallelic sites: variants were excluded from analysis based on depth (total DP < 10,000 or > 226,000), missingness > 5%, passing rate in the whole dataset < 70%, sites out of Hardy–Weinberg equilibrium (HWE; by cohort, controls only, p < 1x10-6) and sites with extreme differential missingness between cases and control samples (overall and by cohort, $p < 1x10^{-6}$). Depth of coverage for all miR-218 variants was >25x.

BED file containing genomic coordinates for miR-218-1,2 precursors (according to miRBase v20 (*32*)) on human genome build 19 (hg19), Chr5:168195173-168195236; Chr4: 20529922-20529986, was created and variants in these regions were than extracted from the whole-genome sequencing data using vcftools (*62*). A replication study comprised of data from the Genomic Translation for ALS Care (GTAC), the ALS Sequencing Consortium and the New York Genome Center (NYGC) ALS Consortium genomes and exomes from ALS cases (5336) and non-ALS controls (21388). QC filtering steps were: (i) setting genotypes with GQ<10 to missing; (ii) removing low-quality sites (QUAL) < 30 for SNPs; (iii) removing sites with missingness > 10%; (iv) depth of coverage >10x with >85% of bases in the miR-218-2; (v) a gender check (to identify mismatches); (vi) related individuals by kinship (3 degrees); (vii) missing

phenotype information; (viii) ancestry harmonization with iterative EIGENSTRAT cycles. Samples excluded if deviated from mean by more than 1SD for the first 4 principal components. Finally, 22 samples overlapping with Project Mine dataset were excluded by KING (*63*). A total of 10,491 samples (3457 ALS cases and 7034 healthy controls) passed all QC. Pass filter rare biallelic variants were included for downstream analyses, tested for Hardy–Weinberg equilibrium and for extreme differential missingness between cases and control samples (overall $p < 1x10^{-6}$).

To further increase sample size, as part of a validation effort, we harvested rare biallelic miR-218-2 variants from 62,784 non-ALS genomes that were available to us through NHLBI's Trans-Omics for Precision Medicine (TOPMed) program.

Association of rare variants, in cases versus controls, in pre-miR-218 genomic region, was evaluated by SKAT-O using 'SKAT' R package (*33*) with sex and the top 10 PCs as covariates for Project MinE discovery cohort and with sex, platform and the top 3 PCs for replication study. Summary statistics from each study cohort was used to carry out region-based rare variant meta-analysis, by SKAT-O using 'MetaSKAT' R package (*34*).

TOPMed covariates information is not available, therefore, joint analysis was used, by one tailed Chi square test with Yate’s correction, instead of a meta-analysis. We considered a variant to be rare if the minor allele frequency (MAF) <0.01.

Immortalized cell lines and transfection

Human embryonic kidney 293T (HEK293T) and HEPG2 cell lines were maintained in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% FBS, 1% L-glutamine and 1% Penicillin-streptomycin (Biological industries) at 37 °C with 5%

CO2 incubation. Cells were seeded onto 24-well plates (Corning) 24 h prior to transfection, in antibiotic- free media and transfected at 70–80% confluence, using Lipofectamine 2000 (Life Technologies) with 800 ng plasmid DNA per 1/24-well plate. For small RNA next generation sequencing (NGS), cells were co-transfected using 1 nM miR-214-3p microRNA mimic (Integrated DNA Technologies, Inc., table S2). Each well was considered as a single technical replicate.

Neuronal cultures

C2 hiPSCs were kindly provided by Dr. Jacob Hanna (Weizmann Institute of Science), cultured and differentiated as previously described (*19*). Mixed populations of motor neurons were gained, with moderate (30-50% motor neuron enrichment). Primary motor neurons were isolated and cultured as previously described (*23*). Briefly, Sprague-Dawley timed-pregnant females were sacrificed at rat embryonic day 14.5 (E14.5), and spinal cords were dissected and dissociated enzymatically with papain (2mg/ml, Sigma). Motor neurons were separated over a gradient of Optiprep (Sigma) and plated on 13mm coverslips (200,000 cells/ coverslip, Thermo scientific), pre-coated with 3µg/ml poly-ornithine (Sigma) and 3µg/ml laminin (Gibco). Motor neurons were cultured with Neurobasal medium (Gibco) supplemented with 2% B27 (Gibco) 2% horse serum (Sigma), X1 Glutamax (Gibco) and 1ng/ml CNTF and GDNF (Peprotech). To inhibit glial overgrowth, 200 µM fluorodeoxyuridine (Sigma) was added after 5 days of in vitro culture (DIV). For RNAseq analysis, primary motor neurons were isolated from time pregnant C57BL/6 mice at embryonic day 13.5 (E13.5). Primary cultured hippocampal neurons were prepared from male and female P0 Sprague-Dawley rat pups (Envigo). CA1 and CA3 were isolated, digested with 0.4 mg ml^{-1} papain (Worthington)

and plated onto glass coverslips percolated with 1:30 Matrigel (Corning). Cultured neurons were maintained in a 5% CO_2 humidified incubator with Neurobasal A medium (Invitrogen) containing 1.25% FBS (Biological Industries), 4% B-27 supplement (Gibco) and 2 mM Glutamax (Gibco) and plated on coverslips in a 24-well plate at a density of 65,000 cells per well. To inhibit glial overgrowth, 200 μM fluorodeoxyuridine (Sigma) was added after 5 DIV. siRNAs were designed as either single chemically modified 2'OMe DsiRNA molecules, or as pools of 20 unmodified DsiRNA molecules (Integrated DNA Technologies, Inc., table S2). Non-targeting scramble DsiRNAs were used as control (NC5). siRNAs were encapsulated in Neuro9 nanoparticles (Precision NanoSystems, Inc.) (*64*). Primary motor neurons were transfected with siRNA at a final concentration of 1μg/ml, on 9 DIV. Sequences are listed in table S2.

Whole-cell patch clamp recordings

Recordings were performed under QImaging QIClick-R-F-M-12 CCD camera in a chamber perfused with Tyrode's medium (150 mM NaCl, 4 mM KCl, 2 mM MgCl2, 2 mM CaCl2, 10 mM D-glucose, 10 mM HEPES; 320 mOsm; pH 7.35; 29 °C) at 0.5 ml / min. Pulled borosilicate glass pipettes (Sutter Instrument BF100-58-10) with resistances 3–5 M, were filled with standard intracellular current clamp recording solution (135 mM potassium gluconate, 4 mM KCl, 2 mM NaCl, 10 mM HEPES, 4 mM EGTA, 4 mM Mg-ATP, 0.3 mM Na-GTP; 280 mOsm; pH 7.3). Recordings were performed using a MultiClamp 700B amplifier, filtered at 3 kHz and digitized at 10 kHz using a Digidata 1440A digitizer (Molecular Devices). Data acquired with pClamp 10 software (Molecular Devices) and analyzed with Matlab (Mathworks). Current clamp recordings were obtained from cells with over shooting action potential and a stable resting membrane

potential. Each recorded neuron received a series of 500ms long current steps ranging from –100 to +500 pA in 20 pA increments. The current threshold for action potential was determined as the minimal current required for evoking an action potential during the current injection step. Spikes frequency was measured as the number of spikes evoked in response to 300pA current injection step.

Calcium imaging

Primary motor neurons were imaged at days 12-13 in vitro (12 DIV). Primary hippocampal neurons were imaged at 15 DIV. Cells plated on coverslips were incubated in 2 μM Fluo-2 calcium indicator (Teflabs) for 45 minutes, in a solution containing: 129 mM NaCl, 4mM KCl, 1 mM MgCl2, 2mM CaCl2, 4.2mM glucose and 10mM HEPES, with pH adjusted to 7.4 with NaOH, and osmolarity to 320 mOsm adjusted with sucrose. Cells were washed twice and imaged at a wave length of 488nm with a Zeiss710 confocal laser scanning microscope, using a 40x objective. On each coverslip, 3 fields containing at least 10 neurons were chosen randomly for capture and analysis. At least 3 different coverslips were used per condition. Events in which $\Delta F/F > 0.5$ were included in the analysis.

