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(54) **Title**: METHOD OF TREATING NEURODEGENERATIVE DISEASES WITH MICRORNA REGULATORS

(57) **Abstract**: The present invention relates to compositions and methods for regulating miRNA function in a population of cells or subject. In particular, the invention relates to regulating miRNA function to treat neurodegenerative conditions associated with aberrant miRNA function. The miRNAs are regulated using miRNA specific inhibitory molecules.

METHOD OF TREATING NEURODEGENERATIVE DISEASES WITH MICRORNA REGULATORS

GOVERNMENTAL RIGHTS

[0001] This invention was made with government support under grants K08NS074194, and R01NS078398, awarded by the National Institute of Neurological Disorders and Stroke (NINDS). The government has certain rights in the invention.

FIELD OF THE INVENTION

[0002] The present invention relates to compositions and methods for regulating miRNA function in a population of cells or subject. More particularly, the invention relates to regulating miRNA function to treat neurodegenerative conditions associated with aberrant miRNA function, such as Amyotrophic Lateral Sclerosis (ALS).

BACKGROUND OF THE INVENTION

[0003] MicroRNAs (miRNAs) are single-stranded, non-coding RNAs that regulate transcription and translation of coding RNAs (mRNA). Since their discovery in 1993, miRNAs have emerged as key regulators in numerous physiological and pathological processes. miRNAs are highly conserved and are about 18-25 nucleotides in length. Typically, miRNAs direct translational repression by binding to the 3' untranslated region (UTR) of mRNAs. Because only partial complementarity is required for miRNA-mRNA interactions, a single miRNA can potentially regulate hundreds of mRNA transcripts.

[0004] Neurodegenerative diseases are those involving progressive loss of structure or function of neurons, including death of neurons. Such diseases include Alzheimer's, Amyotrophic Lateral Sclerosis (ALS), Huntington's, and Parkinson's diseases. Most neurodegenerative diseases have no cure and available therapeutics are targeted at improving symptoms, relieving pain, and slowing degeneration. Neurodegenerative diseases, such as ALS, need novel, innovative approaches to drug

development since many traditional therapeutics have failed or only shown marginal benefits.

[0005] Macrophages are white blood cells derived from monocytes, and microglia are the resident macrophages in the brain and spinal cord. These cells play important roles in innate and acquired immunity. When primed by interferon gamma and activated by a T-helper cell Type I-cytokine, these cells may enter their classically activated M1 state. In this state, macrophages serve to destroy the surrounding insult and amplify tissue damage by releasing reactive oxygen species, nitric oxide, and pro-inflammatory cytokines like TNFα, IL1β, and IL-6. When exposed to T-helper cell Type II-cytokines, macrophages can become alternatively activated (M2). In the M2 state, macrophages resolve inflammation, promote cell growth, and aid with tissue repair by releasing anti-inflammatory cytokines and trophic factors. In neurological diseases like Alzheimer's disease, Parkinson's disease, multiple sclerosis, and ALS, microglia become classically activated and create a prolonged inflammatory atmosphere while promoting continued cell death. Activated glia (astrocytes and microglia) are recognized as part of the pathology of human ALS.

[0006] Accordingly, a need still exists for identifying new therapeutic targets and regulators of physiological and pathological neurodegenerative conditions and diseases. While miRNAs are known regulators of physiological and pathological processes, little is known about their involvement in neurodegenerative conditions or diseases. Compositions and methods exploiting miRNA regulation in neurodegenerative conditions or diseases are needed to further medical research and provide diagnostic and therapeutic resources for such conditions and diseases. The present invention provides compositions and methods for treating conditions and diseases associated with aberrant miRNA function.

REFERENCE TO COLOR FIGURES

[0007] The application file contains at least one photograph executed in color. Copies of this patent application publication with color photographs will be provided by the Office upon request and payment of the necessary fee.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0009] **FIG. 1** graphically illustrates that miR-155 is increased in ALS models and a microglia cell line. (**A**) depicts a plot showing a 7.2 fold increase of miR-155 in spinal cord tissue of ALS rats ($SOD1^{G93A}$ rats) as compared to normal rats ($SOD1^{wt}$) (normalized to U6, $p<0.05$). (**B**) depicts a plot showing a 3.9 fold increase of miR-155 in spinal cord tissue of ALS mice ($SOD1^{G93A}$ mice) as compared to normal age-matched controls ($p<0.05$). (**C**) depicts a plot showing a 1.8 fold increase of miR-155 in spinal cord tissue of ALS mice ($TDP\text{-}43^{A315T}$ mice) compared to normal age-matched controls ($p<0.05$). (**D**) depicts a plot showing 10 week non-transgenic male mice exhibit a step-wise increase of miR-155 in the cerebral cortex one hour after an intraperitoneal LPS injection (N=3 per group; LPS full dose=0.2 mg/kg, $6x10^5$ endotoxin units/kg, from *Escherichia coli* O111:B4, $p<0.05$ for full LPS compared to saline). (**E**) depicts a plot showing LPS (24 hours, 100ng/mL) treatment increased miR-155 14.3 fold in a microglia cell line (BV2).

[0010] **FIG. 2** graphically illustrates that miR-155 is increased in ALS patient samples. miRNAs were isolated from post-mortem spinal cords from controls (n=4) and ALS patients (n=5). miR-155 levels were measured by QPCR. (Bars = avg. +/- SE, $p<0.05$).

[0011] **FIG. 3** graphically illustrates cytokine and chemokine levels in miR-155 knockout mice and ALS mice while highlighting the blunted response in the miR-155 knockout mice as compared to their age-matched controls. (**A**) depicts a plot showing mRNA levels for cytokines IL-6, IL1-β, and TNFα for miR-155 knockout mice. (**B**) depicts a plot showing mRNA levels for chemokines CXCL2, CCR2, CXCR4, CXCR3, CCL2, and CXCL10 for miR-155 knockout mice. (**C**) depicts a plot showing mRNA levels for cytokines IL-6, IL1-β, and TNFα for ALS mice. (**D**) depicts a plot

showing mRNA levels for chemokines CXCL2, CCR2, CXCR4, CXCR3, CCL2, and CXCL10 for ALS mice.

[0012] **FIG. 4** depicts a schematic of the delivery of miRNA inhibitory molecules. A catheter is inserted into the lateral ventricle of an anesthetized mouse. The catheter is connected via plastic tubing to an Alzet osmotic pump that is located in a subcutaneous pocket on the back of the mouse. miRNA inhibitory molecules are delivered continuously via this method for up to 31 days. The pump can be replaced if continued drug delivery is desired.

[0013] **FIG. 5** shows miR-155 anti-sense oligonucleotides de-repress miR-155 related mRNA transcripts *in vivo*. miR-155 sequence (SEQ ID NO: 2) is depicted in black. Antisense oligonucleotides complementary to miR-155 seed sequences are depicted in blue and red.

[0014] **FIG. 6** graphically illustrates that neuroinflammation may be identified through the detection of cell-specific and inflammation markers in the $SOD1^{G93A}$ mouse model. (**A**) depicts a plot illustrating that microglia cell specific marker Iba1 and the astrocyte cell specific marker GFAP have increased expression in ALS (G93A) samples compared to normal samples. (**B**) depicts a plot illustrating that inflammation markers IL-1β and TNFα have increased expression in ALS (G93A) samples compared to normal samples.

[0015] **FIG. 7** shows expression of miR-155 in different cell types. Specifically, miR-155 is shown to be expressed in primary neuroglial cells.

[0016] **FIG. 8** graphically illustrates the longer survival of ALS (G93A) mice treated with antimiR-155 (**A**), and survival of miR-155 knockout/ALS (155-/- G93A) mice (**B**).

[0017] **FIG. 9** graphically illustrates that miR-155 knockout mice have reduced pro-inflammatory cytokine levels when challenged with LPS. (**A**) depicts a plot showing the levels of IL-6 in the cortex. (**B**) depicts a plot showing the levels of IL1β in the cortex. (**C**) depicts a plot showing the levels of TNFα in the cortex. (**D**) depicts a plot showing the levels of IL-6 in the spinal cord (SC). (**E**) depicts a plot showing the levels of TNFα in the spinal cord.

[0018] **FIG. 10** graphically illustrates that miR-155 knockout mice have reduced chemokine levels. (**A**) depicts a plot showing the levels of CCL2 in the cortex. (**B**) depicts a plot showing the levels of CXCL1 in the cortex. (**C**) depicts a plot showing the levels of CXCL10 in the cortex. (**D**) depicts a plot showing the levels of CCL2 in the spinal cord (SC). (**E**) depicts a plot showing the levels of CXCL1 in the spinal cord. (**F**) depicts a plot showing the levels of CXCL10 in the spinal cord.

[0019] **FIG. 11** graphically illustrates that miRNA array changes were confirmed changed in mouse, rat, and human spinal cord samples. (**A**) Using individual Taqman miRNA assays, 11 out of the 12 miRNA microarray hits were confirmed significantly increased in end-stage mouse spinal cord tissue. (n=5-6). (**B**) In rat lumbar spinal cord tissue, 10 out of the 12 miRNAs tested were increased in end-stage $SOD1^{G93A}$ rats over nontransgenic and overexpressing SOD1WT age-matched controls (n=4,4,4). (**C**) In autopsy spinal cord samples from 16 ALS patients and 12 non-ALS controls, 6 miRNAs assayed were significantly increased in the ALS tissue. (**D**) Specifically, both sporadic and familial ALS patients showed a significant increase in miR-155 (n=12 controls; 1.7x increase in n=11 sporadic ALS; 1.7x increase in n=5 familial ALS). SC = spinal cord. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

[0020] **FIG. 12** graphically illustrates that let-7 and miR-155 anti-miRs distribute throughout CNS and derepress target mRNAs. (**A**) Saline, scrambled anti-miR control, or anti-let-7 was infused directly to the lateral ventricle for 28 days with a subdermal osmotic pump. At 42 days, RNA was extracted. (**B**) Using Affymetrix 430 2.0 mouse gene arrays and Sylamer software analysis, cortical mRNA with let-7 binding sites (red and blue lines) were enriched among the upregulated (derepressed) mRNAs for anti-let-7 treated mice as normalized to scrambled-treated mice. (**C**, **D**) mRNA of two confirmed let-7 targets (TGFBR1, NRAS) were significantly increased in anti-let-7 treated mice in all regions assayed ($p<0.05$ TGFBR1; $p<0.01$ NRAS). (**E**) Hela cells were transfected with a miR-155 expression plasmid and a luciferase reporter containing two miR-155 binding sites. After 4 hours, either anti-miR-155 or a scrambled control anti-miR was transfected into cells (1 to 200nM). Quantified 24 hours later, luciferase levels were increased in anti-miR-155 treated cells, indicating inhibition of

miR-155. (**F**) After three daily IP injections of either anti-miR-155 or scrambled anti-miR control, peritoneal macrophages were isolated from adult mice, stimulated with LPS, and RNA was extracted. An Affymetrix microarray of mRNA followed by Sylamer analysis shows derepression of mRNAs that contain the heptamer seed region compliment to miR-155 (red and blue lines). (**G**) Anti-miR-155 was infused into the lateral ventricles of adult mice for 4 weeks. Two weeks later, mRNA was extracted. mRNA levels of SHIP1, a confirmed miR-155 target, were significantly increased in anti-miR-155 treated mice in all regions assayed ($p<0.05$).

[0021] **FIG. 13** depicts micrograph images illustrating that cy3-anti-miR-155 distributes from lateral cerebral ventricle throughout brain and spinal cord. Mice were treated for 2 weeks with an osmotic pump delivering 10ug/day cy3-labeled anti-miR-155 directly into the lateral ventricle. Anti-miR-155 distributes throughout the (**A**) subventicular zone (4x, scale bar = 500um), (**B**) cortical layers (4x, scale bar = 500um), (**C**) hippocampus (10x, scale bar = 200um), and (**D**) spinal cord (10x, scale bar = 200um).

[0022] **FIG. 14** graphically illustrates that survival, but not onset, is extended in anti-miR-155 treated ALS mice. $SOD1^{G93A}$ SJL mice were treated with both osmotic pumps directed to the lateral ventricles and with weekly IP injections starting at 60 days of age. Mice were weighed and scored biweekly using the ALSTDI system. A score of 1 determined onset and was marked by one of the following: 1) failure to fully extend legs when lifted by tail; 2) failure to spread legs past midline when lifted by tail; and 3) marked tremors when lifted by tail. Survival was determined as when the mouse could not right itself within 30 seconds of being placed on either side. (**A-B**) Saline (n=20) and anti-miR-155 treated mice (n=22) had no significant difference in onset as determined by neuroscore or by age at peak weight. (**C-D**) Anti-miR-155 treated mice had a significant extension in survival and a 38% extension in disease duration over saline. (median values shown, log-rank test, *$p<0.01$, **$p<0.01$, ***$p<0.001$).

[0023] **FIG. 15** graphically illustrates that miR-196 is increased in a microglia cell line and in primary cortical samples. (**A**) depicts a plot showing an increase of miR-

196a in nsc-34 cells and primary cortical samples. (**B**) depicts a plot showing an increase of miR-196b in primary cortical samples.

[0024] **FIG. 16** graphically depicts increased expression of miR-196 in autopsy samples from human ALS patients using individual Taqman miRNA assays.

[0025] **FIG. 17** graphically depicts increased miR-196 expression in human samples. (**A**) depicts a plot showing significant increase of miR-196a in ALS human patients. (**B**) depicts a plot showing significant increase of miR-196a in sporadic ALS human patients, but less significant increase in familial ALS patients. (**C**) depicts a plot showing significant increase of miR-196b in ALS human patients. (**D**) depicts a plot showing significant increase of miR-196b in sporadic ALS human patients, but less significant increase in familial ALS patients.

SUMMARY OF THE INVENTION

[0026] One aspect of the invention encompasses a method of treating a neurodegenerative disorder associated with overexpression of miR-155. The method generally comprises administering to a subject having a neurodegenerative disorder, or at risk of developing a neurological disorder associated with overexpression of miR-155 a therapeutically effective amount of a composition comprising a miR-155 agent that decreases the expression of miR-155r.

