HHS Public Access

Author manuscript

Science. Author manuscript; available in PMC 2016 December 18.

Published in final edited form as:

Science. 2015 December 18; 350(6267): 1525–1529. doi:10.1126/science.aad2509.

Loss of motoneuron-specific microRNA-218 causes systemic neuromuscular failure

Neal D. Amin[1,2,3], **Ge Bai**[1,*], **Jason R. Klug**[4], **Dario Bonanomi**[1], **Matthew T. Pankratz**[1], **Wesley D. Gifford**[1,2,5], **Christopher A. Hinckley**[1], **Matthew J. Sternfeld**[1,6], **Shawn P. Driscoll**[1], **Bertha Dominguez**[7], **Kuo-Fen Lee**[7], **Xin Jin**[4], and **Samuel L. Pfaff**[1,†]

[1]Howard Hughes Medical Institute and Gene Expression Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

[2]Medical Scientist Training Program, University of California, San Diego (UCSD), 9500 Gilman Drive, La Jolla, CA 92037, USA

[3]Biomedical Sciences Graduate Program, UCSD, 9500 Gilman Drive, La Jolla, CA 92037, USA

[4]Molecular Neurobiology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

[5]Neurosciences Graduate Program, UCSD, 9500 Gilman Drive, La Jolla, CA 92037, USA

[6]Biological Sciences Graduate Program, UCSD, 9500 Gilman Drive, La Jolla, CA 92037, USA

[7]Peptide Biology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

Abstract

Dysfunction of microRNA (miRNA) metabolism is thought to underlie diseases affecting motoneurons. One miRNA, miR-218, is abundantly and selectively expressed by developing and mature motoneurons. Here we show that mutant mice lacking miR-218 die neonatally and exhibit neuromuscular junction defects, motoneuron hyperexcitability, and progressive motoneuron cell loss, all of which are hallmarks of motoneuron diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy. Gene profiling reveals that miR-218 modestly represses a cohort of hundreds of genes that are neuronally enriched but are not specific to a single neuron subpopulation. Thus, the set of messenger RNAs targeted by miR-218, designated *TARGET*^{218}, defines a neuronal gene network that is selectively tuned down in motoneurons to prevent neuromuscular failure and neurodegeneration.

Motoneurons are a specialized neuronal subpopulation within the central nervous system (CNS) that establish synaptic connections with muscles to regulate movement. Diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), which

†Corresponding author. pfaff@salk.edu.

*Present address: Department of Chemical Physiology, The Scripps Research Institute La Jolla CA 92037 USA

Supplementary Materials: www.sciencemag.org/content/350/6267/1525/suppl/DC1

The supplementary materials contain additional data.

affect motoneurons, appear to share a common pathogenic mechanism based on defective RNA metabolism and biogenesis of microRNAs (miRNAs) (1–5). Consistent with earlier descriptions of miR-218 expression in embryonic motoneurons (6, 7), we found that miR-218 was the most abundant motoneuron miRNA and was enriched ~27-fold, as determined by small RNA sequencing (RNA-seq) of embryonic mouse spinal cord *Hb9::gfp*$^{+}$ motoneurons purified by fluorescence-activated cell sorting (FACS) (Fig. 1A and supplementary materials and methods). The spinal cord is composed of many interneuron subtypes (8). We found that V2a and V3 ventral spinal interneurons did not express a single characteristic miRNA (fig. S1A), indicating that motoneurons may be distinctly reliant on cell type-specific miRNA expression.

We detected miR-218 within visceral and somatic spinal (Fig. 1B and fig. S1B) and brainstem motoneurons (fig. S1C). Robust miR-218 expression was detected postnatally in choline acetyltransferase (ChAT)–positive α and γ motoneurons (Fig. 1C and fig. S1D) but not ChAT^{+} interneurons (fig. S1E). Likewise, miR-218 was selectively expressed in human embryonic motoneurons (fig. S1F). Compared with the extensive catalog of protein markers that delineate motoneuron subtypes (8), miR-218 is notable for its expression spanning motoneuron classes from embryonic stages into adulthood (fig. S1G) and its undetectable expression in other tissues.