Retrograde labeling of neuron subtypes

Motor neurons were back labeled via AAV-GFP injection into the mouse hindlimb Gastrocnemius (FF motor neurons) or Soleus (FR, S motor neurons) muscles. Mice were deeply anesthetized with ketamine/xylazine (0.25 mL, 10% (vol/vol), administered i.p.) before organ perfusion with PBS and 2.5% paraformaldehyde (PFA; as in (*65*)).

miRNA in situ hybridization (ISH)

miR-218 in situ hybridization in human tissues on 5µ FFPE sections followed (*66*). miR-218 ISH mix, 30 pmol/100 µL in hybridization buffer (Enzo Life Sciences), was placed on tissue sections, heated to 60°C for 5 minutes and hybridizated at 37°C overnight. Sections were washed (0.2X SSC with 2% bovine serum albumin) at 55°C for 10 minutes, treated with anti-digoxigenin – alkaline phosphatase conjugate (1:150 dilution in pH 7 Tris buffer; Roche) at 37°C for 30 minutes, develop with NBT/BCIP (ThermoFisher; 34042) until chromogen signal appeared at 15 – 30 minutes, counterstained with nuclear fast red for 3 to 5 minutes, rinsed and mounted with coverslips. Optical Density (OD) was calculated as log of (max intensity/Mean intensity).

miR-218 in situ hybridization in mouse tissues was performed on frozen spinal cord sections as in (*67*). Slides were treated with 2µg/ml Proteinase K, fixed in EDC (Sigma), acetylated in acetic anhydride/ triethanolamine solution and hybridized in a hybridization buffer containing 40nM of 5' + 3' DIG-labeled miR-218 or scrambleLNA probe (Exiqon). Slides were washed, incubated with alkaline phosphatase-conjugated goat-anti-DIG Fab fragments (1:1000, Roche) at 4ºC overnight, developed with BCIP/NBT (Sigma), as in (*68*), mounted with Immu-mount (Shandon) and covered with a glass coverslip.

miR-218 lentiviruses

500bp flanking the pri-miR-218-2 sequence were purchased in pMX vectors (GeneArt, Invitrogen, table S3), and a 625bp fragment of the human pri-miR-218-1 were digested with BamHI-EcoRI and subcloned to pUltra-Hot vector (a gift from Malcolm Moore, Addgene plasmid # 24130) downstream of the human Ubiqiutic C promoter and mCherry-P2A. To generate miR-218 inhibitor encoding lentivirus, an approximately 250bp fragment of the commercial inhibitor, miR-Zip-218 plasmid (SBI), driven by a U6

promoter was subcloned into pGEM vector (Promega) with Fw 5'-CGTACGTAAAGATGGCTGTGAGGGACAG-3' and Rev 5'-CGTACGTAAGAGAGACCCAGTAGAAGCAAAAAG-3' primers, and then into the 3'LTR of pUltra-Hot vector using a SnaBI restriction site. Lentiviral particles were produced in HEK-293T cells, transfected with pUltra-Hot vector and with psPAX2 and pMD2.G packaging plasmids (a gift from Didier Trono, Addgene plasmid numbers 12259 and 12260). Supernatants were concentrated by ultracentrifugation, re-suspended in DMEM media (Gibco) and titrated. Primary neuronal cultures were infected at a multiplicity of infection (MOI) of 1 or of 10, on 1 DIV.

Luciferase assays

The rat KCND2 and KCNH1 3′UTRs were subcloned into psiCHECK2 vector (Promega) and transfected into HEK-293T cells with JET-PEI reagent (Poly Plus) together with miRVec-control and miRVec-218 plasmids, that were a gift from Reuven Agami (NKI, The Netherlands). For each well of a 24-well plate, a 50 ng psiCHECK2 plasmid DNA and 150 ng miRVec plasmid DNA were used; each well represents one technical replicate.72 h post-transfection cells were harvested using the Dual luciferase reporter assay system (Promega).

Protein analysis

Primary motor neurons were harvested on E13.5, plated, transduced with lentivruses and harvested after 5 days in culture. Cell pellet lysed in (50mM Tris pH7.4, 40mM NaCl, 1mM EDTA pH8, 0.5% TritonX-100, 50mM NaF, 10mM Na pyrophosphate, 10mM Na beta-glycerol phosphate, 1x phosphatase inhibitor (Roche) and 1x proteinase inhibitor (Roche)) and equal amounts of protein were loaded per lane for SDS-PAGE. After

electro-transfer, blots were developed with rabbit anti Kv4.2 antibody (Proteintech Group, Rosemont, IL catalog# 21298-1-AP, RRID:AB_10733102, 1:2000), rabbit anti Kv10.1 antibody (Abcam, catalog# ab101174, RRID:AB_10862156, 1:500) and rabbit anti Tubulin Beta-III (TUBB3) (Biolegend, catalog# 802001, RRID:AB_2564645, 1:100,000) using chemiluminescence. Samples were loaded and quantified in duplicates on each gel. Densitometric analysis of specific bands was performed using ImageJ (NIH).

RNA analysis

Total RNA from cultured cells was isolated using DirectZol RNA miniprep Kit or miRNeasy micro Kit (Qiagen) and reverse transcribed using the miScript II RT Kit (Qiagen). Quantitative real-time PCR was performed with the LightCycler480 (Roche) or StepOnePlus (Thermo Fisher Scientific), in >3 independent biological repeats and technical duplicates. For miRNAs we used miScript SYBR Green PCR (Qiagen) or TaqMan microRNA assays (Thermo-Fisher Scientific). mRNA quantified with KAPA SYBR fast qPCR (KAPA biosystems). Hypoxanthine phosphoribosyltransferase 1 (Hprt) and β–actin were used as references for normalization of mRNA expression, and U6 for normalization of miRNA. Primer sequences are described in table S4. For small RNA next generation sequencing (NGS), cDNAs were prepared from 10 ng of total RNA using the QIAseq™ miRNA Library Kit and QIAseq miRNA NGS 48 Index IL (Qiagen). cDNA libraries were pooled and sequenced on a single NextSeq 500 flow cell (Illumina), with 75bp single read. Data was analyzed using the GeneGlobe analysis web tool (Qiagen), and further processed by in-house script for discovery of pre-miRNA sequences (source code: https://github.com/TsviyaOlender/mir-218). miR-218-5p reads were normalized to the reads of miR-214-3p miRNA mimic. For mRNA NGS, cDNAs

were prepared following (*69*) and sequenced on one a single lane in a Hiseq2500 (Illumina) with 50bp single read. Fasta files for each sample were generated by the usage of Illumina CASAVA-1.8.2 software. Reads for each sample were mapped independently using TopHat2 version (v2.0.10) (*70*) against the mouse genome build mm9. Approximately, 85-90% mapping rate was observed. Only uniquely mapped reads were used to determine the number of reads falling into each gene with the HTSeq-count script (0.6.1p1) (*71*). Differentially expressed genes were calculated with the DESeq2 package (v1.4.5) (*72*). Genes that were expressed at least on one sample were considered. Differentially expressed genes, were determined by P-adj < 0.05 and an absolute fold change >1.5. Hierarchical clustering using Pearson dissimilarity and complete linkage was performed in order to explore a pattern of gene expression. Clustering analysis was performed with Matlab software (8.0.0.783).

NanoString

Human spinal cord RNA was extracted using Trizol, tested by NanoDrop (Thermo Scientific) for O.D. of 260/280 between 1.9 - 2.1 and hybridized for 18 hrs. against the nCounter Human v2 miRNA Panel (798 unique human miRNA barcodes) by the nCounter System (NanoString Technologies). Data from 280 fields of view per sample was analyzed with nSolver.