[0027] A further aspect of the invention provides a method of treating a neurodegenerative disorder associated with overexpression of miR-155. The method generally comprises administering to a subject having a neurodegenerative disorder, or at risk of developing a neurological disorder associated with overexpression of miR-155 a therapeutically effective amount of a composition comprising a miR-155 antisense oligonucleotide that decreases the expression of miR-155.

[0028] Yet another aspect of the invention provides a method of treating Amyotrophic Lateral Sclerosis. The method generally comprises administering to a subject having Amyotrophic Lateral Sclerosis, or at risk of developing Amyotrophic Lateral Sclerosis a therapeutically effective amount of a composition comprising a miR-155 antisense oligonucleotide that decreases the expression of miR-155.

[0029] Other features and aspects of the invention are described in more detail herein.

DETAILED DESCRIPTION

[0030] In accordance with the present invention, a process for regulating miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b to treat neurological disorders has been discovered. The present invention also provides compositions and methods based on the treatment. In particular, the present invention provides compositions and methods useful in research, diagnostics, and therapeutics for conditions and diseases associated with disregulation of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. The compositions and methods are directed at modulating the activity of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0031] Various aspects of the invention are described in further detail in the following sections.

I. Compositions

(a) nucleic acid molecules

[0032] One aspect of the invention pertains to isolated nucleic acid molecules that encode miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b or biologically active portions thereof, as well as nucleic acid molecules sufficient for use as hybridization probes to identify miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b -encoding nucleic acids (e.g., miR-155 miRNA, pri-miRNA, pre-mRNA) and fragments for use as PCR primers for the amplification or mutation of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b nucleic acid molecules.

[0033] A nucleic acid molecule of the present invention, or a complement of any of these nucleotide sequences, may be isolated using standard molecular biology techniques and the sequence information provided herein. For instance, using all or a portion of the nucleic acid sequences of SEQ ID NO: 1-6, miR-155 nucleic acid

molecules may be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook et al., eds., Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

[0034] A nucleic acid of the invention may be amplified using cDNA, mRNA or genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified may be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b nucleotide sequences may be prepared by standard synthetic techniques known in the art, such as using an automated DNA synthesizer.

[0035] In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of a nucleotide sequence of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b (such as SEQ ID NO: 1-6), or portion thereof. A nucleic acid molecule which is complementary to a given nucleotide sequence is one which is sufficiently complementary to the given nucleotide sequence that it can hybridize to the given nucleotide sequence, thereby forming a stable duplex.

[0036] Moreover, the nucleic acid molecule of the invention may comprise only a portion of a nucleic acid sequence encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. By way of example, a fragment of the nucleic acid coding sequence may be used as a probe, primer, or a fragment encoding a biologically active portion of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. The nucleotide sequence determined from the cloning of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b allows for the generation of probes and primers designed for use in identifying and/or cloning miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b homologues in other cell types, as well as miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or

miR-196b homologues and orthologs from other species. The probe/primer typically comprises substantially purified oligonucleotides. The oligonucleotides typically comprise a region of nucleotide sequence that hybridizes under stringent conditions to at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more consecutive nucleotides of the sense or antisense sequence of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b (such as SEQ ID NO: 1-6 for miR-155), or of a naturally occurring mutant.

[0037] Probes based on the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b nucleotide sequence may be used to detect transcripts or genomic sequences encoding the same or similar miRNA. The probe comprises a label group attached thereto, such as a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes may be used in diagnostic or screening assays.

[0038] A nucleic acid fragment encoding a "biologically active portion" of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b may be prepared by isolating a portion of a nucleotide sequence having miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b biological activity, expressing the encoded portion of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. For example, a nucleic acid fragment encoding a biologically active portion of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b includes the seed region, or an RNA binding site.

[0039] The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence of the native miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b (e.g. for miR-155, SEQ ID NO: 1-6), due to degeneracy of the genetic code and thus encode the same miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b as that encoded by the native nucleotide sequence.

[0040] In addition, it will be appreciated by those skilled in the art that nucleotide sequence polymorphisms may exist within a population (e.g., the human population). Such genetic polymorphism in the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b coding sequence may exist among individuals within a population due to natural allelic variation. Such natural allelic variations may result in as much as 15% variance in the nucleotide sequence. Any and all such nucleotide variations and resulting polymorphisms in miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b that are the result of natural allelic variation and that do not alter the functional activity of miR-155 are intended to be within the scope of the invention. Thus, e.g., 1%, 2%, 3%, 4%, or 5% of the nucleotide bases in miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b (e.g., 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, or 55) may be replaced by another nucleotide base.

[0041] Moreover, nucleic acid molecules encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b from other species (miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b orthologs/homologues), which have a nucleotide sequence which differs from that of a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b disclosed herein, are intended to be within the scope of the invention.

[0042] In addition to naturally occurring allelic variants of the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b sequence that may exist in the population, the skilled artisan will further appreciate that changes may be introduced by mutation into the native nucleotide sequence, thereby leading to changes in the sequence of the encoded miRNA without altering the functional ability of the miRNA. For example, such mutations may include nucleotide substitutions at "nonessential" nucleotide bases. A "nonessential" nucleotide base is one that may be altered from the wildtype sequence of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b without altering the biological activity, whereas an "essential" nucleotide base is required for biological activity.

[0043] Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b that contain changes in nucleotide bases that may or may not be essential for activity. Such miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b sequences differ from the native sequences. In specific embodiments, the isolated nucleic acid molecule includes a nucleotide sequence encoding miRNA that is at least about 45% identical, 65%, 75%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or more identical to the sequence of SEQ ID NO: 1-6 or 19-26. An isolated nucleic acid molecule encoding miR-155 having a sequence which differs from that of SEQ ID NO: 1-6 may be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of miR-155 (SEQ ID NO: 1-6) such that one or more substitutions, additions or deletions are introduced into the encoded miRNA. Similarly, An isolated nucleic acid molecule encoding miR-196 having a sequence which differs from that of SEQ ID NO: 19-26 may be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of miR-196a (SEQ ID NO: 19, 21-24) or miR-196b (SEQ ID NO: 20, 25, 26) such that one or more substitutions, additions or deletions are introduced into the encoded miRNA. Mutations may be introduced by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis.

(b) miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents

[0044] Another aspect of the invention pertains to miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents. As used herein, the term “miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent” refers to any molecule capable of modulating one or more activities of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. A miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent may respectively modulate one or more

activities of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b by increasing or decreasing expression of the respective miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b in a subject. In some embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent respectively modulates a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b activity by increasing the respective expression of a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b in a subject. In preferred embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent respectively modulates a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b activity by decreasing expression of a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b in a subject.

[0045] Exemplary miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents may include, without limitation, a compound, a drug, a small molecule, a peptide, a nucleic acid molecule, a protein, an antibody, and combinations thereof. miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents may be synthetic or naturally occurring.

[0046] In some embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a compound. In another embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a drug. In yet another embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a small molecule. In another embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a peptide. In another embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a protein. In still another embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is an antibody. In another embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a combination of miR-24, miR-142-3p, miR-142-

5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents capable of respectively modulating miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b activity.

[0047] In preferred embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a nucleic acid molecule. For instance, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b nucleic acid agent may be an antisense oligonucleotide, a ribozyme, a small nuclear RNA (snRNA), a long noncoding RNA (LncRNA), or a nucleic acid molecule which forms triple helical structures.

[0048] In some embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as miRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) Nature 334:585-591)) may be used to catalytically cleave miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b to thereby respectively inhibit activity of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. A ribozyme having specificity for a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b -encoding nucleic acid may be designed based upon the nucleotide sequence of a respective miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b cDNA. For example, miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b may be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel and Szostak (1993) Science 261:1411-1418; Suryawanshi, Scaria, and Maiti (2010) Mol Biosyst. 6:1807-1809.

[0049] In other embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a snRNA. For instance, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b snRNA agent may be a snRNA capable of regulating transcription of a nucleic

acid sequence respectively encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. Alternatively, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b snRNA agent may be a snRNA capable of regulating splicing of a mirtron encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0050] In yet other embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a LncRNA. A miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b LncRNA agent may be a LncRNA capable of regulating transcription of a nucleic acid sequence respectively encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0051] In other embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is a nucleic acid molecule which forms triple helical structures. For example, miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b expression may be modulated by targeting nucleotide sequences complementary to the regulatory region of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b (e.g., the miR-155 coding sequence promoter and/or enhancers) to form triple helical structures that respectively prevent transcription of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b in target cells. See generally, Helene (1991) Anticancer Drug Des. 6(6):569-84; Helene (1992) Ann. N.Y. Acad. Sci. 660:27-36; and Maher (1992) Bioassays 14(12):807-15.

[0052] In preferred embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is an antisense oligonucleotide. Antisense molecules are oligonucleotides comprising nucleic acid sequences complementary to a sense nucleic acid sequence. A miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b antisense oligonucleotide agent comprises nucleic acid sequences complementary to a miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, and may modulate the respective expression of miR-24, miR-142-3p, miR-

142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b by binding to a miRNA respectively encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. The expression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b may be modulated by blocking the respective activity of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, and respectively reducing the effective amount of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b in a cell.

[0053] An antisense oligonucleotide may bind through hydrogen bonds to a sense nucleic acid. As used herein, the term "sense nucleic acid sequence" is a nucleic acid sequence corresponding to an RNA sequence expressed in a cell. For instance, a sense nucleic acid sequence may be an expressed mRNA nucleic acid sequence, or a DNA nucleic acid sequence corresponding to an expressed mRNA nucleic acid sequence. As such, an antisense molecule of the invention comprises a nucleic acid sequence complementary to an expressed miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0054] A miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b may be a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b or a miRNA processing intermediate encoding a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA. As such, an antisense nucleic acid may comprise nucleic acid sequences complementary to a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b or to a miRNA processing intermediate encoding a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA. Non-limiting examples of miRNA processing intermediates encoding a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA include a pre-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, a pri-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, or a mirtron encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In some embodiments, an

antisense oligonucleotide comprises nucleic acid sequences complementary to a pri-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In other embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a mirtron encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In some embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a pre-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In yet other embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0055] An antisense oligonucleotide may comprise nucleic acid sequences complementary to a noncoding region in a miRNA processing intermediate encoding a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA. For instance, an antisense oligonucleotide may comprise nucleic acid sequences complementary to a noncoding region of a pri-miRNA, a pre-miRNA, or a mirtron encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. As used herein, the term “noncoding region” is used to describe nucleic acid sequences that flank a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b sequence in a miRNA processing intermediate encoding a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA.

[0056] In some embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a noncoding region of a pri-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In other embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a noncoding region of a mirtron encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In yet other embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a noncoding region of a pre-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0057] In yet other embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to coding and noncoding regions of a miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In one alternative of the embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to the stem-loop of a pre-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0058] In preferred embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a coding region in a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA. As used herein, the term "coding region" is used to describe a nucleic acid sequence present in a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA. As will be recognized by those of skill in the art, a nucleic acid sequence present in a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b is also present in a pri-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, a pre-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, and a mirtron miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. As such, an antisense oligonucleotide comprising nucleic acid sequences complementary to a nucleic acid sequence present in a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, may be complementary to a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, as well as to a pri-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, a pre-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, and a mirtron encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In some embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a coding region of a pri-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In other embodiments,

an antisense oligonucleotide comprises nucleic acid sequences complementary to a coding region of a mirtron encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In some embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a coding region of a pre-miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In yet other embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0059] An antisense oligonucleotide molecule may comprise nucleic acid sequences complementary to the entire coding region of a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA. Alternatively, an antisense oligonucleotide molecule may comprise nucleic acid sequences complementary to only a portion of the coding or noncoding region of a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA. As such, an antisense oligonucleotide may comprise nucleic acid sequences complementary to 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 80, 90, 100 or more nucleotides of the coding or noncoding region of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In some embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to 4, 5, 6, 7, 8, 9, or 10 nucleotides of the coding or noncoding region of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In other embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides of the coding or noncoding region of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In yet other embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides of the coding or noncoding region of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In yet other embodiments, an antisense oligonucleotide comprises nucleic acid sequences

complementary to 30, 35, 40, 45, 50, 55, 60, 65, 70, 80, 90, 100 or more nucleotides of the coding or noncoding region of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In preferred embodiments, an antisense oligonucleotide comprises nucleic acid sequences complementary to 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides of the coding or noncoding region of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0060] In particularly preferred embodiments, an antisense oligonucleotide of the invention comprises nucleic acid sequences complementary to a seed region of a miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In other particularly preferred embodiments, an antisense oligonucleotide consists of nucleic acid sequences complementary to a seed region of a miRNA encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. The seed region is a 7-8 nucleotide motif in the miRNA that determines specificity of binding of an miRNA to a target mRNA regulated by the miRNA. In most miRNAs, the seed region is within nucleotides 1-9 of the mature miRNA sequence. Antisense oligonucleotides comprising nucleic acid sequences complementary to the seed sequence of a miRNA have been shown to inhibit activity of the miRNA. Such inhibitory activity is described in PCT Publication No. WO 2009/043353, which is herein incorporated by reference in its entirety for its description of modified oligonucleotides targeting miRNA seed sequences.

[0061] As will be recognized by individuals skilled in the art, both arms of a pre-miRNA hairpin encoding miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b may give rise to a mature miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA. For instance, two mature miRNAs that may result from a pre-miRNA encoding a miR-155 miRNA may be miR-155-3p and miR-155-5p. As such, when an antisense nucleic acid comprises nucleic acid sequences complementary to a coding region of miR-155, an antisense nucleic acid may comprise nucleic acid sequences complementary to a miR-155-3p coding region of miR-155, or to a miR-155-5p coding region of miR-155. In some

embodiments, an antisense nucleic acid comprises nucleic acid sequences complementary to a miR-155-3p coding region of miR-155. In preferred embodiments, an antisense nucleic acid comprises nucleic acid sequences complementary to a miR-155-5p coding region of miR-155.