Two mammalian paralogs of miR-218, *miR-218-1* and *miR-218-2*, are embedded within homologous introns of the *Slit2* and *Slit3* genes, respectively. It has been suggested that the motoneuron-specifying transcription factors Isl1 and Lhx3 up-regulate miR-218 by increasing transcription of *Slit2* and *Slit3* (6). We used RNA-seq to investigate Slit2 and Slit3 mRNA expression in motoneurons and discovered that both genes were transcribed using start sites located upstream of exon 6 (Fig. 1D and fig. S2, A and B). This intronic start site was not used by adjacent floor-plate glial cells expressing *Slit2* and *Slit3*. Previously mapped Isl1/2, Lhx3, and Phox2a chromatin immunoprecipitation (ChIP) peaks (9) were observed at conserved hexamer DNA response elements (HxREs) proximal to exon 6 (Fig. 1E), suggesting the presence of intragenic motoneuron-specific promoters (Fig. 1F). To test this hypothesis, we generated a transgenic mouse line, *tg(218-2::eGFP)*, with a 7.4-kb sequence containing putative regulatory elements (fig. S2C). In vivo, enhanced green fluorescent protein (eGFP) was expressed in *tg(218-2::eGFP)* spinal and cranial motoneurons, reproducing the endogenous expression pattern of miR-218 (Fig. 1, G and H, and fig. S2C). These findings demonstrate that primary miR-218 transcripts are under independent activation in motoneurons by promoters distinct from those that generate *Slit2* and *Slit3* full-length transcripts in other tissues.

Studies in vitro and in chick embryos have suggested that miR-218 may contribute to motoneuron specification (6). However, such miRNA knockdown and overexpression studies can yield nonphysiological effects (10). To determine the biological effect of miR-218 disruption in vivo, we used clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene editing (11) to create microdeletions of *miR-218-1* and *miR-218-2* precursor sequences in mice (Fig. 2A and fig. S3, A to D). miR-218 expression was detected in miR-218-1$^{-/-}$ and at lower levels in miR-218-2$^{-/-}$ motoneurons but was undetectable in miR-218-1$^{-/-}$2$^{-/-}$ double knockout (218^{DKO}) motoneurons (Fig. 2B and fig.

S3, E to H). We found that the number and spatial organization of motoneurons in embryonic day 12.5 (E12.5) spinal cords of 218^{DKO} mutants were unchanged compared to controls (fig. S4, A to F). Subsequent developmental events—including axonal exit from the spinal cord, outgrowth, and peripheral pathfinding—were indistinguishable between Hb9: :gfp^+ control and 218^{DKO} embryos (fig. S4G). 218^{DKO} mutants displayed none of the pheno-types characteristic of *Slit2* and *Slit3* disruption (fig. S4, H and I) (12,13), indicating that *Slit2* and *Slit3* function is unaffected by the intronic microdeletions. We conclude that miR-218 is dispensable for in vivo fate specification and gross early development of motoneurons.

However, 218^{DKO} mice were never viable (fig. S5A). 218^{DKO} embryos exhibited akinesia, kyphosis, and weak or absent responses to pain stimulation after caesarean delivery at E18.5 and died within minutes due to an apparent lack of respiration (Fig. 2C and movie S1). These phenotypic defects are similar to those observed in mice carrying null alleles of Agrin, MuSK, ChAT, and other components required for neuromuscular transmission (14). Consequently, we investigated whether the loss of miR-218 affects neuromuscular synaptogenesis, an intricate process in which motor nerves first innervate muscle and subsequently form presynaptic specializations with postsynaptic acetylcholine receptors (AChRs) expressed by developing muscle (15). We examined glycerol-cleared *tg(218-2: :eGFP)* embryos and found that the motor nerves of 218^{DKO} motoneurons reached E14.5 limb tissue (fig. S5B). However, motor axons appeared to lack penetrating, fine intramuscular branches in limbs, the diaphragm, and intercostal muscles (Fig. 2, D and E, and fig. S6, A and B). At E18.5, the majority of a-bungarotoxin–labeled $AChR^+$ clusters lack motor innervation in 218^{DKO} limb muscles (Fig. 2F and fig. S6C), reflecting a gross failure of motoneurons to establish the neuromuscular junctions (NMJs) needed to control body movements.