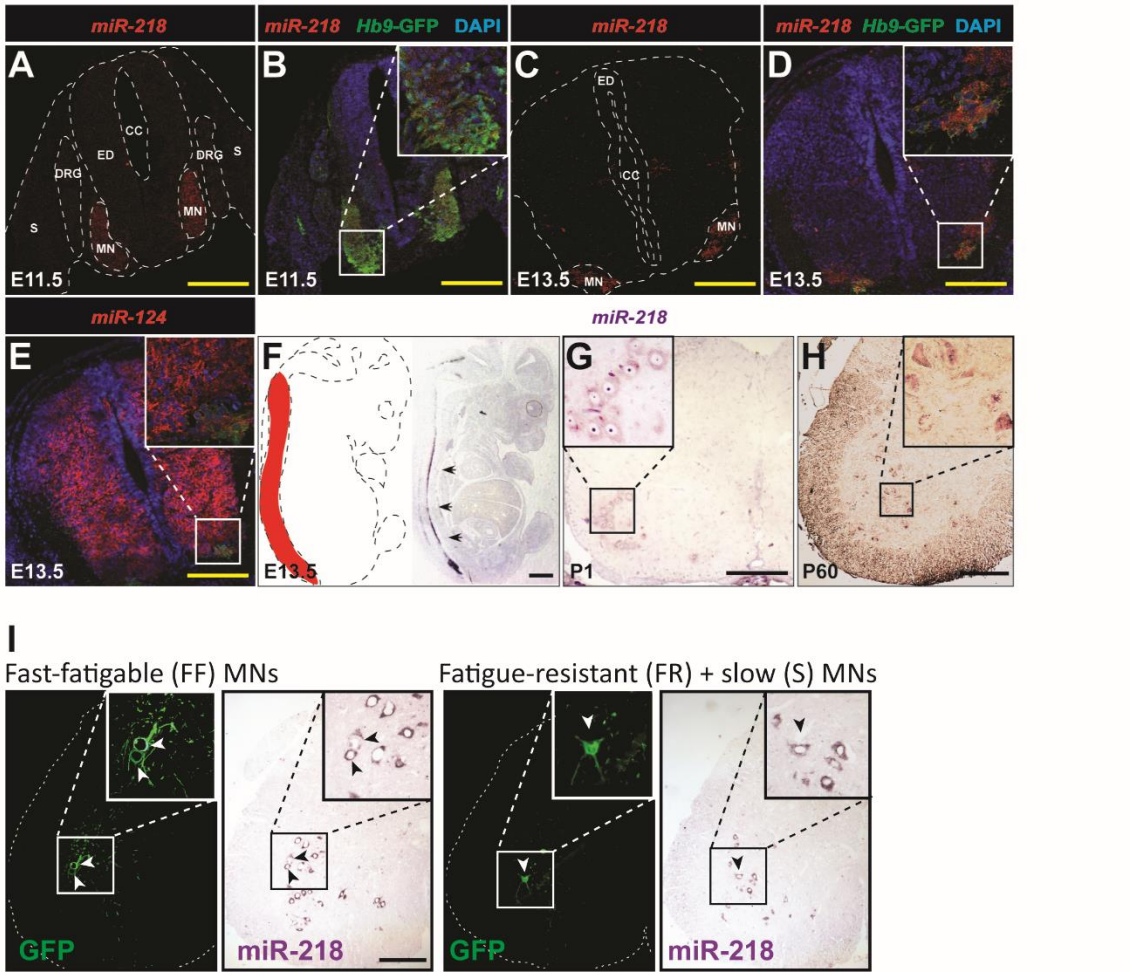

Fig. S1. miR-218 is highly and specifically expressed in human and murine spinal motor neurons. (**A** and **C**) miR-218 fluorescent in situ hybridization on transverse sections of mid-gestation mouse embryos (red) and (**B** and **D**) co-localization of motor neuron reporter transgene Hb9-GFP (green). CC-central canal; ED-ependymal layer; DRG-dorsal root ganglion; MN-motor neurons; S-somites. (**E**) In situ hybridization of pan-neuronal miR-124. Merged micrographs depict nuclei by DAPI counterstaining. (**F**) Alkaline Phosphatase chromogen development of miR-218 in situ hybridization on a mid-sagittal section of E13.5 mouse embryo. Arrowheads indicate the localization of motor neurons. miR-218 in situ hybridization on transverse hemisections of (**G**) newborn or (**H**) adult mouse spinal cord. Inset - large motor perikaryon cluster. (**I**) Transverse hemisections of adult mouse spinal cord, retrogradely labeled by AAV9-GFP injection into the lateral gastrocnemius (FF motor neurons, left) or soleus (FR/S motor neurons, right) and accompanied by miR-218 in situ hybridization. Arrowheads depict soma of GFP-labeled, lumbar motor neurons. Scale bars - 200 µm.

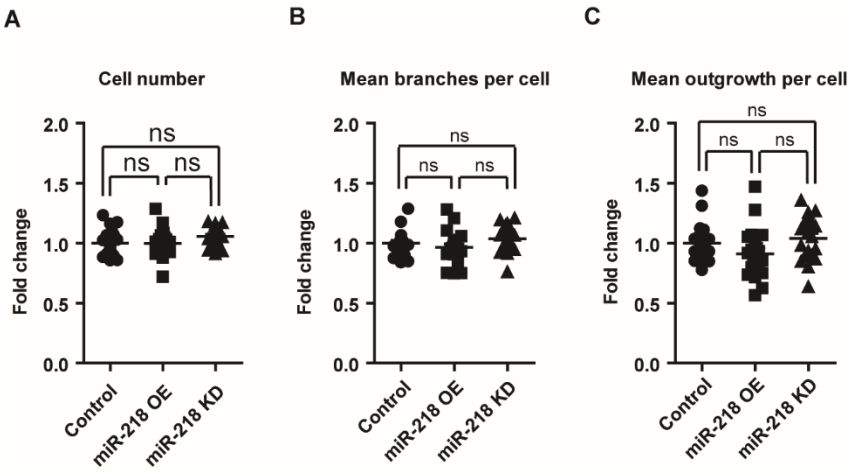

Fig. S2. High content analysis of neuronal morphology after miR-218 perturbation. (**A** to **C**) Primary motor neurons were plated in a 384-well plate, transduced with control, miR-218 OE or miR-218 KD lentiviruses and stained at 4 DIV. Neuron-specific class III beta-tubulin (Tuj1) was used to visualize neuronal processes and DAPI for visualization of nuclei. Phenotypic parameters were quantified using MetaXpress High-Content Image Acquisition and Analysis Software (Molecular Devices) set to identify and measure only cellular processes connected to cell bodies. (A) Cell number. Only nuclei of cells expressing Tuj1 were counted. (B) Mean number of branches per cell. (C) Mean axonal outgrowth per cell. Data normalized to control virus transduced cells. *n*=24 independent wells per experimental condition, in two technical duplicates, each. This experiment was repeated 3 independent times with similar results. Error bars, mean ± SEM. One-way ANOVA followed by Bonferronie's multiple comparison test. *ns* – non-significant.