[0062] The size of a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b antisense agent of the invention can and will vary depending on the target miRNA encoding a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, the size of the nucleic acid sequence complementary to a region of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, and whether the antisense oligonucleotide comprises nucleic acid sequences in addition to the sequences complementary to a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA. An antisense oligonucleotide may be about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45 or about 50 nucleotides in length. In some embodiments, an antisense oligonucleotide is about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or about 15 nucleotides in length. In other embodiments, an antisense oligonucleotide is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or about 25 nucleotides in length. In yet other embodiments, an antisense oligonucleotide is about 25, 26, 27, 28, 29, 30, 35, 40, 45, or about 50 nucleotides in length. In some preferred embodiments, an antisense oligonucleotide is about 5, 6, 7, 8, 9, or about 10 nucleotides in length. In other preferred embodiments, an antisense oligonucleotide is about 19, 20, 21, 22, 23, 24, or about 25 nucleotides in length. In exemplary embodiments, an antisense oligonucleotide is 22 nucleotides in length.

[0063] In certain embodiments, a nucleic acid sequence of an antisense oligonucleotide comprising nucleic acid sequences complementary to a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b miRNA may have one or more mismatched base pairs with respect to its target miRNA or precursor sequence, and remains capable of hybridizing to its target sequence. For instance, a nucleic acid sequence of an antisense oligonucleotide comprising nucleic acid sequences complementary to a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-

146b, miR-155, miR-196a, or miR-196b miRNA may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mismatched base pairs with respect to its target miRNA or precursor sequence, and remains capable of hybridizing to its target sequence.

[0064] In certain embodiments, an antisense oligonucleotide comprises 8-25 nucleotides at least 85% complementary to a miR-155, a miR-196a, or a miR-196b miRNA. In preferred embodiments, an antisense oligonucleotide consists of 8-25 nucleotides at least 85% complementary to a miR-155, a miR-196a, or a miR-196b miRNA. In exemplary embodiments, an antisense oligonucleotide consists of 8-25 nucleotides at least 85% complementary to a mature miR-155, miR-196a, or a miR-196b miRNA. In particularly exemplary embodiments, an antisense oligonucleotide consists of 8-25 nucleotides at least 85% complementary to a miR-155 of SEQ ID NO: 2 or 5, a miR-196a of SEQ ID NO: 19, or a miR-196b of SEQ ID NO: 20.

[0065] In certain embodiments, an antisense oligonucleotide comprises 8-25 nucleotides at least 90% complementary to a miR-155, a miR-196a, or a miR-196b miRNA. In preferred embodiments, an antisense oligonucleotide consists of 8-25 nucleotides at least 90% complementary to a miR-155, a miR-196a, or a miR-196b miRNA. In exemplary embodiments, an antisense oligonucleotide consists of 8-25 nucleotides at least 90% complementary to a mature miR-155, miR-196a, or a miR-196b miRNA. In particularly exemplary embodiments, an antisense oligonucleotide consists of 8-25 nucleotides at least 90% complementary to a miR-155 of SEQ ID NO: 2 or 5, a miR-196a of SEQ ID NO: 19, or a miR-196b of SEQ ID NO: 20.

[0066] In certain embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is complementary to the nucleic acid sequence of the pre-miRNA of SEQ ID NO: 1 or 4. In preferred embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is about 70, 75, 80, 85, 90, 95%, or about 100% complementary to the nucleic acid sequence of the pre-miRNA of SEQ ID NO: 1 or 4.

[0067] In other embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is complementary to the nucleic acid sequence of the pre-miRNA of SEQ ID NO: 1 or 4. In preferred embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is about 70, 75, 80, 85, 90, 95%, or about

100% complementary to the nucleic acid sequence of the pre-miRNA of SEQ ID NO: 1 or 4.

[0068] In yet other embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is complementary to the nucleic acid sequence of a miR-196 pre-miRNA selected from the group of miR-196 pre-miRNAs consisting of SEQ ID NO: 21, 22, 23, 24, 25, and 26. In preferred embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is about 70, 75, 80, 85, 90, 95%, or about 100% complementary to the nucleic acid sequence of a miR-196 pre-miRNA selected from the group of pre-miRNAs consisting of SEQ ID NO: 21, 22, 23, 24, 25, and 26.

[0069] In other embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is complementary to the nucleic acid sequence of a miR-196 pre-miRNA selected from the group of miR-196 pre-miRNAs consisting of SEQ ID NO: 21, 22, 23, 24, 25, and 26. In preferred embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is about 70, 75, 80, 85, 90, 95%, or about 100% complementary to the nucleic acid sequence of a miR-196 pre-miRNA selected from the group of miR-196 pre-miRNAs consisting of SEQ ID NO: 21, 22, 23, 24, 25, and 26.

[0070] In certain embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is complementary to the nucleic acid sequence of the mature miR-155 miRNA of SEQ ID NO: 3 or 6. In preferred embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is about 70, 75, 80, 85, 90, 95%, or about 100% complementary to the nucleic acid sequence of the mature miR-155 miRNA of SEQ ID NO: 3 or 6.

[0071] In yet other embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is complementary to the nucleic acid sequence of the mature miR-155 miRNA of SEQ ID NO: 3 or 6. In preferred embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is about 70, 75, 80, 85, 90, 95%, or about 100% complementary to the nucleic acid sequence of the mature miR-155 miRNA of SEQ ID NO: 3 or 6.

[0072] In other preferred embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is complementary to the seed region of the miR-155 miRNA of SEQ ID NO: 3 or 6. In yet other preferred embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is complementary to the seed region of the miR-155 miRNA of SEQ ID NO: 3 or 6. In certain embodiments, the seed region is nucleotides 1-7 of SEQ ID NO: 3 or 6. In certain embodiments, the seed region is nucleotides 2-8 of SEQ ID NO: 3 or 6. In preferred embodiments, the seed region is nucleotides 1-8 of SEQ ID NO: 3 or 6.

[0073] In preferred embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is complementary to the nucleic acid sequence of the mature miR-155 miRNA of SEQ ID NO: 2 or 5. In other embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is complementary to the nucleic acid sequence of the mature miR-155 miRNA of SEQ ID NO: 2 or 5. In yet other preferred embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is about 70, 75, 80, 85, 90, 95%, or about 100% complementary to the nucleic acid sequence of the mature miR-155 miRNA of SEQ ID NO: 2 or 5.

[0074] In other preferred embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is 100% complementary to the seed region of the miR-155 miRNA of SEQ ID NO: 3 or 6. In yet other preferred embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is 100% complementary to the seed region of the miR-155 miRNA of SEQ ID NO: 3 or 6. In certain embodiments, the seed region is nucleotides 1-7 of SEQ ID NO: 3 or 6. In other embodiments, the seed region is nucleotides 2-8 of SEQ ID NO: 3 or 6. In preferred embodiments, the seed region is nucleotides 1-8 of SEQ ID NO: 3 or 6.

[0075] In other preferred embodiments, an antisense oligonucleotide comprises a nucleic acid sequence that is 100% complementary to the seed region of the miR-155 miRNA of SEQ ID NO: 2 or 5. In yet other preferred embodiments, an antisense oligonucleotide consists of a nucleic acid sequence that is 100% complementary to the seed region of the miR-155 miRNA of SEQ ID NO: 2 or 5. In certain embodiments, the seed region is nucleotides 1-7 of SEQ ID NO: 2 or 5. In

certain embodiments, the seed region is nucleotides 2-8 of SEQ ID NO: 2 or 5. In preferred embodiments, the seed region is nucleotides 1-8 of SEQ ID NO: 2 or 5.

[0076] In exemplary embodiments, an antisense oligonucleotide of the invention comprises a nucleic acid sequence having at least about 80%, more preferably 85%, more preferably 90%, or more preferably 95% identity to the nucleic acid sequence of SEQ ID NO: 16. In preferred embodiments, an antisense oligonucleotide of the invention comprises the nucleic acid sequence of SEQ ID NO: 16.

[0077] In other exemplary embodiments, an antisense oligonucleotide of the invention consists of a nucleic acid sequence having at least about 80%, more preferably 85%, more preferably 90%, or more preferably 95% identity to the nucleic acid sequence of SEQ ID NO: 16. In exemplary embodiments, an antisense oligonucleotide of the invention consists of the nucleic acid sequence of SEQ ID NO: 16.

[0078] An antisense oligonucleotide of the invention may be synthesized using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an oligonucleotide (e.g., an antisense oligonucleotide) may be chemically synthesized using naturally occurring ribonucleotides, deoxyribonucleotides, variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, or combinations thereof. For example, phosphorothioate derivatives and acridine substituted nucleotides may be used. Other examples of modified nucleotides which may be used to generate an antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-

isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-aino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the oligonucleotide may be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation.

[0079] In certain embodiments, antisense oligonucleotides provided herein may include one or more modifications to a nucleobase, sugar, and/or internucleoside linkage, and as such is a modified oligonucleotide. A modified nucleobase, sugar, or internucleoside linkage may be selected over an unmodified form because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for other oligonucleotides or nucleic acid targets, and increased stability in the presence of nucleases. In certain embodiments, a modified nucleoside is a sugar-modified nucleoside. In certain such embodiments, sugar-modified nucleosides may further comprise a natural or modified heterocyclic base moiety or natural or modified internucleoside linkage and may include further modifications independent from the sugar modification. In certain embodiments, a sugar modified nucleoside is a 2'-modified nucleoside, wherein the sugar ring is modified at the 2' carbon from natural ribose or 2'-deoxy-ribose. In certain embodiments, a 2'-modified nucleoside comprises a 2'-substituent group selected from F, O-CH_3, and $OCH_2CH_2OCH_3$. In certain embodiments, a 2'-modified nucleoside has a bicyclic sugar moiety. In certain embodiments, a bicyclic sugar moiety comprises a bridge group between the 2' and the 4' carbon atoms.

[0080] In certain embodiments, a modified oligonucleotide comprises one or more internucleoside modifications. In certain such embodiments, each internucleoside linkage of an oligonucleotide is a modified internucleoside linkage. In certain embodiments, a modified internucleoside linkage comprises a phosphorus atom.

[0081] In certain embodiments, a modified oligonucleotide comprises at least one phosphorothioate internucleoside linkage. In preferred embodiments, each

internucleoside linkage of a modified oligonucleotide is a phosphorothioate internucleoside linkage.

[0082] In certain embodiments, a modified oligonucleotide comprises one or more modified nucleobases. In certain embodiments, a modified oligonucleotide comprises one or more 5-methylcytosines. In certain embodiments, each cytosine of a modified oligonucleotide comprises a 5-methylcytosine.

[0083] In certain embodiments, a modified nucleobase is selected from 5-hydroxymethyl cytosine, 7-deazaguanine and 7-deazaadenine. In certain embodiments, a modified nucleobase is selected from 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone.

[0084] In some embodiments, the antisense molecules of the invention may be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. By way of another example, the deoxyribose phosphate backbone of the nucleic acids may be modified to generate peptide nucleic acids (see Hyrup et al. (1996) Bioorganic & Medicinal Chemistry 4(l):5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers may be performed using standard solid phase peptide synthesis protocols as described in Hyrup et al. (1996) supra; Perry-O'Keefe et al. (1996) Proc. Natl. Acad. Sci. USA 93:14670-675.

[0085] PNAs of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b may be used for therapeutic and diagnostic applications. For example, PNAs may be used as antisense or miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents for sequence-specific modulation of expression by inducing transcription arrest or inhibiting replication. PNAs of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b may also be used in the analysis of single base pair mutations

in a gene by PNA-directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, such as S1 nucleases (Hyrup (1996) supra); or as probes or primers for DNA sequence and hybridization (Hyrup (1996) supra; Perry-O'Keefe et al. (1996) Proc. Natl. Acad. Sci. USA 93: 14670-675).

[0086] In other embodiments, the oligonucleotides of the invention may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) Proc. Natl. Acad. Sci. USA 86:6553-6556; Lemaitre et al. (1987) Proc. Natl. Acad. Sci. USA 84:648-652; PCT Publication No. W0 88/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W0 89/10134). In addition, oligonucleotides may be modified with hybridization-triggered cleavage agents (see, e.g., Krol et al. (1988) Bio/Techniques 6:958-976) or intercalating agents (see, e.g., Zon (1988) Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0087] In exemplary embodiments, an antisense oligonucleotide of the invention is synthesized with a full phosphorothioate backbone with alternating blocks of 2'-MOE and 2'fluoro sugar-modified nucleosides. In a particularly exemplary embodiment, an antisense oligonucleotide of the invention consists of the nucleic acid sequence of SEQ ID NO: 16, and is synthesized with a full phosphorothioate backbone with alternating blocks of 2'-MOE and 2'fluoro sugar-modified nucleosides.

(c) pharmaceutical compositions

[0088] The miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents (also referred to herein as "active compounds") of the invention may be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the agent and a pharmaceutically acceptable carrier. As used herein, the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with

pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds may also be incorporated into the compositions.

[0089] The invention includes methods for preparing pharmaceutical compositions for modulating the expression or activity of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. Such methods comprise formulating a pharmaceutically acceptable carrier with an agent which modulates expression or activity of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. Such compositions can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a pharmaceutically acceptable carrier with an agent which modulates expression or activity of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b and one or more additional active compounds.

[0090] An agent which modulates expression or activity may, for example, be a small molecule. For example, such small molecules include peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the knowledge of the ordinarily skilled artisan. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner

desires the small molecule to have. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram). It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of miR-155, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

[0091] A pharmaceutical composition of the invention may be formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0092] Pharmaceutical compositions suitable for injectable use may include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL (BASF; Parsippany, N.J.), or phosphate buffered saline (PBS). In all cases, a composition may be sterile and may be fluid to the extent that easy syringeability exists. A composition may be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it may be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride, in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0093] Sterile injectable solutions may be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0094] Oral compositions generally may include an inert diluent or an edible carrier. Oral compositions may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions may also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents and/or adjuvant materials may be included as part of the composition. The tablets, pills, capsules, troches, and the like, may contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose; a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[0095] Systemic administration may also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and may include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration may be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art. The compounds may also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0096] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery

systems. Biodegradable, biocompatible polymers may be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0097] The nucleic acid molecules of the invention may be inserted into vectors and used as gene therapy vectors. Gene therapy vectors may be delivered to a subject by, for example, intravenous injection, local administration (U.S. Pat. No. 5,328,470) or by stereotactic injection (see, e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector may include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded.