Although 218^{DKO} mutants had normal numbers of motoneurons at E12.5 (Fig. 2G), 218^{DKO} mutants had 18 to 36% fewer motoneurons at cervical, thoracic, and lumbar spinal cord segments at E18.5, indicating neurodegenerative cell loss (Fig.2H and fig. S7,A and B). To examine whether the physiology of the remaining motoneurons was altered in mice lacking miR-218, we assessed fictive locomotion (16) and performed intra-cellular recordings of *Hb9: :*gfp^+ lateral motor column a motoneurons from E18.5 lumbar spinal slices (fig. S8A). Left-right and flexor-extensor activation of motor roots were normal in 218^{DKO} spinal cords, and motoneuron resting membrane potentials, capacitances, resistances, and holding currents were similar between control and 218^{DKO} motoneurons (fig. S8, B to J). However, action potentials were elicited by a rheobase current that was 4.4 times lower in 218^{DKO} motoneurons than in controls (Fig. 2, I and J), indicating membrane hyperexcitability. Thus, miR-218 does not appreciably contribute to early motoneuron development, but it is critical for the regulation of neuromuscular synapses, membrane excitability, and motoneuron survival.

These phenotypic defects suggest that motoneuron-specific gene regulation depends on the post-transcriptional repression of miR-218 target mRNAs. To extend beyond miRNA target identification studies performed in vitro using cancer cell lines (6, 17), we identified miR-218 gene targets within motoneurons by performing polyadenylated ($polyA^+$) RNA-seq

of FACS-isolated Hb9::*gfp*$^{+}$ motoneurons from wild-type (WT) and 218^{DKO} E12.5 spinal cords, before the apparent onset of defects (fig. S9A). Using Sylamer (18), we determined that 6-, 7-, and 8–base pair (bp) 3′ untranslated region (3′UTR) complementary seed matches to miR-218 were enriched within genes expressed higher in 218^{DKO} motoneurons versus controls (fig. S9, B to E). This finding reflects the widespread derepression of miR-218 target genes in 218^{DKO} motoneurons.

We identified 333 genes with predicted miR-218 binding sites [TargetScan 6.2 (19)] that were significantly derepressed in 218^{DKO} versus WT motoneurons (Fig. 3A). We name this coordinately regulated gene set, likely to be under direct miR-218–mediated repression, *TARGET*^{218}. *TARGET*^{218} genes are enriched for neurotransmission and neurotransmitter transport processes (fig. S9, F and G). The most highly up-regulated *TARGET*^{218} gene, *Slc1a2* (266% increase), is a glutamate re-uptake transporter known to be modulated by riluzole, the only medication approved for the treatment of ALS (20). On average, *TARGET*^{218} genes were expressed 61.1% higher in 218^{DKO} motoneurons, and 47 of these genes were expressed at least twofold higher (table S1). The wide breadth of target genes affected in 218^{DKO} motoneurons suggests that miR-218 shapes expression of an extensive genetic network rather than merely modulating a small group of individual genes within a single molecular pathway.

Other miRNA gene regulatory networks have been shown to reinforce the repression of differentiation programs to confer robustness to cell fate decisions (21–23). However, the lack of cell specification errors in 218^{DKO} embryos suggests that miR-218 might have a previously unrecognized regulatory role. To evaluate whether the *TARGET*^{218} gene network was expressed higher or lower in motoneurons compared with other spinal neuronal subpopulations, we performed gene profiling of FACS-purified interneuron subpopulations labeled by genetic reporters: GABAergic-V1 (En1:Cre), glutama-tergic-V2a (Chx10:Cre), and glutamatergic-V3 (Sim1:Cre) spinal interneurons (Fig. 3B and fig. S10, A to C). We found that ~80% of *TARGET*^{218} genes are expressed lower in WT motoneurons versus each of the V1, V2a, and V3 interneurons (Fig. 3C). Moreover, the majority (69.1%) of *TARGET*^{218} genes are expressed lower in WT motoneurons versus all three spinal interneuron sub-populations (Fig. 3D). These findings suggest that miR-218 represses a gene network shared across interneuron subpopulations but not specific to a single one. Furthermore, hierarchical clustering revealed that 218^{DKO} motoneurons express *TARGET*^{218} genes at levels closer to those of V1, V2a, and V3 interneurons than to those of WT motoneurons (Fig. 3E). Thus, rather than reinforcing a preexisting low target gene expression in motoneurons, miR-218 establishes the low *TARGET*^{218} expression observed in motoneurons relative to interneurons.