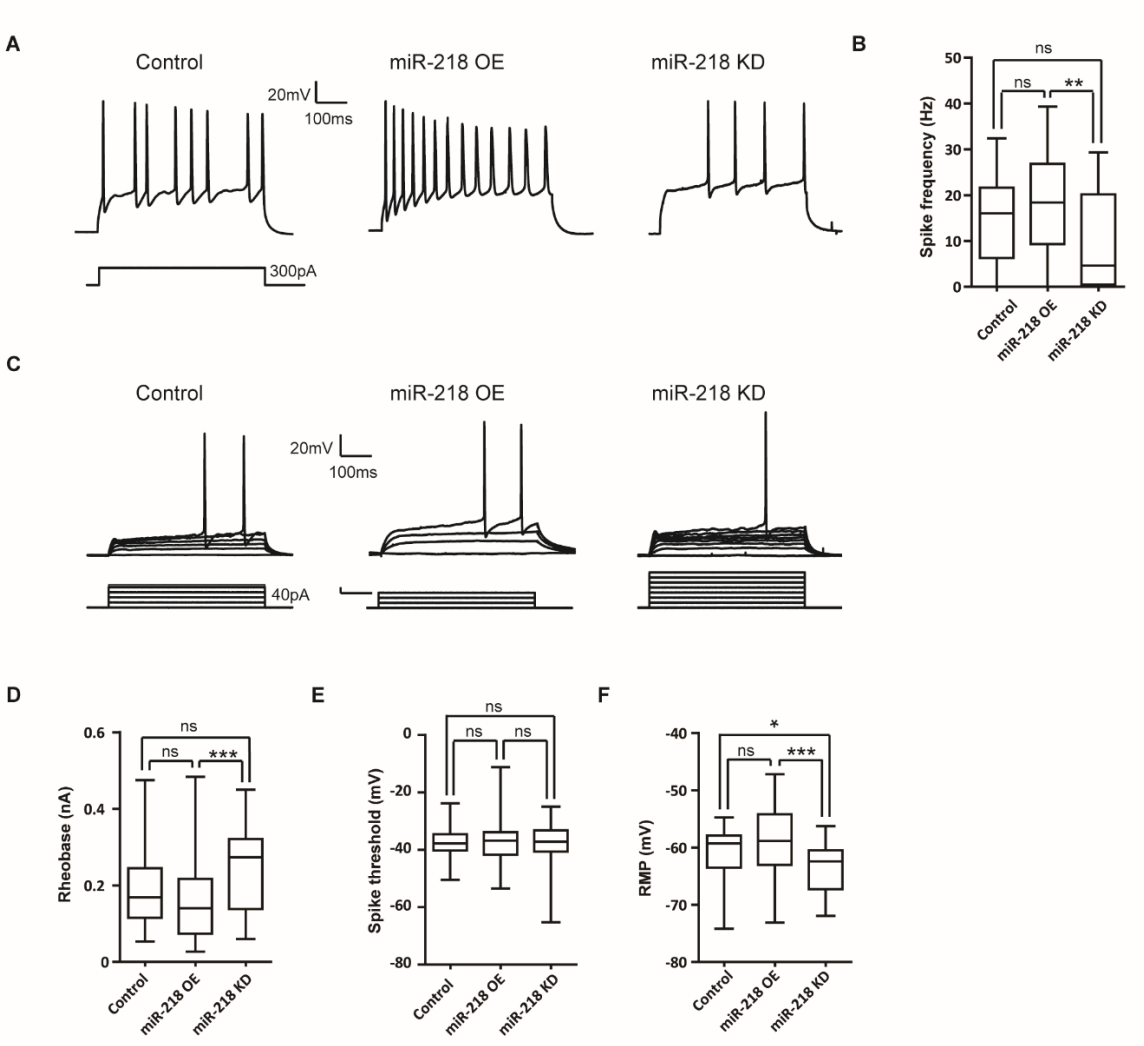

Fig. S3. miR-218 regulates intrinsic excitability. (**A**) Representative traces depicting repetitive firing, following miR-218 perturbation. Recordings of rat hippocampal neurons, transduced with lentiviruses encoding mCherry alone (control), miR-218 overexpression (miR-218 OE) or a miR-218 knockdown (miR-218 KD) inhibitor, following injection of 300 pA depolarizing current for 500 ms. Injected current amplitude and duration are illustrated in the lower pane. (**B**) Quantification of firing rates. (**C**) Representative traces depicting rheobase - current input required to generate action potential. (**D**) Quantification of rheobase; (**E**) spike threshold; (**F**) resting membrane potential (RMP). Control, n=44; miR-218 OE, n=65; miR-218 KD, n=30. Data was combined from 4 independent experiments. Kruskal-Wallis test and Dunn's multiple comparison test. Error bars, mean ±SEM, * P<0.05; ** P<0.01; *** P<0.001; *ns* - non-significant.

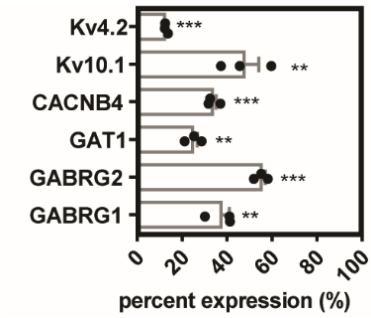

Fig. S4. qPCR validation of miR-218 target KD. Targeting and non-targeting control siRNAs (Integrated DNA Technologies, Inc.) were encapsulated in Neuro9™ nanoparticles (Precision NanoSystems, Inc.). Primary motor neurons were transfected with siRNA at a final concentration of 1μg/ml, at 9 DIV. At 12 DIV RNA was extracted and expression of miR-218 targets was analyzed by qPCR. Data normalized to average expression of HPRT and β–actin. *n*=3 independent wells per experimental condition, in two technical duplicates, each. Error bars, mean ± SEM. Two-sided student t-test. ** $P<0.01$; *** $P<0.001$.

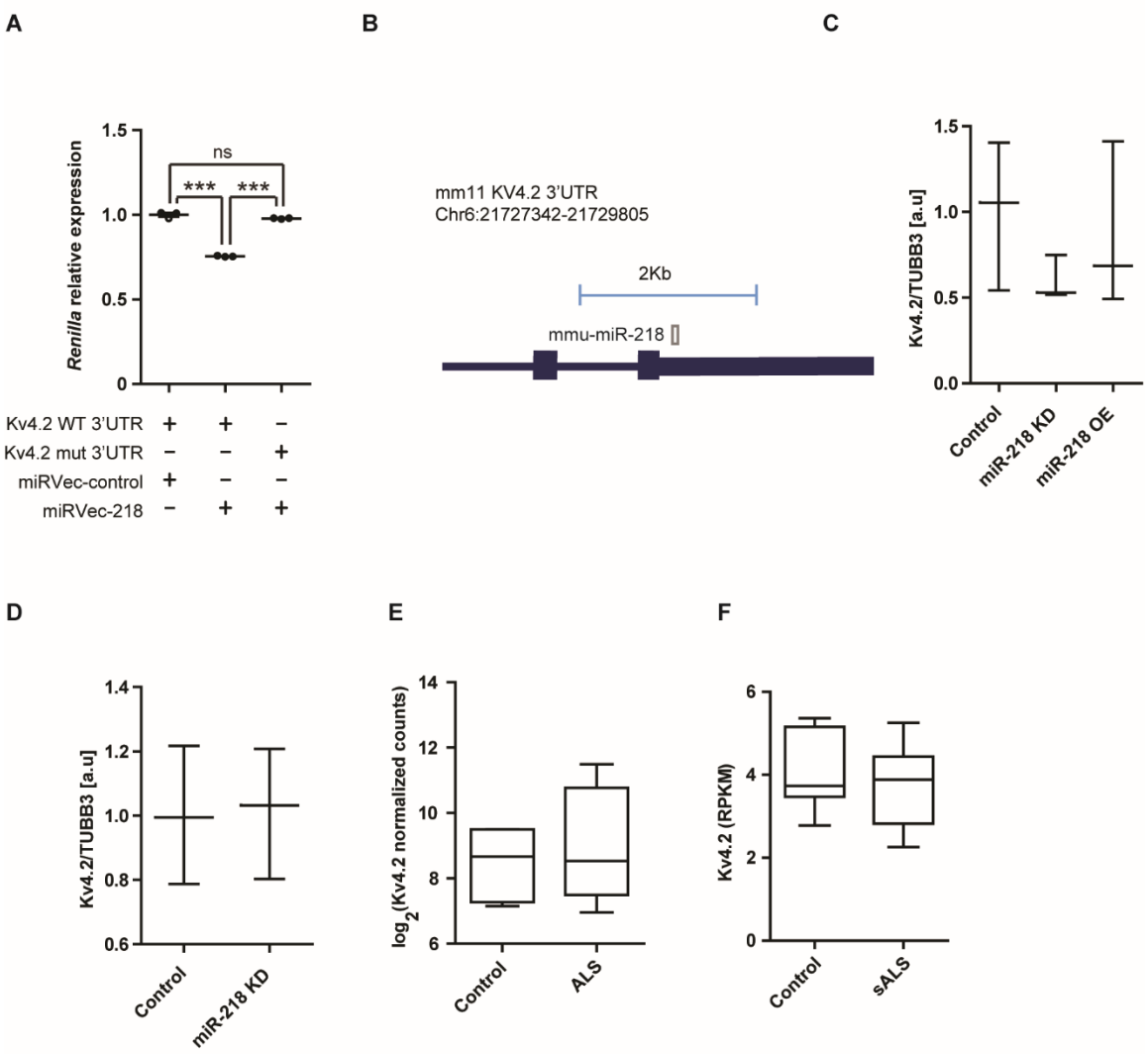

Fig. S5. Evaluation of miR-218 upstream of the mRNA encoding for the potassium channel *Kv4.2* (*Kcnd2*). (**A**) Relative Renilla luminescence upstream of a wild-type Kv4.2 3′UTR or a mutated 3′UTR that is insensitive to miR-218, normalized to co-expressed firefly luciferase and to a negative control miRNA vector. n=3 independent wells per experimental condition. One-way ANOVA followed by Bonferronie's multiple comparison test. Error bars, mean ± SEM. *** *P*<0.001. (**B**) AGO2 CLEAR-CLIP experiment did not identify a miR-218:Kv4.2 3'UTR chimera in mouse cortex (*29*), as opposed to the miR-218:Kv10.1 3'UTR chimera in main Fig. 3J. (**C** and **D**) Western blot studies of Kv4.2 protein expression, in primary rat motor neurons, presented as boxplot (median, upper / lower quartiles & extreme points), upon (C) miR-218 knockdown (miR-218 KD, n=3) or overexpression (miR-218 OE, n=3), relative to Control (n=3). One-way ANOVA followed by Newman-Keuls multiple comparison test, and a validation study of (D)