[0098] The gene therapy vectors of the invention may be either viral or non-viral. Examples of plasmid-based, non-viral vectors are discussed in Huang et al. (1999) Nonviral Vectors for Gene Therapy. A modified plasmid is one example of a non-viral gene delivery system. Peptides, proteins (including antibodies), and oligonucleotides may be stably conjugated to plasmid DNA by methods that do not interfere with the transcriptional activity of the plasmid (Zelphati et al. (2000) BioTechniques 28:304-315). The attachment of proteins and/or oligonucleotides may influence the delivery and trafficking of the plasmid and thus render it a more effective pharmaceutical composition.

II. Methods

[0099] In other aspects, the present invention encompasses a method of treating a subject with a neurodegenerative disorder resulting from a dysregulated miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b activity. A dysregulated miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b activity may result from overexpression or underexpression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In some embodiments, a method of the invention comprises

treating a neurodegenerative disorder resulting from underexpression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In preferred embodiments, a method of the invention comprises treating a neurodegenerative disorder resulting from overexpression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0100] Methods of the invention include administering compositions of the present invention to a subject for the treatment of a neurological disorder. In one embodiment, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to a subject for the treatment of a neurodegenerative disorder or symptom. In one embodiment, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to a subject for the treatment of ALS. In another embodiment, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to a subject or population of cells to respectively regulate the function of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

(a) neurodegenerative disorders

[0101] Conditions that would benefit from miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b therapy (e.g. treatment with miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents) may include any condition, symptom, disorder or disease that respectively involves dysregulation of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. For instance, exemplary conditions that may benefit include neurodegenerative movement disorders, neurodegenerative conditions relating to memory loss, dementia, pain disorders, sleep disorders, Alzheimer's disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's disease, Parkinson's disease, multiple sclerosis, AIDS dementia, epilepsy or retinal diseases, presenile dementia, senile dementia, progressive supranuclear palsy (PSP), Pick's disease, primary progressive aphasia, frontotemporal dementia, corticobasal dementia, dementia with

Lewy bodies, Down's syndrome, multiple system atrophy, Hallervorden-Spatz syndrome, stroke, head or spinal trauma, or asphyxia; conditions associated with head trauma, spinal trauma, general anoxia, hypoxia, including fetal hypoxia, hypoglycemia, hypotension, as well as similar injuries seen during procedures from embole, hyperfusion or hypoxia; movement related neurodegenerative disorders such as dyskinesias, tremor, dystonia, chorea, athetosis, tic disorders, blepharospasm, hemiballysmus, myoclonus, focal dystonias, torticollis, restless leg syndrome and asterixis, as well as others known in the art or yet to be discovered. Exemplary symptoms of neurodegenerative disorders include cognitive impairments such as difficulties with concentration, attention, memory, and poor judgment. A skilled artisan will appreciate that specific diseases are defined by specific symptoms. For example, ALS is recognized by muscle weakness, spreading atrophy to other parts of the body, increasing problems with moving, swallowing, and speaking or forming words. The major hallmarks of Alzheimer's include amyloid plaques, neurofibrillary tangles, and loss of connections between neurons responsible for memory and learning. In Huntington's disease, uncontrolled movements, loss of intellectual faculties, and emotional disturbance are often observed. Parkinson's disease involves motor system disorders, which are the result of the loss of dopamine-producing brain cells. The four primary symptoms of Parkinson's disease are tremor, or trembling in hands, arms, legs, jaw, and face; rigidity, or stiffness of the limbs and trunk; bradykinesia, or slowness of movement; and postural instability, or impaired balance and coordination. Symptoms of multiple sclerosis include blurred or double vision, red-green color distortion, or even blindness in one eye, extreme muscle weakness, difficulty with coordination and balance, impairment to walking or even standing, partial or complete paralysis, paresthesias, transitory abnormal sensory feelings such as numbness, prickling, or "pins and needles" sensations and pain, speech impediments, tremors, dizziness, hearing loss, cognitive impairments, and depression. Additionally, dementia, appearing in many specific neurodegenerative diseases, is a collection of symptoms relating to impaired intellectual functioning such as ability to solve problems and maintain

emotional control; personality changes and behavioral problems, such as agitation, delusions, and hallucinations; memory loss and impaired language skills.

[0102] In preferred embodiments, the neurodegenerative disorder is Amyotrophic Lateral Sclerosis.

[0103] In some embodiments, methods of the invention may be utilized to treat a population of cells that would benefit from miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b therapy. Such cells include those in a subject as well as those removed from a subject for therapeutic treatment, cultured cells, those used in gene therapy practices, and any other cell that may benefit from miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b therapy.

(b) administration

[0104] The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of, or susceptible to a disorder, or having a disorder associated with aberrant miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b expression or activity. Generally, methods of the present invention include administering to a subject a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition respectivelycomprising at least one miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent. For instance, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents may be administered (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more miR-155 agents may be administered). In some embodiments, 1, 2, 3, 4, or 5 miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents are administered. In other embodiments, 5, 6, 7, 8, 9, 10 or more miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents are administered. In one embodiment, one miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is administered. In another embodiment, two miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b

agents are administered. In yet another embodiment, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent is delivered in combination with additional therapeutic agents known in the art. miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents may be as described in **Section I(b)**.

[0105] In certain embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered in combination with at least one additional therapeutic agent. In certain embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered sequential to an additional therapeutic agent. In other embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered prior to the administration of an additional therapeutic agent. In certain embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered prior to and after the administration of an additional therapeutic agent. In other embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered at the same time as at least one therapeutic agent. In certain embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition may be administered without additional therapeutic agents.

[0106] Additional therapeutic agents may include those used in immunotherapy, gene transfer therapy, stem cell and progenitor cell based cellular replacement therapy, antisense oligonucleotide therapy, antioxidant therapy, antidepressant therapy, antibody therapy, autophagy control therapy, drug therapy (small-molecule inhibitor of kynurenine 3- monooxygenase JM6), and any therapeutic agent known in the art or yet to be discovered.

[0107] A miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition of the invention may be administered to a subject by several different means. For instance, compositions may generally be administered

in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired.

[0108] Methods of administration include any method known in the art or yet to be discovered. Exemplary administration methods include intravenous, intraocular, intratracheal, intratumoral, oral, rectal, topical, intramuscular, intraarterial, intrahepatic, intrathoracic, intrathecal, intracranial, intraperitoneal, intrapancreatic, intrapulmonary, or subcutaneously. A composition of the invention may also be administered directly by infusion into central nervous system fluid. One skilled in the art will appreciate that the route of administration and method of administration depend upon the intended use of the compositions, the location of the target area, and the condition being treated, in addition to other factors known in the art such as subject health, age, and physiological status.

[0109] In a preferred embodiment, the oligonucleotide may be administered parenterally. The term “parenteral” as used herein describes administration into the body via a route other than the mouth, especially via infusion, injection, or implantation, and includes intradermal, subcutaneous, transdermal implant, intracavernous, intravitreal, intra-articular or intrasynovial injection, transscleral, intracerebral, intrathecal, epidural, intravenous, intracardiac, intramuscular, intraosseous, intraperitoneal, intravenous, intrasternal injection, or nanocell injection. Formulation of pharmaceutical compositions is discussed in, for example, Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. (1975), and Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y. (1980).

[0110] In some embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition of the invention is administered parenterally. When a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered parenterally, delivery methods are preferably those that are effective to circumvent the blood-brain barrier, and are effective to deliver agents to the central nervous system. For example, delivery methods may include the use of nanoparticles. The particles may be of any

suitable structure, such as unilamellar or plurilamellar, so long as the antisense oligonucleotide is contained therein. Positively charged lipids such as N-[1-(2,3-dioleoyloxi)propyl]-N, N,N-trimethyl-amoniummethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known in the art. See, e.g., U.S. Pat. Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.; 4,911,928 to Wallach; 4,917,951 to Wallach; 4,920,016 to Allen et al.; 4,921,757 to Wheatley et al.; etc.

[0111] In preferred embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition of the invention is administered into the central nervous system. Methods of administering a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition of the invention to the central nervous system are known in the art. For instance, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition of the invention may be administered in a bolus directly into the central nervous system. A miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition may be administered to the subject in a bolus once or multiple times. In some preferred embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition may be administered in a bolus once. In other preferred embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition may be administered in a bolus multiple times. When administered multiple times, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition may be administered at regular intervals or at intervals that may vary during the treatment of a subject. In some embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered multiple times at intervals that may vary during the treatment of a subject. In other embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered multiple times at regular intervals.

[0112] In another preferred embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion into the central nervous system. Non-limiting examples of methods that may be used to deliver a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition into the central nervous system by continuous infusion may include pumps, wafers, gels, foams and fibrin clots. In a preferred embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is delivered into the central nervous system by continuous infusion using an osmotic pump. An osmotic minipump contains a high-osmolality chamber that surrounds a flexible, yet impermeable, reservoir filled with the targeted delivery composition-containing vehicle. Subsequent to the subcutaneous implantation of this minipump, extracellular fluid enters through an outer semi-permeable membrane into the high-osmolality chamber, thereby compressing the reservoir to release the targeted delivery composition at a controlled, pre-determined rate. The targeted delivery composition, released from the pump, may be directed via a catheter to a stereotaxically placed cannula for infusion into the cerebroventricular space. In an exemplary embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is delivered into the central nervous system by continuous infusion using a pump as described in the Examples.

[0113] Compositions of the invention are typically administered to a subject in an amount sufficient to provide a benefit to the subject. This amount is defined as a "therapeutically effective amount." A therapeutically effective amount may be determined by the efficacy or potency of the particular composition, the neurodegenerative disorder being treated, the duration or frequency of administration, the method of administration, and the size and condition of the subject, including that subject's particular treatment response. A therapeutically effective amount may be determined using methods known in the art, and may be determined experimentally, derived from therapeutically effective amounts determined in model animals such as the mouse, or a combination thereof. Additionally, the route of administration may be considered when determining the therapeutically effective amount. In determining the

therapeutically effective amounts, one skilled in the art may also consider the existence, nature, and extent of any adverse effects that accompany the administration of a particular compound in a particular subject.

[0114] In some embodiments, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered in a bolus into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition may be administered to the subject in an amount of about 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, or about 100 mg/kg or more. In one embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered in a bolus into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 0.1, 0.2, 0.3, 0.4, 0.5, or about 1 mg/kg. In another embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered in a bolus into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or about 10 mg/kg. In yet another embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered in a bolus into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 18, 19, or about 20 mg/kg. In another embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered in a bolus into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 20, 21, 22, 23,

24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. In an additional embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered in a bolus into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 50, 60, 70, 80, 90, or about 100 mg/kg. In a preferred embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered in a bolus into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 23, 24, 25, 26, or about 27 mg/kg.

[0115] In some embodiments, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition may be administered to the subject in an amount of about 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, or about 100 µg/day or more. In one embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 0.1, 0.2, 0.3, 0.4, 0.5, or about 1 µg/day. In another embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion into the central nervous system, the v composition is administered to the subject in an amount of about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or about 10 µg/day. In yet another embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous

infusion into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 18, 19, or about 20 µg/day. In another embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 µg/day. In an additional embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 50, 60, 70, 80, 90, or about 100 µg/day. In a preferred embodiment, when a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion into the central nervous system, the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered to the subject in an amount of about 17, 18, 19, 20, 21, 22, or about 23 µg/day.

[0116] One of skill in the art will also recognize that the duration of the administration by continuous infusion can and will vary, and will depend in part on the subject, the neurodegenerative disorder, and the severity, progression and improvement of the condition of the subject, and may be determined experimentally. In some embodiments, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96,

97, 98, 99, 100 days or longer. In one embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion for 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 days or longer. In another embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion for 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65 days or longer. In yet another embodiment, a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition is administered by continuous infusion for 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95 days or longer. Longer continuous infusions of the antisense oligonucleotide may also be envisioned using existing pump technology as is known in the art.

[0117] When a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b composition of the invention is an antisense oligonucleotide, molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b or the coding sequence of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b inhibiting the respective biological activity of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. The hybridization may be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An antisense nucleic acid molecule of the invention may be administered by direct injection at a tissue site. Alternatively, antisense nucleic acid molecules may be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules may be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens.

The antisense nucleic acid molecules may also be delivered by direct infusion into a subject. The antisense nucleic acid molecules may also be delivered to cells using gene therapy vectors known in the art. To achieve sufficient intracellular concentrations of the antisense molecules, vectors in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

(c) treating a subject

[0118] A method of the invention comprises treating a subject by administering to the subject a therapeutically effective amount of a composition comprising a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent that decreases the expression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. As used herein, "subject" may refer to a living organism having a central nervous system. In particular, subjects may include, but are not limited to, human subjects or patients and companion animals. Exemplary companion animals may include domesticated mammals (e.g., dogs, cats, horses), mammals with significant commercial value (e.g., dairy cows, beef cattle, sporting animals), mammals with significant scientific value (e.g., captive or free specimens of endangered species), or mammals which otherwise have value. Suitable subjects may also include: mice, rats, dogs, cats, ungulates such as cattle, swine, sheep, horses, and goats, lagomorphs such as rabbits and hares, other rodents, and primates such as monkeys, chimps, and apes. In some preferred embodiments, a subject is a human. In other preferred embodiments, a subject is a rat. In yet other preferred embodiments, a subject is a mouse. In an exemplary embodiment, a subject is a $SOD1^{G93A}$ B6/SJL mouse. Subjects may be of any age including newborn, adolescent, adult, middle age, or elderly.