To evaluate miRNA-mediated repression in an unbiased manner, we bioinformatically evaluated the statistical enrichment of binding sites for all miRNAs across the transcriptome of investigated cell types. Using Sylamer (18), we determined the hypergeometric statistical enrichment of 7-bp 3′UTR sequences complementary to known miRNA seed sequences (miRNA seed matches) in transcripts expressed higher or lower in motoneurons versus interneurons (Fig. 4A). We found that 3′UTR seed matches to miR-218 are significantly enriched in transcripts expressed lower in WT motoneurons versus pooled interneurons (Fig. 4A), individual spinal interneuron subpopulations, and even distantly located cortical neuron

subpopulations (Fig. 4B) (24). 3′UTR seed matches to miR-218 were not found to be enriched in genes expressed higher or lower in 218^{DKO} motoneurons (Fig. 4C). The 3′UTR seed match to miR-124 [a pan-neuronal miRNA abundantly expressed in motoneurons and other CNS neurons (25)], but not 3′UTR seed matches to miR-218, was overrepresented in transcripts expressed lower in motoneurons versus purified motoneuron progenitors differentiated from embryonic stem cells (Fig. 4, B and C). We conclude that: (i) miR-218 targets a genetic network shared across functionally and spatially distinct neuronal cell types; (ii) the low relative expression of this gene network in motoneurons is established by miR-218; and (iii) although miR-124 and miR-218 are coexpressed in motoneurons, their regulatory roles differ—miR-124 represses a neuronal progenitor-associated gene network, whereas miR-218 represses a gene network coordinately expressed by other spinal and cortical neuronal subpopulations.

We have identified a neuronal gene network, $TARGET^{218}$, that is selectively repressed in motoneurons by a single miRNA. When this network is derepressed in 218^{DKO} mice, motoneurons exhibit severe NMJ defects, hyperexcitability, and cell loss—the pathological hallmarks of motoneuron diseases such as ALS and SMA (1,26–28). The link between miR-218 and motoneuron diseases likely extends beyond phenotypic similarities. Patients suffering from motoneuron diseases carry genetic mutations in ubiquitously expressed RNA processing factors (e.g., TDP-43, FUS, SMN) or expansion repeats in C9ORF72 that sequester RNA binding proteins (1, 2), but the biological mechanisms that magnify the effects of these changes on motoneurons are unclear. miRNA-dependent regulation—and specifically the repression of miR-218's genetic network-might be particularly sensitive to defects in these RNA metabolic pathways thought to underlie motoneuron disease. Investigations of the pathways affecting miR-218's biogenesis and the modulation of its genetic network may be critical to under stand and tackle these devastating diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank K. Lettieri, N. White, and M. Gullo for mouse husbandry; Y. Dayn at the Salk Transgenic Core for oocyte injections; C. O'Connor and C. Fitzpatrick at the Salk Flow Cytometry Core for cell sorting; and M. Ku at the Salk Next Generation Sequencing Core for RNA-seq. Salk Core facilities are supported by the National Cancer Institute (grant P30 CA014195), the National Institute of Neurological Disorders and Stroke (NINDS) (grant P30 NS072031), The Leona M. and Harry B. Helmsley Charitable Trust, and the H.A. and Mary K. Chapman Charitable Foundation. N.D.A. is supported by a NINDS fellowship (F31-NS080340-03) and the UCSD Medical Scientist Training Program (grant T32-GM007198). S.L.P. is a Howard Hughes Medical Institute investigator and the Benjamin H. Lewis chair in neuroscience at the Salk Institute. This research is supported by grants from the NIH (R01GM088278, R21NS084254, R01NS054154, R01NS044420, R01NS060833, and R01AG0476669), The Marshall Heritage Foundation, Sol Goldman Trust, and The Clayton Foundation. The Gene Expression Omnibus accession number is GSE75601. N.D.A., S.L.P., and the Salk Institute have filed the following U.S. provisional patent applications: no. 62/168,755 (30 May 2015) and no. 62/166,677 (26 May 2015).

References and Notes

1. Paez-Colasante X, Figueroa-Romero C, Sakowski SA, Goutman SA, Feldman EL. Nat Rev Neurol. 2015; 11:266–279. [PubMed: 25896087]