miR-218 KD (n=3), relative to Control (n=3). Two-sided student t-test. (**E**) Kv4.2 mRNA expression, as log2 normalized counts, from NGS study of induced ALS motor neurons (n=4 different donors in duplicates) or non-neurodegeneration controls (n=3 different donors in duplicates; (*30*)). Box-Plots depict median, upper / lower quartiles & extreme points, DESeq analysis. Non-significant difference. (**F**) Kv4.2 mRNA expression, as Reads Per Kilobase Million (RPKM) from NGS study of laser capture microdissection-enriched surviving motor neurons from lumbar spinal cords of patients with sALS with rostral onset and caudal progression (n=12) and non-neurodegeneration controls (n=9; (*21*), GSE76220). Box-Plots depict median, upper / lower quartiles & extreme points, two-sided student's t-test. Non-significant difference.

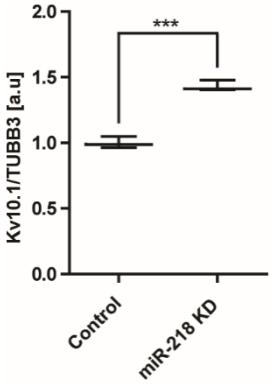

Fig. S6. Kv10.1 (KCNH1) protein quantification by Western blot after miR-218 KD. A western blot validation study of Kv10.1 protein expression upon miR-218 KD (n=3), in primary rat motor neurons, relative to Control (n=3). Box-Plots depict median, upper / lower quartiles & extreme points, two-sided student t-test. *** $P<0.001$.

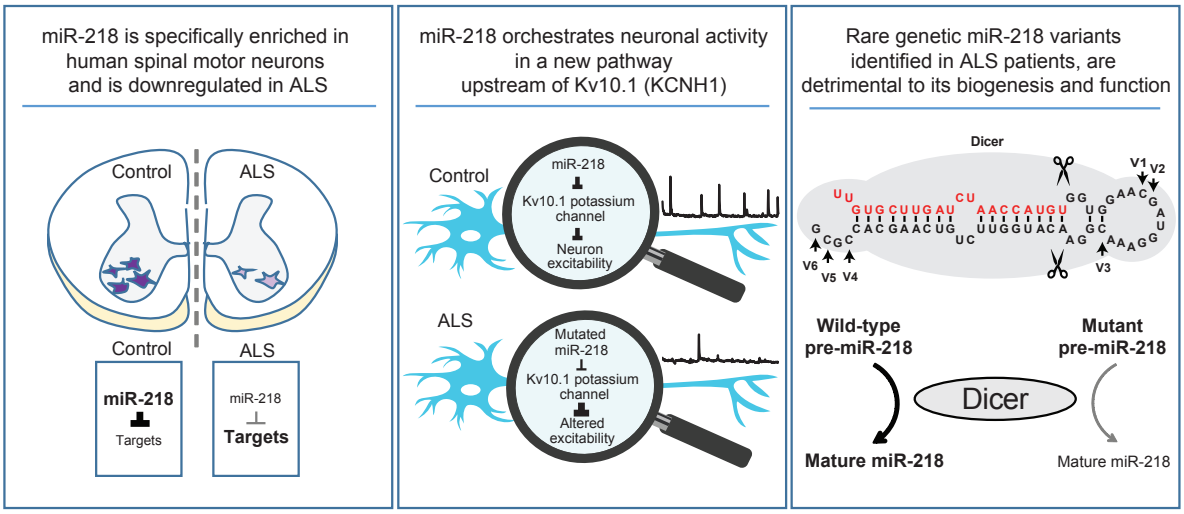

Fig. S7. A summary diagram of key observations. (**Left**) Human pathology: miR-218 expression is reduced in ALS ventral horns, because of both molecular downregulation and of motor neuron loss. Accordingly, mRNA targets of miR-218 are reciprocally upregulated (de-repressed). miR-218 might serve as a biomarker of motor neuron mass. (**Center**) miR-218 regulates neuronal activity upstream of the voltage gated potassium channel Kv10.1 (Kcnh1). (**Right**) Human genetics: rare genetic variants identified in patients with ALS, in the sequence of the pre-miR-218-2 gene, dysregulate miR-218 biogenesis and impair regulation of neuron activity. miR-218 contributes to motor neuron specificity and its function may be particularly susceptible to failure in motor neuron diseases. Therefore, the work connects human genetics and human neuropathology to motor neuron-specific functions via a small non coding RNA.

Table S1. Identified hsa-miR-218-2 variants.

Variant	Position (GRCh37/hg19)	Ref	Alt	dbSNP ID	Minor Allele Frequency						
					Discovery		Replication		TOPMed	Total	
					cases (n= 4281)	controls (n=1838)	cases (n=3457)	controls (n=7034)	controls (n=62784)	cases (n=7738)	controls (n=71656)
V1	Chr5:168,195,208 (-)	C	A	rs751147155	2.3×10^{-4}	0				1.3×10^{-4}	0
V2	Chr5:168,195,207 (-)	G	A	rs374716278	2.3×10^{-4}	0	2.9×10^{-4}	0	0.5×10^{-4}	2.6×10^{-4}	0.4×10^{-4}
V3	Chr5:168,195,199 (-)	C	T	rs773402384	0	5.4×10^{-4}				0	0.1×10^{-4}
V4	Chr5:168,195,176 (-)	C	T	rs368624931	2.3×10^{-4}	0	2.9×10^{-4}	0		2.6×10^{-4}	0
V5	Chr5:168,195,174 (-)	C	T	rs140638702	9.3×10^{-4}	5.4×10^{-4}	11.6×10^{-4}	4.3×10^{-4}	3.2×10^{-4}	10.3×10^{-4}	3.3×10^{-4}
V6	Chr5:168,195,173 (-)	G	A	rs780192152	4.7×10^{-4}	0	0	1.4×10^{-4}	1.1×10^{-4}	2.6×10^{-4}	1.1×10^{-4}
V7	Chr5:168,195,198 (-)	G	A	rs770316009					0.5×10^{-4}	0	0.4×10^{-4}
V8	Chr5:168,195,196 (-)	A	G	rs372358902					0.5×10^{-4}	0	0.4×10^{-4}
V9	Chr5:168,195,175 (-)	G	A	rs758061075					0.2×10^{-4}	0	0.1×10^{-4}
Total					**21.0×10^{-4}**	**10.9×10^{-4}**	**17.4×10^{-4}**	**5.7×10^{-4}**	**5.9×10^{-4}**	**19.4×10^{-4}**	**6.0×10^{-4}**

Ref, Reference allele; Alt, Alternate allele.

Table S2. DsiRNA sequences used in the study.