[0119] A subject may be at risk for developing a neurodegenerative disorder resulting from overexpression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. As such, in some embodiments, treating a neurodegenerative disorder prevents a disorder from developing in a subject at risk of developing a neurological disorder resulting from overexpression of miR-24, miR-142-

3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. Subjects at risk for a neurological disorder which is caused or contributed to by overexpression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b or activity may be identified by, for example, any or a combination of diagnostic or prognostic assays for detecting miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b mutation or activity. A prophylactic agent may be administered prior to the manifestation of symptoms characteristic of the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b aberrancy, such that a disease or disorder is prevented, or delayed in its progression.

[0120] A subject may also be diagnosed as having a neurodegenerative disorder resulting from overexpression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. In some embodiments, treating a neurodegenerative disorder treats a disorder in a subject having a neurological disorder resulting from overexpression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by overexpression of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b.

[0121] Treating a subject using a method of the invention may extend the survival of the subject. Alternatively, treating a subject using a method of the invention may extend the disease duration of the subject.

[0122] In some embodiments, treating a subject extends the survival of the subject. A method of the invention may extend the survival of a subject by days, weeks, months, or years, when compared to the survival of a subject that was not treated using a method of the invention. As will be recognized by individuals skilled in the art, the number of days, months, or years that a method of the invention may extend the survival of a subject can and will vary depending on the subject, the neurological disorder, and the condition of the subject when treatment was initiated among other factors. By way of non-limiting example, treating a $SOD1^{G93A}$ B6/SJL mouse may extend the survival of the mouse by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,

16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or about 30 days or more, when compared to the survival of a $SOD1^{G93A}$ B6/SJL mouse that was not treated using a method of the invention.

[0123] In other embodiments, treating a subject extends the disease duration of a subject. As used herein, the term "disease duration" is used to describe the length of time between onset of symptoms and death caused by the disease. A method of the invention may extend the disease duration of a subject by days, weeks, months, or years, when compared to the survival of the subject that was not treated using a method of the invention. The number of days, months, or years that a method of the invention may extend the disease duration of a subject can and will vary depending on the subject, the neurological disorder, and the condition of the subject when treatment was initiated among other factors. By way of non-limiting example, treating a $SOD1^{G93A}$ B6/SJL mouse may extend the disease duration of the mouse by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or about 30 days or more, when compared to the survival of a $SOD1^{G93A}$ B6/SJL mouse that was not treated using a method of the invention. Alternatively, treating a $SOD1^{G93A}$ B6/SJL mouse may extend the disease duration of the mouse by about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50% or more, when compared to the disease duration of a $SOD1^{G93A}$ B6/SJL mouse that was not treated using a method of the invention.

III. Kits

[0124] In still other aspects, the present invention provides articles of manufacture and kits containing materials useful for treating the conditions described herein. The article of manufacture may include a container of a composition as described herein with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition having an active agent which is effective for treating, for example, conditions that benefit from miR-24, miR-142-3p, miR-142-5p,

miR-146a, miR-146b, miR-155, miR-196a, or miR-196b therapy. The active agent is at least one miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent of the invention and may further include additional bioactive agents known in the art for treating the specific condition. The label on the container may indicate that the composition is useful for treating specific conditions and may also indicate directions for administration.

DEFINITIONS

[0125] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. All patents, applications, published applications and other publications are incorporated by reference in their entirety. In the event that there is a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

[0126] As used herein, “administering” is used in its broadest sense to mean contacting a subject with a composition of the invention.

[0127] As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% (65%, 70%, preferably 75%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A non-limiting example of stringent hybridization conditions are hybridization in 6x sodium chloride/sodium citrate (SSC) at about 45°C., followed by one or more washes in 0.2.x SSC, 0.1% SDS at 50-65°C. (e.g., 50°C. or 60°C. or 65°C). Preferably, the isolated nucleic acid molecule of the invention that hybridizes under stringent conditions corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to a RNA or DNA molecule having a nucleotide sequence that occurs in a human cell in nature (e.g., encodes a natural protein).

[0128] As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA

or miRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule may be single-stranded or double-stranded.

[0129] An "isolated nucleic acid molecule" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated, even if subsequently reintroduced into the natural system. Such polynucleotides may be part of a vector or other composition and still be isolated in that such vector or composition is not part of its natural environment.

[0130] A "nucleic acid vector" is a nucleic acid sequence designed to be propagated and or transcribed upon exposure to a cellular environment, such as a cell lysate or a whole cell. A "gene therapy vector" refers to a nucleic acid vector that also carries functional aspects for transfection into whole cells, with the intent of increasing expression of one or more genes or proteins. In each case, such vectors usually contain a "vector propagation sequence" which is commonly an origin of replication recognized by the cell to permit the propagation of the vector inside the cell. A wide range of nucleic acid vectors and gene therapy vectors are familiar to those skilled in the art.

[0131] A miRNA is a small non-coding RNA molecule which functions in transcriptional and post-transcriptional regulation of gene expression. A miRNA functions via base-pairing with complementary sequences within mRNA molecules, usually resulting in gene silencing via translational repression or target degradation. A mature miRNA is processed through a series of steps from a larger primary RNA transcript (pri-miRNA), or from an intron comprising a miRNA (mirtron), to generate a stem loop pre-miRNA structure comprising the miRNA sequence. A pre-miRNA is then cleaved to generate the mature miRNA.

[0132] Primary miRNA transcripts are transcribed by RNA polymerase II and may range in size from hundreds to thousands of nucleotides in length (pri-mRNA). Pri-miRNAs may encode for a single miRNA but may also contain clusters of several

miRNAs. The pri-miRNA is subsequently processed into an about 70 nucleotide hairpin (pre-miRNA) by the nuclear ribonuclease III (RNase III) endonuclease, Drosha. Thus, isolated nucleic acid molecules of the invention have various preferred lengths, depending on their intended targets. When targeted to pri-miRNA, preferred lengths vary between 100 and 200 nucleotides, e.g., 100, 120, 150, 180 or 200 nucleotides. In the cytoplasm, a second RNAse III, Dicer, together with its dsRBD protein partner, cuts the pre-miRNA in the stem region of the hairpin thereby liberating an about 21 nucleotide RNA-duplex. Thus, isolated polynucleotides of about 80, 70, 60, 50, 40, 30, 25, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, or 6 nucleotides in length are also considered in one embodiment of the invention.

[0133] As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain and/or common functional activity. For example, amino acid or nucleotide sequences which contain a common structural domain having about 65% identity, preferably 75% identity, more preferably 85%, 95%, or 98% identity are defined herein as sufficiently identical.

[0134] As used interchangeably herein, a “miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b activity”, “biological activity of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b” or “functional activity of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b” refers to an activity exerted by a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b, respectively on a miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b responsive cell, target mRNA, or target protein as determined *in vivo* or *in vitro*, according to standard techniques. A miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b activity may be a direct activity such as an association with a second protein or mRNA. A miR-24, miR-142-3p, miR-142-5p, miR-

146a, miR-146b, miR-155, miR-196a, or miR-196b activity may be an indirect activity such as a cellular signaling activity mediated by interaction of the miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b protein with a second protein or mRNA.

[0135] The term "sample" refers to a cell, a population of cells, biological samples, and subjects, such as mammalian subjects. The term "biological sample" refers to tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject.

[0136] As used herein, "subject" refers to a living organism having a central nervous system. In particular, subjects may include, but are not limited to, human subjects or patients and companion animals. Exemplary companion animals may include domesticated mammals (e.g., dogs, cats, horses), mammals with significant commercial value (e.g., dairy cows, beef cattle, sporting animals), mammals with significant scientific value (e.g., captive or free specimens of endangered species), or mammals which otherwise have value. Suitable subjects may also include: mice, rats, dogs, cats, ungulates such as cattle, swine, sheep, horses, and goats, lagomorphs such as rabbits and hares, other rodents, and primates such as monkeys, chimps, and apes. In some embodiments, subjects may be diagnosed with a fibroblastic condition, may be at risk for a fibroblastic condition, or may be experiencing a fibroblastic condition. Subjects may be of any age including newborn, adolescent, adult, middle age, or elderly.

[0137] The term "miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent" refers to any molecule capable of respectively modulating miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b activity. Exemplary miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents may include, without limitation, a compound, drug, small molecule, peptide, oligonucleotide, protein, antibody, and combinations thereof. miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agents may be synthetic or naturally occurring. A miR-24,

miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b agent may be a molecule identified in a screening assay as described herein.

[0138] The term "miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b indicator" refers to any molecule capable of detecting, respectively, the presence of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. A suitable miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b indicator may be a compound, drug, small molecule, peptide, oligonucleotide, protein, antibody, and combinations thereof.

[0139] As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of pathological processes mediated by dysregulation of miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-155, miR-196a, or miR-196b. The specific amount that is therapeutically effective may be readily determined by ordinary medical practitioners, and may vary depending on factors known in the art, such as the type of disorder being treated, the subject's history and age, the stage of the disorder, and administration of other agents in combination.

[0140] As used herein, a "pharmaceutical composition" includes a pharmacologically effective amount of a therapeutic agent of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically effective amount" or simply "effective amount" refers to that amount of an agent effective to produce the intended pharmacological, therapeutic or preventive result. For example, if a given clinical treatment is considered effective when there is at least a 15% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of an agent for the treatment of that disorder or disease is the amount necessary to effect at least a 15% reduction in that parameter.

[0141] The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers may include, but are not limited to , saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally,

pharmaceutically acceptable carriers may include, but are not limited to, pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents may include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, may generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate to delay absorption in the gastrointestinal tract.

[0142] As used herein, "percent complementarity" means the percentage of nucleotides of a modified oligonucleotide that are complementary to a microRNA. Percent complementarity may be calculated by dividing the number of nucleotides of the modified oligonucleotide that are complementary to nucleotides at corresponding positions in the microRNA by the total length of the modified oligonucleotide.

[0143] As used herein, "oligonucleotide" means a polymer of linked nucleosides, each of which may be modified or unmodified, independent from one another.

[0144] As used herein, "anti-miR" means an oligonucleotide having a nucleotides sequence complementary to a microRNA. In certain embodiments, an anti-miR is a modified oligonucleotide.

[0145] As used herein, "internucleoside linkage" means a covalent linkage between adjacent nucleosides.

[0146] As used herein, "linked nucleosides" means nucleosides joined by a covalent linkage.

[0147] As used herein, "nucleobase" means a heterocyclic moiety capable of non-covalently pairing with another nucleobase.

[0148] As used herein, "nucleoside" means a nucleobase linked to a sugar.

[0149] As used herein, "nucleotide" means a nucleoside having a phosphate group or other internucleoside linkage forming group covalently linked to the sugar portion of a nucleoside.

[0150] As used herein, "modified oligonucleotide" means an oligonucleotide having one or more modifications relative to a naturally occurring terminus, sugar, nucleobase, and/or internucleoside linkage.

[0151] As used herein, "modified internucleoside linkage" means any change from a naturally occurring internucleoside linkage.

[0152] As used herein, "phosphorothioate internucleoside linkage" means a linkage between nucleosides where one of the non-bridging atoms is a sulfur atom.

[0153] As used herein, "modified sugar" means substitution and/or any change from a natural sugar.

[0154] As used herein, "modified nucleobase" means any substitution and/or change from a natural nucleobase.

[0155] As used herein, "5-methylcytosine" means a cytosine modified with a methyl group attached to the 5′ position.

[0156] As used herein, "2′fluoro sugar" means a sugar having a fluorine modification at the 2′ position.

[0157] As used herein, "2′-O-methyl sugar" or "2′-OMe sugar" means a sugar having an O-methyl modification at the 2′ position.

[0158] As used herein, "2′-O-methoxyethyl sugar" or "2′-MOE sugar" means a sugar having an O-methoxyethyl modification at the 2′ position.

[0159] As used herein, "2′-O-fluoro" or "2′-F" means a sugar having a fluoro modification at the 2′ position.

[0160] As used herein, "bicyclic sugar moiety" means a sugar modified by the bridging of two non-geminal ring atoms.

[0161] As used herein, "locked nucleic acid (LNA) sugar moiety" means a substituted sugar moiety having a (CH_2)-O bridge between the 4' and 2' furanose ring atoms.

[0162] In practicing the present invention, many conventional techniques in molecular biology, microbiology, and recombinant DNA may be used. These techniques are well known and are explained in, for example, Current Protocols in Molecular Biology, Volumes I, II, and III, 1997 (F.M. Ausubel ed.); Sambrook et al.,

1989, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; DNA Cloning: A Practical Approach, Volumes I and II, 1985 (D. N. Glover ed.); Oligonucleotide Synthesis, 1984 (M.L. Gait ed.); Nucleic Acid Hybridization, 1985, (Hames and Higgins eds.); Transcription and Translation, 1984 (Hames and Higgins eds.); Animal Cell Culture, 1986 (R.I. Freshney ed.); Immobilized Cells and Enzymes, 1986 (IRL Press); Perbal, 1984, A Practical Guide to Molecular Cloning; the series, Methods in Enzymology (Academic Press, Inc.); Gene Transfer Vectors for Mammalian cells, 1987 (J.H. Miller and M.P. Calos eds., Cold Spring Harbor Laboratory); and Methods in Enzymology, Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively).

EXAMPLES

[0163] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Materials and methods for Examples 1-10

[0164] *SOD1 Mice and Rats:* The $SOD1^{G93A}$ mouse model is based on a dominantly inherited ALS-causing mutation and is the most commonly used ALS model. $SOD1^{G93A}$ mice and rats develop normally. At 4 months of age, both $SOD1^{G93A}$ mice and rats begin to lose weight and develop atrophy in hind limb muscles. This phenotype progresses rapidly with paralysis occurring at 157 +/- 9 days of age in the mice. Spinal cords from these mice show motor neuron loss as well as astrocyte and microglial activation. The SOD1wt expressing transgenic lines have no overt

phenotype. As in many neurodegenerative diseases, mutations in SOD1 cause disease by a gain of a toxic function rather than a loss of normal SOD1 function.