2. Volonte C, Apolloni S, Parisi C. CNS Neurol Disord Drug Targets. 2015; 14:194–207. [PubMed: 25613506]
3. Morlando M, et al. EMBO J. 2012; 31:4502–4510. [PubMed: 23232809]
4. Kawahara Y, Mieda-Sato A. Proc Natl Acad Sci USA. 2012; 109:3347–3352. [PubMed: 22323604]
5. Haramati S, et al. Proc Natl Acad Sci USA. 2010; 107:13111–13116. [PubMed: 20616011]
6. Thiebes KP, et al. Nat Commun. 2015; 6:7718. [PubMed: 26212498]
7. Kapsimali M, et al. Genome Biol. 2007; 8:R173. [PubMed: 17711588]
8. Alaynick WA, Jessell TM, Pfaff SL. Cell. 2011; 146:178–178.e1. [PubMed: 21729788]
9. Mazzoni EO, et al. Nat Neurosci. 2013; 16:1219–1227. [PubMed: 23872598]
10. Vidigal JA, Ventura A. Trends Cell Biol. 2015; 25:137–147. [PubMed: 25484347]
11. Wang H, et al. Cell. 2013; 153:910–918. [PubMed: 23643243]
12. Ma L, Tessier-Lavigne M. J Neurosci. 2007; 27:6843–6851. [PubMed: 17581972]
13. Long H, et al. Neuron. 2004; 42:213–223. [PubMed: 15091338]
14. Turgeon B, Meloche S. Physiol Rev. 2009; 89:1–26. [PubMed: 19126753]
15. Darabid H, Perez-Gonzalez AP, Robitaille R. Nat Rev Neurosci. 2014; 15:703–718. [PubMed: 25493308]
16. Kwan AC, Dietz SB, Webb WW, Harris-Warrick RM. J Neurosci. 2009; 29:11601–11613. [PubMed: 19759307]
17. Lu YF, Zhang L, Waye MM, Fu WM, Zhang JF. Exp Cell Res. 2015; 334:173–182. [PubMed: 25857406]
18. van Dongen S, Abreu-Goodger C, Enright AJ. Nat Methods. 2008; 5:1023–1025. [PubMed: 18978784]
19. Garcia DM, et al. Nat Struct Mol Biol. 2011; 18:1139–1146. [PubMed: 21909094]
20. Cheah BC, Vucic S, Krishnan AV, Kiernan MC. Curr Med Chem. 2010; 17:1942–1959. [PubMed: 20377511]
21. Bartel DP. Cell. 2009; 136:215–233. [PubMed: 19167326]
22. Tsang J, Zhu J, van Oudenaarden A. Mol Cell. 2007; 26:753–767. [PubMed: 17560377]
23. Herranz H, Cohen SM. Genes Dev. 2010; 24:1339–1344. [PubMed: 20595229]
24. Molyneaux BJ, et al. Neuron. 2015; 85:275–288. [PubMed: 25556833]
25. Makeyev EV, Zhang J, Carrasco MA, Maniatis T. Mol Cell. 2007; 27:435–448. [PubMed: 17679093]
26. Fischer LR, et al. Exp Neurol. 2004; 185:232–240. [PubMed: 14736504]
27. Wainger BJ, et al. Cell Reports. 2014; 7:1–11. [PubMed: 24703839]
28. Liu H, et al. Sci Rep. 2015; 5:12189. [PubMed: 26190808]