Oligo name	S-strand	AS-strand
has-miR-214-3p mimic	5'-phos-rArCrArGrCrArGrGrCrArCrArGrArCrArGrGrCmAmGmU-3'	5'-mUmGrCmCrUmGrUmCrUmGrUmGrCmCrUmGrCmUrGrU-3'
NC5 siRNA	5'-rCrGrCrGrArCrUrArUrArCrGrCrGrCrArArUrArUmGmGrU-3'	5'-rUmCrCmArUmArAmArGmUrAmGrGmArAmArCmArCmUrAmCA-3'
Rat Cacnb4 siRNA	5'-mGmUrCmUrAmCrCrUrGrArGmCrAmUrGmUrUmGrArArArUrCmAA-3'	5'-rUrUmGrArUrUrUrCmArAmCrAmUrGrCrUrCrArGrGrUrAmGrAmCmUmU-3'
Rat Kcnd2 siRNA	5'-mUmGrCmArAmGrArArCrUrCmArGmUrAmCrAmArUrUrCrArGmAT-3'	5'-rArUmCrUrGrArArUmUrGmUrAmCrUrGrArGrUrUrCrUrUmGrCmAmCmG-3'
Rat Kcnh1 siRNA	5'-mGmCrUmGrAmGrArGrGrArUmCrAmUrUmUrCmArArArArCrAmAA-3'	5'-rUrUmUrGrUrUrUrUmGrAmArAmUrGrArUrCrCrUrCrUrCmArGmCmUmU-3'
Rat Slc6a1 siRNA	5'-rCrCrArArArUrGrArCrArGrArUrGrGrGrCrUrArGrArCrAAG-3' 5'-rCrCrCrArUrGrUrArGrCrArArArGrCrGrUrArUrArUrGrUCT-3' 5'-rArCrCrUrUrArUrArGrArGrCrArCrArArArCrGrUrUrArArGGG-3' 5'-rGrGrArGrArArGrArCrUrCrArUrArGrCrArGrArGrArArCTA-3' 5'-rCrArArArUrGrArUrArUrCrArCrArArCrUrArGrArGrCrACG-3' 5'-rCrCrUrUrArArUrArArCrUrArUrGrGrUrGrArArUrArArCTG-3' 5'-rGrCrArArArCrArGrCrCrUrArUrCrCrArGrArArGrArGrCTC-3' 5'-rArGrCrArUrCrArArArGrCrArCrArCrUrGrUrArGrArGrArATG-3' 5'-rCrCrArGrArGrGrGrArArGrArArGrArUrArArUrUrGrUrATT-3' 5'-rGrCrArCrArCrCrArGrUrUrCrUrUrGrCrArArUrArArGrCTA-3'	5'-rCrUrUrGrUrCrUrArGrCrCrCrArUrCrUrGrUrCrArUrUrUrGrGrUrG-3' 5'-rArGrArCrArUrArUrArCrGrCrUrUrUrGrCrUrArCrArUrGrGrGrArG-3' 5'-rCrCrCrUrUrArArCrGrUrUrGrUrGrCrUrCrUrArUrArArArGrGrUrUrA-3' 5'-rUrArGrUrUrCrUrCrUrGrCrUrArUrGrArGrUrCrUrUrCrUrCrCrUrA-3' 5'-rCrGrUrGrCrUrCrUrArGrUrUrGrUrGrArUrArUrCrArUrUrUrGrArU-3' 5'-rCrArGrUrUrArUrUrCrArCrCrArUrArGrUrUrArUrArArGrGrArU-3' 5'-rGrArGrCrUrCrUrUrCrUrGrGrArUrArGrGrCrUrGrUrUrUrGrCrUrG-3' 5'-rCrArUrUrCrUrCrUrArCrArGrUrGrUrGrCrUrUrGrArUrGrCrUrCrU-3' 5'-rArArUrArCrArArUrUrArUrCrUrUrCrUrUrCrCrCrUrCrUrGrGrGrC-3' 5'-rUrArGrCrUrUrArUrUrGrCrArArGrArArCrUrGrGrGrUrGrUrGrCrUrG-3'

	5'- rGrCrArGrUrArUrArUrUrUrCrArUrG rUrUrUrGrArArArGCA-3' 5'- rGrArArCrArUrUrUrArUrUrArCrArU rUrGrUrCrArUrCrATC-3' 5'- rGrArUrUrUrArUrUrCrUrCrArGrArA rCrArCrCrArArArGrUAA-3' 5'- rArGrArArArGrArUrArArArUrUrGrUrArU rUrArUrCrArUrArUAT-3' 5'- rCrArGrUrUrCrUrUrGrCrArArArUrArA rGrCrUrArUrCrUrCCC-3' 5'- rCrArCrCrArArArGrUrArArArArUrUrUrA rUrCrUrCrUrArUrArATA-3' 5'- rGrArUrUrGrCrUrCrUrGrGrGrArArG rCrUrArCrArArCrUCT-3' 5'- rCrArArGrUrCrUrUrUrUrUrUrGrArG rArUrArArArUrGrCAG-3' 5'- rCrArArArUrGrArUrArUrCrArCrArA rCrUrArGrArArGrCrACG-3' 5'- rUrCrGrArUrGrUrUrCrUrUrUrGrArU rGrGrArArArArCrUGA-3'	5'- rUrGrCrUrUrUrCrArArArCrArUrGrArA rArUrArUrArCrUrGrCrArU-3' 5'- rGrArUrGrArUrGrArCrArArUrGrUrAr ArUrArArArUrGrUrUrCrArG-3' 5'- rUrUrArCrUrUrGrGrUrGrUrUrCrUrGrA rGrArArUrArArArUrCrCrU-3' 5'- rArUrArUrArUrGrArUrArArUrArCrAr ArUrUrArUrCrUrUrCrUrUrC-3' 5'- rGrGrGrArGrArUrArGrCrUrUrArUrUr GrCrArArGrGrArArCrUrGrGrU-3' 5'- rUrArUrArUrArGrArGrArUrArArArUr UrUrArCrUrUrGrGrUrGrUrU-3' 5'- rArGrArGrUrUrGrUrArGrCrUrUrCrCrC rArGrArGrCrArArUrCrArG-3' 5'- rCrUrGrCrArUrUrUrArUrCrUrCrArArA rArArArGrArCrUrUrGrUrU-3' 5'- rCrGrUrGrCrUrCrUrArGrUrUrGrUrGrA rUrArUrCrArUrUrUrGrArU-3' 5'- rUrCrArGrUrUrUrUrCrCrArUrCrArArA rGrArArCrArUrCrGrArGrC-3'
Rat Gabrb2 siRNA	5'- rArCrCrUrArCrUrUrCrCrUrGrArArU rGrArUrArArGrArAGT-3' 5'- rGrArArUrArUrGrCrUrUrUrGrGrUrC rArArCrUrArCrArUCT-3' 5'- rGrCrArCrUrCrUrUrGrArGrArUrArA rArArArArArUrGrArGAT-3' 5'- rCrUrGrArArGrUrCrArArUrArUrGrG rArCrUrArCrArCrCTT-3' 5'- rGrUrGrGrCrArGrUrArGrGrArArUrG rArArCrArUrUrGrATA-3' 5'- rCrArArCrUrGrArUrGrArCrArUrUrG rArGrUrUrUrUrArCTG-3' 5'-	5'- rArCrUrUrCrUrUrArUrCrArUrUrCrArG rGrArArGrUrArGrGrUrGrU-3' 5'- rArGrArUrGrUrArGrUrUrGrArCrCrArA rArGrCrArUrArUrUrCrCrA-3' 5'- rArUrCrUrCrArUrUrUrUrUrUrArUrCrU rCrArArGrArGrUrGrCrUrG-3' 5'- rArArGrGrUrGrUrArGrUrCrCrArUrArU rUrGrArCrUrUrCrArGrArA-3' 5'- rUrArUrCrArArUrGrUrUrCrArUrUrCrC rUrArCrUrGrCrCrArCrArG-3' 5'- rCrArGrUrArArArArCrUrCrArArUrGrU rCrArUrCrArGrUrUrGrUrA-3' 5'-