[0165] *TDP-43 Mice*: TDP-43 was identified biochemically as a component of aggregates associated with ALS and Frontotemporal Dementia. Mutations in TDP-43 are causative for about 5% of autosomal dominant familial ALS. The mice develop progressive weakness and difficulty walking associated with loss of motor neurons and ubiquitinated inclusions in motor neurons and layer V cortical neurons. Spinal cords and brains from these mice show neuron loss, as well as astrocyte and microglial activation. Since there is a large gender disparity in phenotype with variable survival time for females, all of the TDP-43 mouse data presented here focus on TDP-43 male mice.

[0166] *miR-155 Knockout Mice:* miR-155 knockout mice have a decreased response to immune system stimuli. Also, miR-155 knockouts are deficient in their response to inflammation in the brain, a response mediated by glia (microglia and astrocytes).

Example 1. miRNA detected in samples of ALS models.

[0167] miRNA was isolated from 6 symptomatic $SOD1^{G93A}$ and 6 age-matched SOD1wt rat lumbar spinal cords and subjected to ABI Taqman based microarray analysis. Of *the* 585 miRNAs screened, 186 were not amplified well or were too low abundance. Among the 399 miRNAs that were reliably assayed with adequate abundance and reproducibility in the arrays, 14 miRNA were changed greater than two-fold between $SOD1^{G93A}$ and SOD1wt rat samples. Individual QPCR analysis provides a more accurate assessment and confirmed 10 out of the 14 array hits (**Table 1**). These 10 miRNAs include 4 members of the miR-200 family (miR-141, miR200a, miR-200b, miR-200c), a group of miRNAs studied mostly in the context of epithelial to mesenchymal transition (part of tumor metastasis), 3 miRNAs involved in regulation/differentiation of stem cells (miR-291a-3p, miR-292-3p and miR-184), 3 highly correlated inflammatory-mediating miRNAs (miR-142, miR-146, and miR-155). Nine of ten of the changes represent increases in miRNAs in disease.

Table 1. Validation of Microarray miRNA Changes in SOD1^{G93A} ALS Spinal Cords.		
	Microarray	**Confirmatory Assay**
miR-19a	2.3	1.9
miR-141	6.2	2.7*
miR-142	4.0	4.6*
miR-146	2.4	3.3*
miR-155	7.4	7.2*
miR-184	0.49	0.47*
miR-200a	3.5	4.0*
miR-200b	4.3	4.7*
miR-200c	4.0	2.6*
miR-205	2.0	0.76
miR-291a	4100	160*
miR-292	46	9.7*
miR-501	17	11.2
miR-687	2.7	0.88
*p<0.05		

Example 2. miR-155 is increased in ALS.

[0168] First, it was shown that miR-155 is expressed in primary neuroglial cells (**FIG. 7**). To determine the relevance of miRNA expression in neurodegenerative model systems, the levels of miR-155 were analyzed in ALS mice and rats, a microglial cell line, and human samples. In some cases, mice or cells were treated with LPS, which is a bacteria-produced endotoxin that strongly stimulates the immune system.

[0169] Specifically, spinal cord tissue was isolated from 6 late stage SOD1^{G93A} ALS model rats and SOD1wt age-matched controls; 4 late stage SOD1^{G93A} ALS model mice and normal age-matched controls; and 4 TDP-43^{A315T} ice and normal age-matched controls. The samples were analyzed by QPCR for levels of miR-155.

Also, cerebral cortex tissue was isolated from 10-week non-transgenic male mice 1 hour after an intraperitoneal LPS injection (n=3 per group; LPS full dose = 0.2 mg/kg, $6x10^5$ endotoxin units/kg, $p<0.05$ for full LPS compared to saline). Microglia cells were treated *in vitro* with 100 ng/mL of LPS and miRNA was isolated 24 hours after treatment. The miRNA was analyzed by QPCR for levels of miR-155.

[0170] The QPCR results indicated miR-155 was significantly increased in ALS rodent models ($SOD1^{G93A}$ rats (**FIG. 1A**), $SOD1^{G93A}$ mice (**FIG. 1B**), and $TDP\text{-}43^{A315T}$ mice (**FIG. 1C**), in a microglial cell line following LPS stimulation (**FIG. 1E**), and in the brain of mice following LPS *administration* intraperitoneally (**FIG. 1D**). Also, miR-155 was increased in human ALS spinal cord compared to control spinal cord (**FIG. 2**). Therefore, the change in miRNA observed in animal models and cell culture systems is relevant to human ALS.

Example 3. miR-155 knockout decreases LPS induced immune response in brain.

[0171] To determine if miR-155 is involved in neuroinflammation, an immune response was induced in miR-155 knockout mice using LPS. Specifically, miR-155 knockout mice were treated with 0.2 mg/kg of LPS intraperitoneally. Three hours after treatment with LPS, brain and spinal cord tissues were harvested. In miR-155 knockout mice, a reduction in LPS stimulated brain cytokine and chemokine responses was observed (**FIG. 3A, B**; **FIG. 9A-E**; and **FIG. 10A-F**). This data shows that miR-155 is involved in central nervous system inflammation.

Example 4. ALS models have increased levels of inflammation markers.

[0172] To determine if ALS is associated with increased levels of inflammation markers, cytokine and chemokine levels were analyzed in $SOD1^{G93A}$, TDP-43, and normal (wild type) mice. Specifically, *mRNA* was isolated from these mice and analyzed using QPCR for IL-6, IL-1β, TNFα, CCL2, CXCL2, CXCL10, CCR2, CXCR3, and CXCR4. As shown in **FIG. 3C**, **3D** and **6B**, ALS mouse models have increased levels of inflammation markers.

[0173] Similarly, neuroinflammation is identified through the detection of cell-specific and inflammation *markers* in the $SOD1^{G93A}$ mouse model. Microglia cell specific marker Iba1 and the astrocyte cell specific marker GFAP have increased expression in ALS (G93A) samples compared to normal samples (**FIG. 6A**).

[0174] Changes in chemokine and cytokine mRNAs in the ALS models may be relevant to miR-155 inhibition. In particular, miR-155 may be involved in regulating these cytokines and chemokines.

Example 5. Delivery of miRNA inhibitory molecules.

[0175] Since some miRNA inhibitory molecules do not cross the blood brain barrier, an alternative delivery method is to infuse the inhibitory molecule directly into the cerebral spinal fluid (CSF) as demonstrated in **FIG. 4** for a mouse model. This procedure minimizes exposure of the inhibitory molecule to tissues or cells that would not benefit from treatment. For example, decreased miR-155 inhibition in the peripheral T cells, B cells, and macrophages may limit any potential toxicity to the immune system of inhibiting miR-155. Inhibitory molecules delivered by CSF infusion will distribute widely in the central nervous system and penetrate deep within tissues, decreasing the target miRNA.

Example 6. Inhibitory molecules inhibit miR-155 function *in vivo*.

[0176] Antisense nucleotides that inhibit miRNA bind to, but do not destroy, the miRNA. Therefore, reading out miRNA levels after adding antisense nucleotides does not determine whether the antisense nucleotides have inhibited the target miRNA. A miRNA typically regulates 200-300 mRNAs; most of the regulated mRNAs have binding sites in the 3' UTR *that* overlap to some extent with the seed region. The seed region is a 7-8 nucleotide motif in the miRNA that determines specificity. To determine whether the antisense nucleotides inhibited miR-155, saline, a mismatch control, or antisense nucleotides that inhibit miR-155 (referred to as antimiR-155 oligos) were administered intraperitoneally (25 mg/kg/day) in mice. The mismatch control here is identical to the antimiR-155 oligo except for a 6 base pair mismatch to miR-155 in the

seed region. The seed region of a miRNA is particularly important for binding to the mRNA. After 3 days, peritoneal macrophages were isolated and stimulated with LPS. Messenger RNA was then isolated from the macrophages and mRNA levels were determined by microarray analysis. mRNA from control and mice treated with antimiR-155 oligo were compared. mRNA were then ranked from most upregulated to least upregulated. If the antimiR-155 oligos were having the intended effect of inhibiting the mRNAs regulated by miRNA-155, then an enrichment of upregulated mRNAs that contain the 7 base seed region that, in part, determines binding to 3'UTR of particular mRNA, is expected. In other words, binding of a miRNA to a 3' UTR typically represses mRNA levels, thus inhibition of miR-155 should upregulate mRNAs with miR-155 binding sites. Sylamer is a program that assists in searching for a particular heptamer RNA sequence among a list of mRNAs. As demonstrated in **FIG. 5**, the mRNAs that contain a 3' UTR predicted to interact with miR-155 were enriched among the most upregulated transcripts in macrophages treated with the antimiR-155 oligos. The mismatch oligo showed no enrichment of mRNA transcripts. The grey lines in **FIG. 5** represent the seed regions for other miRNAs. There is no enrichment among the upregulated transcripts. These data show that an *in vivo* delivered antisense oligo affects miR-155 targets.

Example 7. miR-155 inhibitory molecules treat ALS.

[0177] miR-155 increases during disease course in $SOD1^{G93A}$ rats, $SOD1^{G93A}$ mice, $TDP\text{-}43^{A315T}$ mice, and in human ALS spinal cord tissue. To determine if reduction of miR-155 would be protective for ALS, reduction of miR-155 in an animal ALS model is analyzed. Specifically, to determine if decreasing miR-155 in an animal model would be protective for ALS, miR-155 was inhibited with antimiR-155 oligo (25µg/day), a mismatch *control* (25µg/day), or saline infused into the CSF using an osmotic pump (see, **FIG. 4**). Mice were treated with 42 day pumps. Grip strength, rotarod, and loss of weight were used as measures of onset of the disease. A logrank (Mantel-Cox) test was preformed on survival data and each of the disease-onset measures including weight loss, grip strength, and time on rotarod. Percent still alive

was the primary measure for disease onset. As shown in **FIG. 8A**, mice treated with antimiR-155 survived longer than mice treated with a mismatch control, or saline.

Introduction for Examples 8-10

[0178] Since their discovery in 1993, microRNAs (miRNAs) have emerged as key regulators in numerous physiological and pathological processes. miRNAs are highly conserved, single-*stranded*, non-coding RNA molecules ~22 nucleotides in length. miRNAs repress gene expression by inhibiting translation of and/or facilitating the degradation of their target mRNAs via binding to the 3' UTR. Because only partial complementarity is required for miRNA-mRNA interactions, a single miRNA can potentially regulate hundreds of mRNA transcripts. Testing the potential therapeutic opportunity of dysregulated miRNAs in any particular disease requires not only a careful analysis of the miRNA expression changes in the target tissues, but also a method to modulate miRNA function in disease models.

[0179] Amyotrophic Lateral Sclerosis (ALS; also known as Lou Gehrig's Disease) is a fatal adult-onset neurodegenerative disease characterized by the selective loss of motor neurons in the spinal cord and brain leading to stiffness, severe muscle weakness, and death due to respiratory failure. Riluzole, the only FDA-approved treatment, prolongs survival by only three to six months. Therefore, discovering novel therapeutic targets is of critical importance.

[0180] Among the list of dysregulated miRNAs in ALS model mice and human ALS samples, miR-155 appeared to be an excellent therapeutic target because of its abundance and fold change in *ALS* and its reproducibility across species and various ALS models. It has also been previously identified as increased in peripheral monocytes from ALS model mice and ALS patients. However, as with many changes in the ALS model, whether the miR-155 increase positively or negatively affects ALS remained untested and required development of a method to inhibit miRNAs both in peripheral blood cells as well as in the central nervous system (CNS).

[0181] Antisense *oligonucleotides* can be used to inhibit miRNA function by binding tightly through Watson-Crick base pairing. This miRNA inhibition strategy has

been successful in the periphery but has not been readily applied to the CNS. Anti-miRs do not cross the blood brain barrier. In the examples below, miRNAs in the CNS were targeted by delivering anti-miRs directly to the cerebral spinal fluid as previously described for mRNA inhibitors (Smith et al., 2006 J Clin Invest 116:2290-2296). The examples below demonstrate the ability of these anti-miRs to inhibit their cognate miRNA target throughout the CNS. Most importantly, these miR-155 inhibitors were used to test whether the increased miR-155 affects ALS disease phenotype and is thus a viable therapeutic target.

Materials and methods for Examples 8-10.

Animals

[0182] Animals were provided food and water ad libitum, and cages were changed once a week. All *mice* were maintained in a 12-hour light/12-hour dark cycle and received routine veterinary monitoring. Post-surgical mice were single-housed as to protect the sutures and tubing.

Human tissues

[0183] Spinal cord autopsy tissue was obtained from 16 ALS patients (9 male, 7 female; age range 34-79, *mean* 64; from Washington University School of Medicine, Massachusetts General Hospital) and 12 non-ALS control patients (6 male, 6 female; age range 51-80, mean 70; from National Disease Research Interchange). Postmortem intervals ranged from 1 to 40 hours.

miRNA quantification

[0184] RNA was extracted from flash frozen tissue using miRNEasy kits per manufacturer's instructions (*Qiagen*, Hilden, Germany). RNA integrity and yield were assessed by Nanodrop before normalizing to 150ng/μL for microarray assay or 2ng/μL for miRNA individual analysis. miRNA microarrays were performed without amplification using low density Rodent MicroRNA A+B cards sets 3.0 (Applied Biosystems, Foster City, CA) run with a 7900HT quantitative real time polymerase chain

reaction (qPCR) machine for 40 cycles. Analysis was conducted on SDSv2.2 software with automatic thresholding. Microarray hits were confirmed with individual TaqMan miRNA assays (Applied Biosystems) as per the manufacturer's instructions. All rodent miRNAs were normalized to endogenous U6, and human to total RNA input. qPCR samples were quantified in technical duplicates on an Applied Biosystems 7500fast Real-Time PCR System.

mRNA quantification

[0185] For mRNA Affymetrix microarray and Sylamer analysis, RNA was diluted to 100ng/μL and sent to Expression Analysis, Inc. (Durham, NC) for running the Affymetrix Mouse Genome 430 2.0 array (Santa Clara, CA). Analysis was then conducted as previously *described* by others. For RT-qPCR of mRNA targets, RNA was normalized to 20ng/μL using Nanodrop. Primers and probes for mRNA targets were purchased from Integrated DNA Technologies (Coralville, IA) and are detailed in **Table 2**. 40ng total RNA was quantified with Express One-Step Superscript Kits (Invitrogen, Carlsbad, CA) as per the manufacturer's protocol. One-step qRT-PCR was conducted on a 7500 fast Real Time PCR System (Applied Biosystems). RNA was quantified as technical duplicates and normalized to GAPDH.