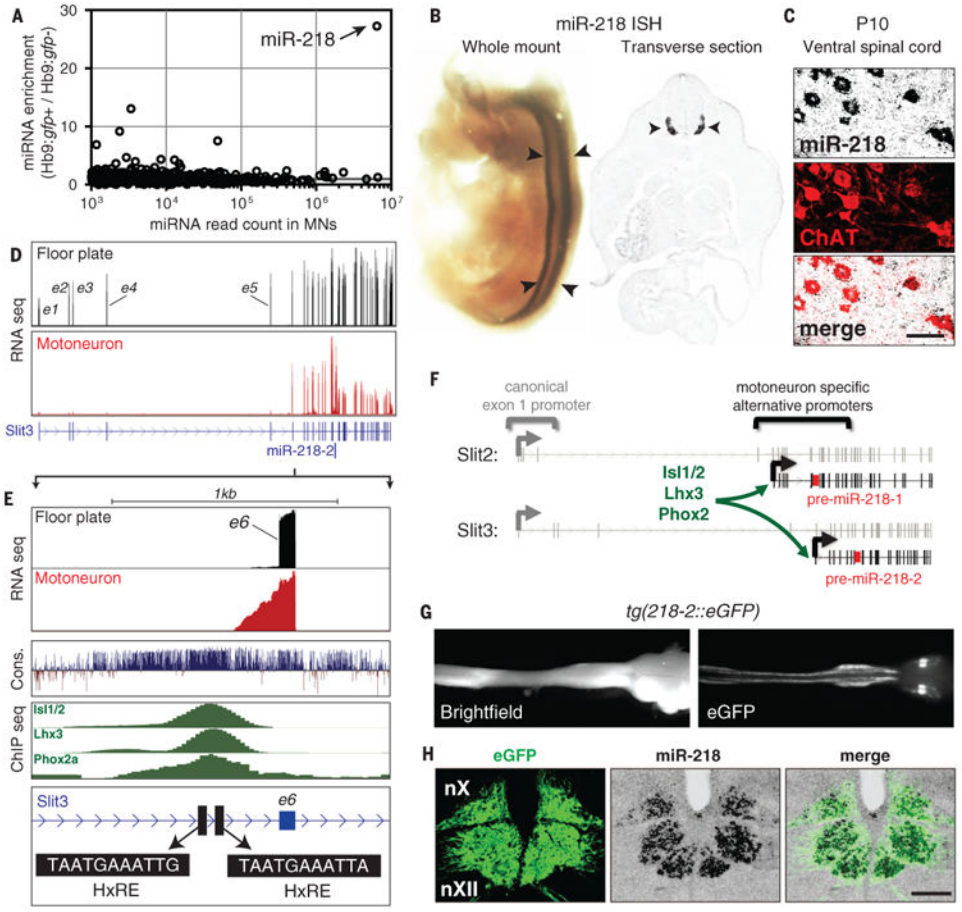

Fig. 1. Abundant and specific expression of miR-218 in spinal and cranial motoneuron subtypes

(**A**) Murine miRNA expression (*x* axis) versus enrichment [E10.5 Hb9::*gfp*$^{+}$ motoneurons (MNs) versus Hb9::*gfp*$^{-}$ non-motoneurons, *y* axis] (*n* = 2 sorting and sequencing experiments). (**B**) miR-218 in situ hybridization (ISH) in whole-mount and transverse sections at E11.5 (arrowheads identify motor columns). (**C**) miR-218 colo-calizes with ChAT^{+} motoneurons at P10. (**D** and **E**) PolyA^{+} RNA-seq reads from E12.5 floor plate and motoneurons, evolutionary conservation (Cons.), and motoneuron-specifying transcription factor ChIP peaks and HxRE DNA binding motifs at the *Slit3* locus containing *pre-miR-218-2*. (**F**) Transcription of miR-218 in motoneurons by alternative promoters. (**G** and **H**) Images from *tg(218-2::eGFP)* mice. (G) Expression of eGFP in spinal and brainstem motoneurons of the CNS and (H) in miR-218^{+} motor nuclei nX and nXII. Scale bars: (C) 50 μm; (H) 200 μm.