	rCrArUrCrArGrArArGrCrArGrUrArArUrGrGrGrArCrUrUGG-3' 5'-rUrArCrUrCrArGrCrArCrUrCrUrUrGrArGrArUrArArArArAAA-3' 5'-rGrUrCrCrUrGrArCrGrArUrGrArCrCrArCrArArUrCrArATA-3' 5'-rGrArUrArArGrArArGrUrCrArUrUrUrGrUrArCrArUrGrGAG-3' 5'-rArGrArCrUrGrUrCrCrUrArCrArArUrGrUrArArUrCrCrCTT-3' 5'-rArCrUrUrCrArUrCrCrUrGrCrArGrArCrArUrArCrArUrGCC-3' 5'-rUrCrArArUrGrArCrCrCrUrArGrUrArArUrArUrGrUrCrGCT-3' 5'-rGrGrArArUrArUrGrCrUrUrUrGrGrUrCrArArCrUrArCrATC-3' 5'-rGrGrArArUrGrArArCrArUrUrGrArUrArUrCrGrCrCrArGCA-3' 5'-rCrCrArCrArUrCrArGrArArGrCrArGrUrArArUrGrGrGrACT-3' 5'-rArGrArArArGrCrUrGrCrUrArArUrGrCrCrArArCrArArCGA-3' 5'-rGrGrArCrUrArCrArCrCrUrUrGrArCrCrArUrGrUrArUrUTC-3' 5'-rArGrArArUrCrArCrArArCrUrArCrArGrCrUrGrCrCrUrGCA-3' 5'-rArGrArUrUrGrUrCrCrCrUrArArGrCrUrUrUrArArGrCrUGA-3'	rCrCrArArGrUrCrCrCrArUrUrArCrUrGrCrUrUrCrUrGrArUrGrUrG-3' 5'-rUrUrUrUrUrArUrCrUrCrArArGrArGrUrGrCrUrGrArGrUrArArG-3' 5'-rUrArUrUrGrArUrUrGrUrGrGrUrCrArUrCrGrUrCrArGrGrArCrArG-3' 5'-rCrUrCrCrArUrGrUrArCrArArArUrGrArCrUrUrCrUrUrArUrCrArU-3' 5'-rArArGrGrGrArUrUrArCrArUrUrGrUrArGrGrArCrArGrUrCrUrCrU-3' 5'-rGrGrCrArUrGrUrArUrGrUrCrUrGrCrArGrGrArUrGrArArGrUrArG-3' 5'-rArGrCrGrArCrArUrArUrUrArCrUrArGrGrGrUrCrArUrUrGrArCrA-3' 5'-rGrArUrGrUrArGrUrUrGrArCrCrArArArGrCrArUrArUrUrCrCrArG-3' 5'-rUrGrCrUrGrGrCrGrArUrArUrCrArArUrGrUrUrCrArUrUrCrCrUrA-3' 5'-rArGrUrCrCrCrArUrUrArCrUrGrCrUrUrCrUrGrArUrGrUrGrGrCrC-3' 5'-rUrCrGrUrUrGrUrUrGrGrCrArUrUrArGrCrArGrCrUrUrUrCrUrCrA-3' 5'-rGrArArArUrArCrArUrGrGrUrCrArArGrGrUrGrUrArGrUrCrCrArU-3' 5'-rUrGrCrArGrGrCrArGrCrUrGrUrArGrUrUrGrUrGrArUrUrCrUrGrA-3' 5'-rUrCrArGrCrUrUrArArArGrCrUrUrArGrGrGrArCrArArUrCrUrGrG-3'
Rat Gabrg1 siRNA	5'-rGrUrCrUrArCrCrArGrUrUrArArArArUrCrUrGrArArUrCAT-3' 5'-rArArArUrCrUrGrArArUrCrArUrUrUrGrGrUrCrArUrArUGA-3' 5'-rCrUrGrGrCrUrUrArGrUrCrCrArArU	5'-rArUrGrArUrUrCrArGrArUrUrUrUrArArCrUrGrGrUrArGrArCrUrU-3' 5'-rUrCrArUrArUrGrArCrCrArArArUrGrArUrUrCrArGrArUrUrUrUrA-3' 5'-rArArGrUrGrArUrUrArUrArUrUrGrGr

	rArUrArArUrCrArCTT-3'	ArCrUrArArGrCrCrArGrArU-3'
	5'-rCrUrArCrCrArGrUrUrArArArArUrCrUrGrArArUrCrArUTT-3'	5'-rArArArUrGrArUrUrCrArGrArUrUrUrUrArArCrUrGrGrUrArGrArC-3'
	5'-rArCrUrGrGrArArUrUrUrUrCrArArGrCrUrArUrGrGrArUAC-3'	5'-rGrUrArUrCrCrArUrArGrCrUrUrGrArArArArUrUrCrCrArGrUrGrG-3'
	5'-rCrArGrUrArArUrUrGrArArArCrUrGrArUrGrUrUrUrArUGT-3'	5'-rArCrArUrArArArCrArUrCrArGrUrUrUrCrArArUrUrArCrUrGrUrG-3'
	5'-rGrUrGrArGrGrUrUrGrArUrArUrUrCrUrUrGrUrUrArCrUAA-3'	5'-rUrUrArGrUrArArCrArArGrArArUrArUrCrArArCrCrUrCrArCrUrC-3'
	5'-rCrCrUrArArGrGrUrUrUrCrUrUrArCrGrUrGrArCrArGrCAA-3'	5'-rUrUrGrCrUrGrUrCrArCrGrUrArArGrArArArCrCrUrUrArGrGrUrA-3'
	5'-rCrUrUrGrCrArUrUrArUrUrUrUrArCrUrArGrUrArArCrAAT-3'	5'-rArUrUrGrUrUrArCrUrArGrUrArArArArUrArArUrGrCrArArGrGrU-3'
	5'-rGrArArUrUrArUrUrCrArGrGrUrUrArArUrUrUrArCrCrUAA-3'	5'-rUrUrArGrGrUrArArArUrUrArArCrCrUrGrArArUrArArUrUrCrUrU-3'
	5'-rGrGrCrUrArUrGrArUrArArCrArArArCrUrUrCrGrUrCrCAG-3'	5'-rCrUrGrGrArCrGrArArGrUrUrUrGrUrUrArUrCrArUrArGrCrCrUrU-3'
	5'-rUrGrArCrArArCrCrCrUrCrArGrUrArCrArArUrCrGrCrUAG-3'	5'-rCrUrArGrCrGrArUrUrGrUrArCrUrGrArGrGrGrUrUrGrUrCrArUrA-3'
	5'-rCrCrUrUrUrGrUrArGrGrGrUrUrArArGrArArArUrUrCrAAC-3'	5'-rGrUrUrGrArArUrUrUrCrUrUrArArCrCrCrUrArCrArArArGrGrCrA-3'
	5'-rGrGrArGrUrCrArArArCrUrArGrArGrGrArGrUrGrArGrGTT-3'	5'-rArArCrCrUrCrArCrUrCrCrUrCrUrArGrUrUrUrGrArCrUrCrCrArC-3'
	5'-rGrGrArArCrUrCrArArGrGrArArArUrCrUrGrArUrGrCrGCA-3'	5'-rUrGrCrGrCrArUrCrArGrArUrUrUrCrCrUrUrGrArGrUrUrCrCrUrG-3'
	5'-rCrCrCrArCrArGrUrArArUrUrGrArArArCrUrGrArUrGrUTT-3'	5'-rArArArCrArUrCrArGrUrUrUrCrArArUrUrArCrUrGrUrGrGrGrUrC-3'
	5'-rCrCrArUrGrArArCrArArCrArUrUrUrCrUrArUrGrCrCrUCA-3'	5'-rUrGrArGrGrCrArUrArGrArArArUrGrUrUrGrUrUrCrArUrGrGrGrA-3'
	5'-rCrCrArArUrArUrArArUrCrArCrUrUrCrGrArUrUrUrCrATA-3'	5'-rUrArUrGrArArArUrCrGrArArGrUrGrArUrUrArUrArUrUrGrGrArC-3'
	5'-rGrGrArUrGrGrGrCrUrArUrUrUrUrCrArCrArArUrUrCrArGAC-3'	5'-rGrUrCrUrGrArArUrUrGrUrGrArArArUrArGrCrCrCrArUrCrCrGrU-3'

	5'- rArArCrArUrGrGrArGrUrArCrArCrG rArUrArGrArCrArUCA-3'	5'- rUrGrArUrGrUrCrUrArUrCrGrUrGrUrA rCrUrCrCrArUrGrUrUrUrA-3'

rA rC rG rU = RNA; mA mC mG mU = 2’OMe RNA ; A G C T = DNA; phos = phosphate.