Table 2. Primer and probes were purchased from IDTDNA, and oligonucleotides were synthesized by Regulus Therapeutics with the sequences listed below.

NRAS Forward Primer	GTGTCCAGTATGTCCAGCAG (SEQ ID NO: 7)
NRAS Reverse Primer	GACGATCCAGCTAATCCAGAAC (SEQ ID NO: 8)
NRAS Probe	/56-FAM/TGCTTTCGG/ZEN/TAAGAATCCTCTATGGTGG/3IABkFQ (SEQ ID NO: 9)
TGFBR1 Forward Primer	CGATGGATCAGAAGGTACAAGA (SEQ ID NO: 10)
TGFBR1 Reverse Primer	GCGCTGACATCTATGCAATG (SEQ ID NO: 11)
TGFBR1 Probe	/56-FAM/AGCGTCGAG/ZEN/CAATTTCCCAGAACA/3IABkFQ/ (SEQ ID NO: 12)

SHIP1 Forward Primer	GAGAATGAAGAGGGAAGTGAAGG (SEQ ID NO: 13)
SHIP1 Reverse Primer	GGGTCAGAGATAATGGGCTTTAG (SEQ ID NO: 14)
SHIP1 Probe	/56-FAM/AGCTGGTGGTACGGTTTGGAGAG/3IABkFQ/ (SEQ ID NO: 15)
anti-miR-155	CCCCUAUCACAATUAGCAUUAA (SEQ ID NO: 16)
anti-let-7	AACUAUACAACCTACUACCUCA (SEQ ID NO: 17)
Scrambled anti-miR	CCCCAUUACCAATTACGUAATA (SEQ ID NO: 18)

Anti-miR sequences

[0186] All anti-*miRs* were synthesized with a full phosphorothioate backbone with alternating blocks of 2'-MOE and 2' fluoro sugar-modified nucleosides. Sequences are denoted in **Table 2**.

Anti-miR-155 in vitro luciferase assay

[0187] microRNA antisense oligonucleotides were screened for *in vitro* function as described by Esau 2008, Methods 44:155-160. Briefly, Hela cells were co-transfected with a miR-155 expressing plasmid and with a luciferase reporter with perfect 2x miR-155 *complementary* sequences at the 3'UTR. Four hours later, anti-miR-155 was added to the media at concentrations ranging from 1 to 200nM. Twenty-four hours later, luciferase activity was determined and plotted.

Isolation of peritoneal macrophages

[0188] Adult C57Bl6 mice were given an intraperitoneal (IP) injection of Thioglycollate followed by 3 *daily* IPs of 25mg/kg anti-miR or saline. On day 4, peritoneal macrophages were isolated, plated in tissue culture plates, and stimulated with LPS for 24 hours (20ng/mL). RNA was then extracted and analyzed by Sylamer analysis.

Alzet Osmotic Pump Surgeries

[0189] Osmotic pumps were prepared per manufacturer protocol (pump model 2004 for 28 day *experiments*; 2006 for 42 day experiments; Alzet, Cupertino, CA). For implantation, mice were anesthetized in a chamber with 5% isoflurane/oxygen mixture and confirmed to be unconscious before being placed in Kopf Model 940 small animal stereotaxic apparatus, fitted with ear bars. The catheter was oriented at 2.00mm lateral and 1.1mm posterior to bregma.

Cy3-anti-miR-155 histology

[0190] Adult C57Bl6 mice were implanted with a subdermal Alzet osmotic pump to deliver 10µg/day of cy3-labeled anti-miR-155 directly into the lateral ventricle (Isis Pharmaceuticals, Carlsbad, CA). After two weeks of treatment, mice were perfused with PBS and 4% paraformaldehyde (PFA). Brain and spinal cord tissues were post-fixed in PFA for 24 hours and then submerged in 30% sucrose for two days. The spinal cord was embedded in O.C.T. (VWR, Radnor, PA) before it and the brain were sliced at 40µm. Tissue was then washed in PBS and mounted with Fluoromount G (Southern Biotech, Birmingham, AL) and coverslipped. Slides were observed at 4x and 10x objectives using a Nikon Eclipse 80i microscope fitted with a Photometrics CoolSnap EZ camera. All images were taken at ambient temperature with a Cy3 filter. For image acquisition and formatting, NIS Elements 3.0 (Nikon) and Adobe Photoshop v12.0 were used.

Treatment and analysis of SJL SOD1^{G93A} ALS mice

[0191] All SJL SOD1^{G93A} were from Prize4Life and were previously confirmed to have high and consistent SOD1^{G93A} copy counts. Mice were implanted with osmotic pumps at 60 days containing 20µg/day anti-miR-155, 20µg/day scrambled control, or saline. Pumps were removed at 102 days. Weekly IP injections (25mg/kg) began at 60 days and continued until the *mouse* reached end-stage. For the first two weeks only, mice received an additional IP injection. Mice were weighed and assessed for neurological score biweekly in blinded fashion starting at 60 days. An ALSTDI

neurological score of 1 was given: 1) when the mouse was no longer able to fully extend its legs; 2) when the mouse could no longer spread its legs past midline when lifted by its tail; or 3) when significant hind limb tremors were present upon being lifted by its tail. End-stage was defined as when the mouse was no longer able to right itself within 30 seconds after being placed on either side. All involved in the administration and monitoring of the animals were blinded with separate people involved in injecting and scoring the mice. Blinding was broken only once all analyses were completed.

Statistical analysis

[0192] Data are presented as mean ± SEM. All statistical tests were conducted with Graphpad *Prism* 6 Software. All gene expression data were analyzed with a 2-tailed Student t test. All survival and onset data were analyzed using both the Mantel-Cox Log-Rank test and the Gehan-Breslow-Wilcoxon test.

Example 8. miR-155 is significantly upregulated in rodent and patient ALS spinal cord tissue.

[0193] To identify miRNA therapeutic targets for ALS, miRNA changes were measured in both the rodent ALS model and in human ALS autopsy samples. Using Taqman miRNA microarrays that assayed 673 miRNAs, miRNA expression levels were measured in both end-stage mouse and rat spinal cord tissue as compared to their age matched controls. Twelve *miRNAs* were identified as significantly increased in both ALS models (**Table 3**). Using individual miRNA assays, eleven miRNAs were confirmed increased in the mouse, nine in the rat, and six in patient ALS autoposy tissues (**FIG. 11A-C**). Specifically, the most researched amongst these hits, miR-155, was significantly increased in both familial and sporadic ALS spinal cord tissue (**FIG. 11D**).

Table 3. Twelve miRNAs are altered in end-stage ALS mouse and rat spinal cords by microarray. miRNAs were isolated from end-stage $SOD1^{G93A}$ mouse and rat spinal cords and from age-matched non-transgenic (NonTG) controls (n=3-4). For rats, miRNAs were also isolated from age-matched SOD1WT control spinal cords (n=3). 673 miRNAs were assayed using Applied Biosystem's TaqMan Rodent Array A+B Cards v3.0. 434 miRNAs were amplified and were quantified. For mouse, 6 miRNAs were decreased and 37 miRNAs were increased greater than 2 fold at $p<0.05$. From these 43 miRNAs, 12 were also changed >50% in the rat samples at $p<0.20$. RQ = relative quantification.

	Mouse		Rat			
	RQ: $SOD1^{G93A}$/NonTG	T-test	RQ: $SOD1^{G93A}$/NonTG	T-test	RQ: $SOD1^{G93A}$/$SOD1^{WT}$	T-test
miR-17	2.66	0.00	1.99	0.04	11.58	0.00
miR-19b	2.51	0.00	1.99	0.10	9.20	0.02
miR-20a	2.33	0.01	1.79	0.10	20.60	0.01
miR-24-2-5p	2.44	0.02	1.78	0.06	3.22	0.00
miR-106a	2.59	0.01	2.11	0.01	11.14	0.00
miR-142-3p	3.36	0.04	3.81	0.06	24.84	0.02
miR-142-5p	3.27	0.05	10.61	0.17	2.94	0.34
miR-146a	8.86	0.00	3.89	0.02	8.79	0.01
miR-146b	3.02	0.01	1.84	0.04	2.95	0.01
miR-155	2.08	0.01	20.08	0.20	58.75	0.19
miR-223	3.52	0.05	1.89	0.14	10.25	0.02
miR-338-3p	2.27	0.01	1.75	0.16	5.33	0.03

Example 9. Anti-miRs delivered through ventricular osmotic pumps distribute widely in CNS and derepress target mRNAs.

[0194] To determine whether anti-miRs could inhibit targets broadly in the CNS, a well-characterized miRNA inhibitor of let-7 was used. After treating animals with anti-let-7 with an osmotic *pump* directed to the lateral ventricles (**FIG. 12A**), mRNAs with 3'-UTR let-7 binding sites were globally derepressed in cortical tissue (**FIG. 12B**). Analysis of a subset of these mRNAs demonstrated derepression of targets in parietal

cortex, midbrain, occipital cortex, cerebellum, cervical and lumbar spinal cord (**FIG. 12C, D**).

[0195] Having established that anti-miRs can target the CNS, including the spinal cord, an anti-miR-155 oligonucleotide was developed to test whether this inhibitor would affect the ALS rodent model. In HeLa cells transfected with miR-155 and a luciferase reporter containing a 3' UTR recognized by miR-155, the novel anti-miR-155 derepressed luciferase and led to increased luciferase activity (**FIG. 12E**). To next test efficacy of miR-155 inhibition peripherally *in vivo*, three daily IP injections of anti-miR-155 (25mg/kg) were administered in non-transgenic mice. An mRNA profile from LPS-stimulated peritoneal macrophages from anti-miR-155 treated mice showed global derepression of mRNAs with a miR-155 3'-UTR binding site (**FIG. 12F**). Finally, to test efficacy in CNS tissue, 28-day osmotic pumps with saline, scrambled control, or anti-miR-155 that infused directly into the lateral ventricles, were implanted. SHIP1, a well-studied and confirmed miR-155 target, was then quantified across the CNS. SHIP1 was significantly increased over saline in all areas assayed (**FIG. 12G**). In a separate experiment, mice were treated with cy3-labeled anti-miR-155 osmotic pumps for two weeks and saw clear cellular uptake throughout the brain and spinal cord (**FIG. 13**).

Example 10. Anti-miR-155 significantly extends survival and disease duration in the SOD1^{G93A} mouse model.

[0196] To test the therapeutic potential of antagonizing miR-155 in ALS, a large cohort of SOD1^{G93A} mice were treated at 60 days of age with continuous intraventricular infusion of anti-miR-155 (n=22), scrambled control anti-miR (n=21), or saline (n=20). To account for any peripheral role of miR-155, the CNS treatment was supplemented with weekly *anti*-miR or saline IP injections.

[0197] Beginning at 60 days of age, weights and ALSTDI neuroscores were recorded biweekly. Onset was determined in two ways: age when weight peaked and age when an ALSTDI neuroscore of 1 was observed. Treatment and monitoring continued until the animal reached end-stage, defined as when the animal was unable to right itself within 30 seconds *after* being placed on its side. There was no significant

change in disease onset as defined by either an ALSTDI score of 1 (**FIG. 14A**) or by age at weight peak (**FIG. 14B**). However, survival was significantly increased in only the anti-miR-155 treated mice (**FIG. 14C**). These mice had a 9.5 day extension in survival over saline (p=0.006, **Table 3**). There was no significant difference in survival between scrambled-treated and saline-treated animals. The controls in these Examples agreed with published survival data for $SOD1^{G93A}$ B6/SJL animals (129.5 vs 128.9 days). Furthermore, disease duration was only significantly increased in the anti-miR-155 treated animals. These mice had a disease duration 14.5 days longer than saline (38% extension, p=5.0x10-4 log rank test) and 11 days longer than scrambled (27% extension, p=2.7x10-3). There was no significant difference in disease duration between scrambled-treated and saline-treated animals (**FIG. 14D, Table 4**).

Table 4. Anti-miR-155 treated mice have extended survival and disease duration over both saline and scrambled treated mice. Median onset values are denoted for saline (n=20), scrambled (n=21), and anti-miR-155 (n=22) treated mice as determined by both age when the mouse reached weight peak and by a neuroscore of 1. There was no significant change between either onset condition by both Mantel-Cox log-rank and Gehan-Breslow-Wilcoxon tests. However, survival and disease duration were significantly extended in only the anti-miR-155 treated mice over both saline and scrambled mice as determined by both log-rank and Wilcoxon tests.

Onset (days)	**Weight**	**p-value**	**Log-Rank**	**Wilcoxon**
Saline	91.5	Sal - Scr	0.10	0.26
Scrambled	90	Sal - 155	0.13	0.12
anti-miR-155	87	Scr - 155	0.82	0.49
	Neuroscore			
Saline	112.5	Sal - Scr	0.60	0.29
Scrambled	115	Sal - 155	0.21	0.19
anti-miR-155	115	Scr - 155	0.54	0.81
Survival (days)	**Survival**	**p-value**	**Log-Rank**	**Wilcoxon**
Saline	129.5	Sal - Scr	0.51	0.24
Scrambled	133	Sal - 155	0.007**	0.007**
anti-miR-155	139	Scr - 155	0.018*	0.035*
	Disease Duration			
Saline	38.5	Sal - Scr	0.081	0.15
Scrambled	42	Sal - 155	<0.001***	0.003**
anti-miR-155	53	Scr - 155	0.038*	0.047*

Discussion for Examples 8-10

[0198] To determine the therapeutic potential of targeting miRNAs in ALS, a list of miRNAs changed in the $SOD1^{G93A}$ rodent model was first generated. Six of these miRNA changes are also upregulated in familial and sporadic human ALS spinal cord. However, for each of these changed miRNAs, whether the change contributes to disease progression or *represents* a compensatory/beneficial response remained unclear. To test the importance of the miRNA for disease, an antisense oligonucleotide strategy was developed to inhibit miRNAs in the brain and spinal cord. For miR-155, it was demonstrated that inhibiting miR-155 slowed disease progression in the $SOD1^{G93A}$ model. Because miR-155 is increased in human sporadic ALS, these data highlight the miR-155 pathway as an exciting, new therapeutic target for ALS broadly.