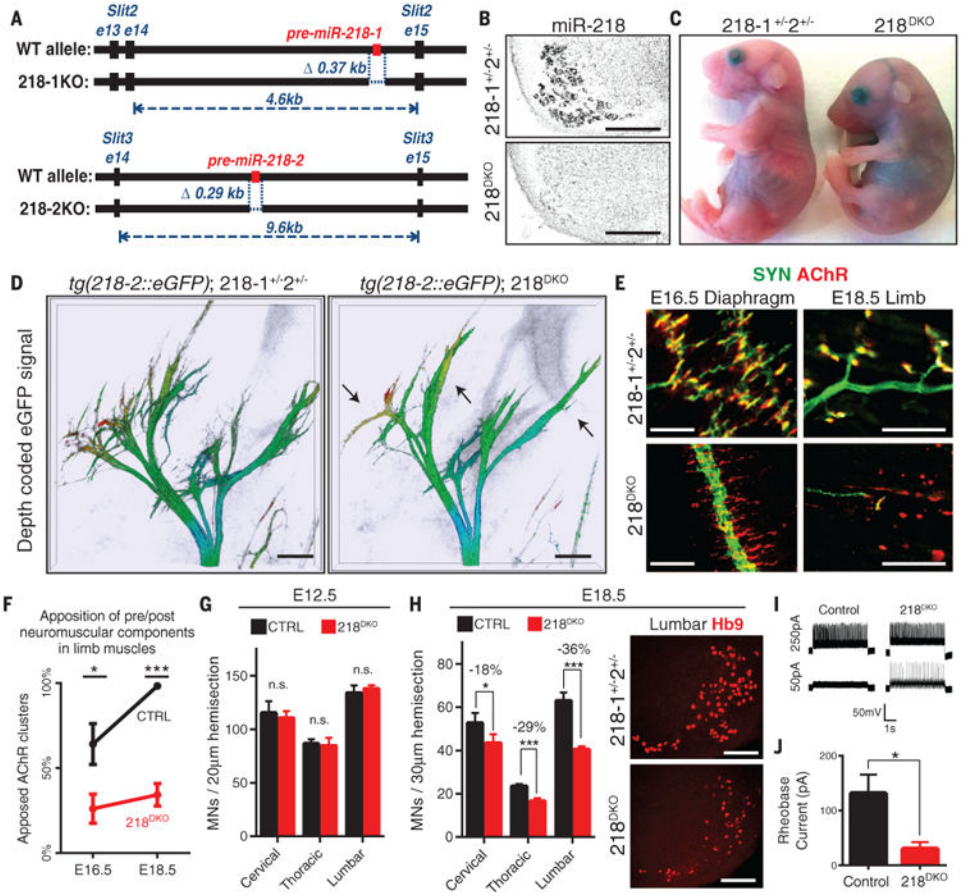

Fig. 2. The loss of miR-218 results in systemic neuromuscular failure, motoneuron cell loss, and hyperexcitability

(**A**) CRISPR-Cas9–mediated multiplexed microdeletions of *pre-miR-218-1* and *pre-miR-218-2* from the mouse genome. (**B**) miR-218 in situ hybridization signal in control and 218^{DKO} E18.5 spinal cords. (**C**) Cesarean-delivered 218^{DKO} E18.5 embryos exhibit flaccid paralysis and die within minutes. (**D**) Decreased intramuscular branching (arrows) of E14.5 motor nerves in *tg(218-2::eGFP)*;218^{DKO} embryos (deep peroneal nerve). (**E** and **F**) In 218^{DKO} embryos, (E) NMJs exhibit abnormal morphology, and (F) most limb AChR^{+} clusters are aneural (n = 3 embryos). (**G** and **H**) Motoneuron counts at E12.5 (n = 4 and 3 embryos) and E18.5 (n = 4 embryos) across spinal segments. (**I**) Representative traces of control and 218^{DKO} motoneurons after intracellular current injection. (**J**) Rheobase quantification (n = 9 and 5 motoneurons). Statistics: In (F), (G), and (H), error bars indicate SD, and results of two-tailed t tests are shown. In (J), error bars indicate SEM, and nonparametric Mann-Whitney t test results are shown. *P< 0.05; ***P< 0.001; n.s., not significant. Scale bars: (B), (D), and (H) 150 µm; (E) 50 µm.