Table S3. Synthetic miR-218 sequences used for cloning into pMA-T vectors.

Variant	Synthetic gene sequence
WT miR-218	5'- CTGTGTCTCCCGCGGATCCGCGCTGGGGTGGGGCACAAGGGCAGCAGGGC TGCAATCTTCGGAAGTGTTCCAGTGGAACCCCACTCCTGATACTAATCACG CTCAGTGGGGGCCTGCTCCGGCTTCCGCTTCTCCACGCTGCTTCCTCTGAG CGCTCCTGTCCTCTCTCTGACGCTGCTTCCTGACCTTGACTCTGACCAGTC GCTGCGGGGCTTTCCTTTGTGCTTGATCTAACCATGTGGTGGAACGATGGA AACGGAACATGGTTCTGTCAAGCACCGCGGAAAGCACCGTGCTCTCCTGC AGCATGGCCCGCCACCGCCGCCACCACCGCTGGACACCTCTCCTCTGCTCT GGAGCACCGCAGCCCACCACCTGCCAGACCCACCTCTCCCAGTCTCAACT CACCAAAGGCAGGAGGGTAGGAGTCTCAAAGGATGCAGATACAGAGGGA AATGGGTCTGGAGAAGCACCCTGGCCGGAATTCCGGGGAAAGAGAT-3'
V2	5'- CTGTGTCTCCCGCGGATCCGCGCTGGGGTGGGGCACAAGGGCAGCAGGGC TGCAATCTTCGGAAGTGTTCCAGTGGAACCCCACTCCTGATACTAATCACG CTCAGTGGGGGCCTGCTCCGGCTTCCGCTTCTCCACGCTGCTTCCTCTGAG CGCTCCTGTCCTCTCTCTGACGCTGCTTCCTGACCTTGACTCTGACCAGTC GCTGCGGGGCTTTCCTTTGTGCTTGATCTAACCATGTGGTGGAACAATGGA AACGGAACATGGTTCTGTCAAGCACCGCGGAAAGCACCGTGCTCTCCTGC AGCATGGCCCGCCACCGCCGCCACCACCGCTGGACACCTCTCCTCTGCTCT GGAGCACCGCAGCCCACCACCTGCCAGACCCACCTCTCCCAGTCTCAACT CACCAAAGGCAGGAGGGTAGGAGTCTCAAAGGATGCAGATACAGAGGGA AATGGGTCTGGAGAAGCACCCTGGCCGGAATTCCGGGGAAAGAGAT-3'
V5	5'- CTGTGTCTCCCGCGGATCCGCGCTGGGGTGGGGCACAAGGGCAGCAGGGC TGCAATCTTCGGAAGTGTTCCAGTGGAACCCCACTCCTGATACTAATCACG CTCAGTGGGGGCCTGCTCCGGCTTCCGCTTCTCCACGCTGCTTCCTCTGAG CGCTCCTGTCCTCTCTCTGACGCTGCTTCCTGACCTTGACTCTGACCAGTC GCTGCGGGGCTTTCCTTTGTGCTTGATCTAACCATGTGGTGGAACGATGGA AACGGAACATGGTTCTGTCAAGCACCGTGGAAAGCACCGTGCTCTCCTGC AGCATGGCCCGCCACCGCCGCCACCACCGCTGGACACCTCTCCTCTGCTCT GGAGCACCGCAGCCCACCACCTGCCAGACCCACCTCTCCCAGTCTCAACT CACCAAAGGCAGGAGGGTAGGAGTCTCAAAGGATGCAGATACAGAGGGA AATGGGTCTGGAGAAGCACCCTGGCCGGAATTCCGGGGAAAGAGAT-3'
Vall	5'- CTGTGTCTCCCGCGGATCCGCGCTGGGGTGGGGCACAAGGGCAGCAGGGC TGCAATCTTCGGAAGTGTTCCAGTGGAACCCCACTCCTGATACTAATCACG CTCAGTGGGGGCCTGCTCCGGCTTCCGCTTCTCCACGCTGCTTCCTCTGAG CGCTCCTGTCCTCTCTCTGACGCTGCTTCCTGACCTTGACTCTGACTAGTCG CTGCGGGGCTTTCCTTTGTGCTTGATCTAACCATGTGGTGGAAAAATGGAA ACGGAACATGGTTCTGTCAAGCACTGTAGAAAGCACCGTGCTCTCCTGCA GCATGGCCCGCCACCGCCGCCACCACCGCTGGACACCTCTCCTCTGCTCTG GAGCACCGCAGCCCACCACCTGCCAGACCCACCTCTCCCAGTCTCAACTC ACCAAAGGCAGGAGGGTAGGAGTCTCAAAGGATGCAGATACAGAGGGAA ATGGGTCTGGAGAAGCACCCTGGCCGGAATTCCGGGGAAAGAGAT-3'

Vdead	5'- CTGTGTCTCCCGCGGATCCGCGCTGGGGTGGGGCACAAGGGCAGCAGGGC TGCAATCTTCGGAAGTGTTCCAGTGGAACCCCACTCCTGATACTAATCACG CTCAGTGGGGGCCTGCTCCGGCTTCCGCTTCTCCACGCTGCTTCCTCTGAG CGCTCCTGTCCTCTCTCTGACGCTGCTTCCTGACCTTGACTCTGACCAGTC GCTGCGGGAACCCAATTTGTGCTTGATCTAACCATGTGGTGGAACGATGG AAACGGAACATGGTTCTGTCAAGCACCGCGGAAAGCACCGTGCTCTCCTG CAGCATGGCCCGCCACCGCCGCCACCACCGCTGGACACCTCTCCTCTGCTC TGGAGCACCGCAGCCCACCACCTGCCAGACCCACCTCTCCCAGTCTCAAC TCACCAAAGGCAGGAGGGTAGGAGTCTCAAAGGATGCAGATACAGAGGG AAATGGGTCTGGAGAAGCACCCTGGCCGGAATTCCGGGGAAAGAGAT-3'

pre-miR-218 sequence (Blue); variants (Red)

Table S4. Primers used for quantitative real-time PCR.

Gene	Forward (5' to 3')	Reverse (5' to 3')
miR-218-5p	TTGTGCTTGATCTAACCATGT	NA
RNU6B (U6)	GATGACACGCAAATTCGTGAA	NA
hHPRT	ACTTTGCTTTCCTTGGTCAGGCAGT	CGTGGGGTCCTTTTCACCAGCA
hGAPDH	TTCTTTTGCGTCGCCAGCCGA	GTGACCAGGCGCCCAATACGA
hHB9	GGAGCACCAGTTCAAGCTCA	AATCTTCACCTGGGTCTCGGT
hISL1	CAGTCCAGAGAGACACGACG	AATTGACCAGTTGCTGAAAAGC
hChAT	AAGGAAGGTCCACACCTCTG	TCAGACACCAAGTGTCGCAT
Rat Hprt	CGAGATGCTATGAAGGAGATGG	GTAATCCAGCAGGTCAGCAAAG
Rat Gapdh	CCTTTAGTGGGCCCTCGG	GCCTGGAGAAACCTGCCAAG
Rat Kcnd2	CAAGTTCACCAGCATCCCT	CCCGAAAATCTTCCCTGCTAT
Rat Kcnh1	ACCTGATTCTCACCTACAATCTG	TCTTCCGTTTCATCCTCTCCT
Rat Cacnb4	CACCGTATCCCACAGCAAT	GAGGTCATTAGGCTTCGTCTT
Rat Slc6a1	CCCAGGGTGGCATTTATGTCT	GGGTGTGAAAAAGGACCAGC
Rat Gabrb2	AAGATGCGCCTGGATGTCAA	ATGCTGGAGGCATCATAGGC
Rat Gabrg1	GTCTTCCTTTTCTCTCCTTCCC	TCATCTTCATCATCTGCTTTATCAATG

Data file S1. Individual-level data for miR-218 expression.

Provided in Excel.

Data file S2. NanoString nCounter data for miRNAs measured in lumbar ventral horns.

Provided in Excel.

Data file S3. Source data for Kv10.1 (KCNH1) Western blot studies.

Provided in Excel.

Data file S4. Source data for Kv4.2 (KCND2) Western blot studies.

Provided in Excel.