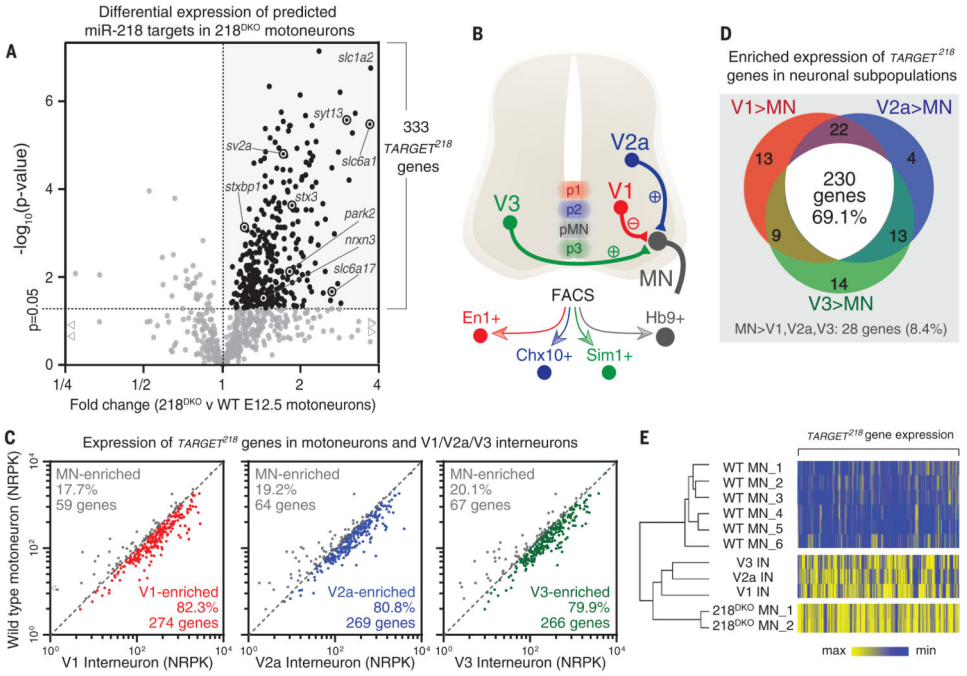

Fig. 3. miR-218 represses an extensive genetic network in motoneurons

(**A**) Volcano plot (mRNA fold difference versus *P* value) of 218^{DKO} versus WT motoneurons of genes expressing at least 10 normalized reads per kilobase and possessing strong miR-218 binding sites [context + score ≤ −0.15, TargetScan6.2 (19)] (n = 6 and 2 sorting and sequencing experiments). Of these genes, 333 (designated *TARGET*^{218} genes) are significantly derepressed in 218^{DKO} motoneurons. *TARGET*^{218} genes involved in neurotransmitter transport are labeled. (**B**) Motoneurons and V1, V2a, and V3 interneuron subpopulations derive from adjacent progenitor domains (p1, p2, pMN, p3) and were labeled with transgenes or Cre-reporters for FACS-isolation and RNA-seq. *TARGET*^{218} genes are expressed at low levels in motoneurons relative to each of V1, V2a, and V3 interneurons. NRPK, normalized reads per kilobase. Most *TARGET*^{218} genes are expressed lower in motoneurons versus V1, V2a, and V3 interneurons. (**E**) Hierarchical clustering of *TARGET*^{218} gene expression in WT motoneurons (WT MN, six replicates), 218^{DKO} motoneurons (218^{DKO} MN, two replicates), and interneuron subpopulations (V1 IN, V2a IN, and V3 IN).

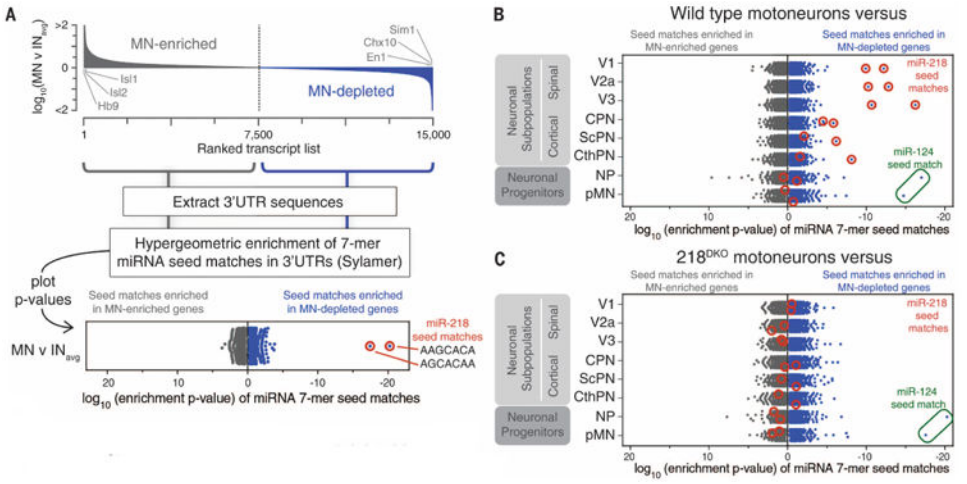

Fig. 4. miR-218 represses a neuronal gene network in motoneurons

(**A**) Seven-nucleotide oligomer seed matches for miR-218 (AGCACAA and AAGCACA, red circles), but not those of other miRNAs, are significantly and specifically enriched in the 3′UTRs of genes expressed low in motoneurons relative to an average of V1, V2a, and V3 interneuron populations (IN_{avg}). (**B**) Genes expressed lower in WT motoneurons versus individual spinal and cortical (24) neuronal subpopulations were most enriched for miR-218 seed matches. (**C**) miR-218 seed matches are not enriched in genes expressed higher or lower in 218^{DKO} motoneurons versus other neuronal populations. Genes expressed lower in WT (B) or 218^{DKO} (C) motoneurons versus neuronal progenitors were most enriched for the seed match to miR-124 (GTGCCTT). CPN, callosal; ScPN, subcerebral; CthPN, subplate neurons; NP, mouse embryonic stem (mES) cell–derived neuronal progenitors; pMN, FACS-purified $Olig2^{+}$ mES cell–derived neuronal progenitors.