[0199] Antisense oligonucleotides can function as potent inhibitors of miRNAs (anti-miRs), but it remained unclear whether these inhibitors could achieve broad CNS distribution similar to antisense oligonucleotide inhibitors of mRNA. First, anti-let-7 was assayed because this oligonucleotide is known to be a potent miRNA inhibitor in the periphery. let-7 is not known to be important in ALS and is best known for its role in cell cycle regulation, cell differentiation, and cancer. Following 28-day infusion with anti-let-7 to the cerebral lateral ventricle in mice, robust derepression of let-7 targets was seen throughout the brain and spinal *cord* as determined by both global Sylamer analysis and targeted mRNA assays. While other studies have demonstrated anti-miR function in limited sections of cortical tissue, these data establish a method of widespread inhibition of miRNAs and are the first to show inhibition of miRNAs in the spinal cord. Next, a novel anti-miR against miR-155 was developed, previously identified as significantly upregulated in ALS. This anti-miR also showed widespread functional distribution in both the peripheral and central compartments. Anti-miRs have been used safely and effectively in the periphery in recent human clinical trials (NCT00688012 and NCT00979927; 13), are known to be effective in non-human primates, and CSF infusion of antisense oligonucleotides designed to lower mRNA have recently been applied to patients with ALS. Thus, CSF delivery of anti-miRs has the real potential to translate to clinical trials.

[0200] In order to test the therapeutic potential of inhibiting dysregulated miRNAs in neurodegenerative disease, a large cohort of ALS-model mice were treated with the anti-miR-155 described in the Examples above. Mice that received the combined central and peripheral treatment lived 10 days longer and had a 38% extension in disease duration. The data presented here establish that the increased miR-155 negatively contributes to ALS disease progression in the $SOD1^{G93A}$ mouse model. While a number of drugs tested in the $SOD1^{G93A}$ rodent model subsequently failed in human clinical trials, it *could* be argued that the Examples herein maintain high translational relevancy. Only those miRNAs increased in both the rodent ALS model and in patient ALS tissue were considered, and miR-155 was also confirmed to be significantly increased in both familial and sporadic ALS. Also, enrollment for a clinical trial can be determined by pre-screening miR-155 levels in peripheral blood cells. Next, a targeted therapy was developed whose efficacy can be read out in peripheral cells throughout treatment. Finally, antisense oligonucleotides have been tested in a Phase I trial in SOD1 human ALS, suggesting this type of strategy to be safe and realistic.

[0201] The data presented in the Examples herein clearly identify miR-155 as a promising therapeutic target for ALS and other neurodegenerative syndromes, yet some key preclinical issues need to be addressed. It will be important to test how CNS inhibition alone or peripheral blood cell inhibition alone affects survival in this model since this will have direct implications for which compartment is the most important to target with a miR-155 therapy. In addition, how the timing of miR-155 inhibition will affect survival needs to be examined. Also, the data presented herein define other miRNAs that are increased in ALS spinal cord tissue. Interestingly, all 6 of these patient-confirmed upregulated miRNAs (miR-24, miR-142-3p, miR-142-5p, miR-146a, miR-146b, and miR-155) are well studied in the context of immunity and inflammation. Each of these targets may be potential candidates for therapies for neurodegenerative disease, and testing them will require a similar approach to the one taken here. Another miRNA identified here may have a more potent effect either alone or in concert with other miRNA targets. Finally, to determine mechanism of action and to determine if neuroinflammation is blunted, a large cohort of mice may need to be treated and

sacrificed in age-matched fashion throughout disease for analysis of molecular and cellular targets.

[0202] A therapeutically relevant method to inhibit microRNAs broadly in the brain and spinal cord is demonstrated for the first time. Additionally, the first published list of altered microRNAs in ALS spinal cord tissue is supplied, and the first microRNA inhibition experiment to prolong survival in a neurodegeneration model is demonstrated.

Example 11. miR-196 is increased in ALS.

[0203] In humans and in mice, miR-196 miRNAs include miR-196a and miR-196b miRNAs. Expression of both miR-196a and miR-196b was found to be increased in a microglia cell line, and in primary cortical samples (**FIG. 15**). In addition, increased miR-196 levels were also confirmed in autopsy samples from human ALS patients (**FIG. 16**). Increased expression of miR-196a and miR-196b was especially significant in patients with sporadic ALS (**FIG. 17**).

CLAIMS

What is claimed is:

1. A method of treating a neurodegenerative disorder associated with overexpression of miR-155, the method comprising administering to a subject having a neurodegenerative disorder, or at risk of developing a neurological disorder associated with overexpression of miR-155 a therapeutically effective amount of a composition comprising a miR-155 agent that decreases the expression of miR-155.

2. The method of claim 1, wherein the neurodegenerative disorder is selected from the group consisting of Amyotrophic Lateral Sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, dementia, and combinations thereof.

3. The method of claim 1, wherein the neurodegenerative disorder is Amyotrophic Lateral Sclerosis.

4. The method of claim 1, wherein treating the subject extends survival of the subject.

5. The method of claim 1, wherein treating the subject extends the disease duration of the subject.

6. The method of claim 1, wherein the miR-155 agent is selected from the group consisting of nucleic acid molecule, protein, polypeptide, small molecule, and combinations thereof.

7. The method of claim 1, wherein the miR-155 agent is an antisense oligonucleotide.

8. The method of claim 7, wherein the antisense oligonucleotide comprises 8-25 nucleotides at least 85% complementary to miR-155.

9. The method of claim 7, wherein the antisense oligonucleotide comprises 8-25 nucleotides at least 90% complementary to miR-155.

10. The method of claim 7, wherein the antisense oligonucleotide comprises a nucleotide sequence that is 100% complementary to a miR-155 seed sequence.

11. The method of claim 7, wherein the antisense oligonucleotide comprises a nucleic acid sequence at least 85% complementary to a mature miR-155.

12. The method of claim 7, wherein the antisense oligonucleotide comprises a nucleic acid sequence at least 90% complementary to a mature miR-155.

13. A method of treating a neurodegenerative disorder associated with overexpression of miR-155, the method comprising administering to a subject having a neurodegenerative disorder, or at risk of developing a neurological disorder associated with overexpression of miR-155 a therapeutically effective amount of a composition comprising a miR-155 antisense oligonucleotide that decreases the expression of miR-155.

14. The method of claim 13, wherein the neurodegenerative disorder is selected from the group consisting of Amyotrophic Lateral Sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, dementia, and combinations thereof.

15. The method of claim 13, wherein the neurodegenerative disorder is Amyotrophic Lateral Sclerosis.

16. The method of claim 13, wherein treating the subject extends survival of the subject.

17. The method of claim 13, wherein treating the subject extends the disease duration of the subject.

18. The method of claim 13, wherein the antisense oligonucleotide comprises 8-25 nucleotides at least 85% complementary to miR-155.

19. The method of claim 13, wherein the antisense oligonucleotide comprises 8-25 nucleotides at least 90% complementary to miR-155.

20. The method of claim 13, wherein the antisense oligonucleotide comprises a nucleotide sequence that is 100% complementary to a miR-155 seed sequence.

21. The method of claim 13, wherein the antisense oligonucleotide comprises a nucleic acid sequence at least 85% complementary to a mature miR-155.

22. The method of claim 13, wherein the antisense oligonucleotide comprises a nucleic acid sequence at least 90% complementary to a mature miR-155.

23. A method of treating Amyotrophic Lateral Sclerosis, the method comprising administering to a subject having Amyotrophic Lateral Sclerosis, or at risk of developing Amyotrophic Lateral Sclerosis a

therapeutically effective amount of a composition comprising a miR-155 antisense oligonucleotide that decreases the expression of miR-155.

24. The method of claim 23, wherein treating the subject extends survival of the subject.

25. The method of claim 23, wherein treating the subject extends the disease duration of the subject.

26. The method of claim 23, wherein the antisense oligonucleotide comprises 8-25 nucleotides at least 85% complementary to miR-155.

27. The method of claim 23, wherein the antisense oligonucleotide comprises 8-25 nucleotides at least 90% complementary to miR-155.

28. The method of claim 23, wherein the antisense oligonucleotide comprises a nucleotide sequence that is 100% complementary to a miR-155 seed sequence.

29. The method of claim 23, wherein the antisense oligonucleotide comprises a nucleic acid sequence at least 85% complementary to a mature miR-155.

30. The method of claim 23, wherein the antisense oligonucleotide comprises a nucleic acid sequence at least 90% complementary to a mature miR-155.

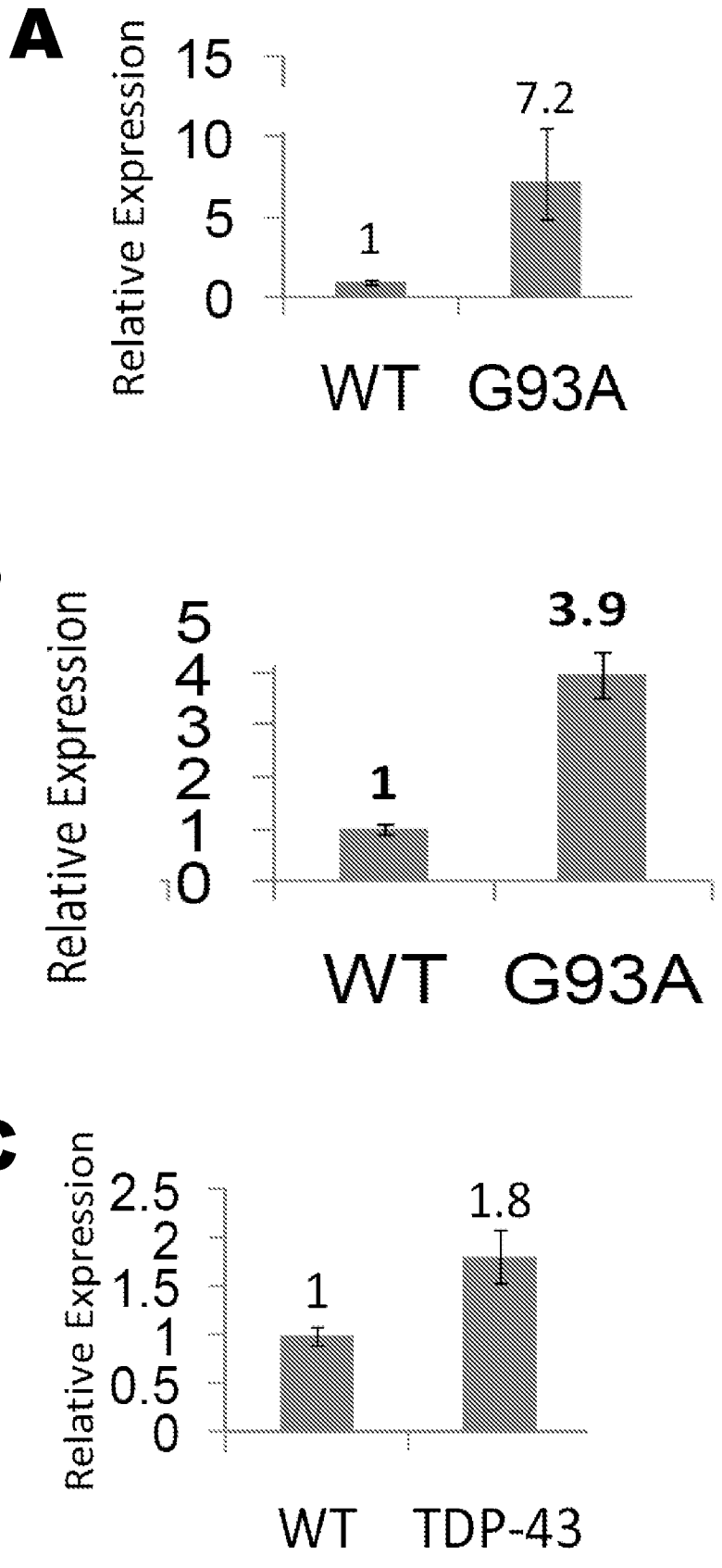
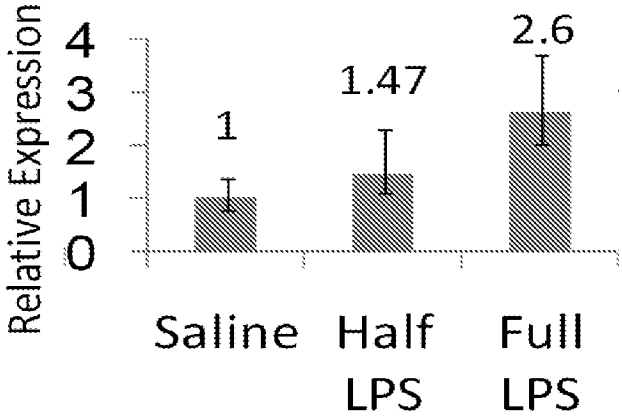

A
Relative Expression
15
10
5
0
1
7.2
WT
G93A
B
Relative Expression
5
4
3
2
1
0
1
3.9
WT
G93A
C
Relative Expression
2.5
2
1.5
1
0.5
0
1
1.8
WT
TDP-43

FIG. 1

D


Relative Expression
4
3
2
1
0
1
1.47
2.6
Saline
Half LPS
Full LPS

E

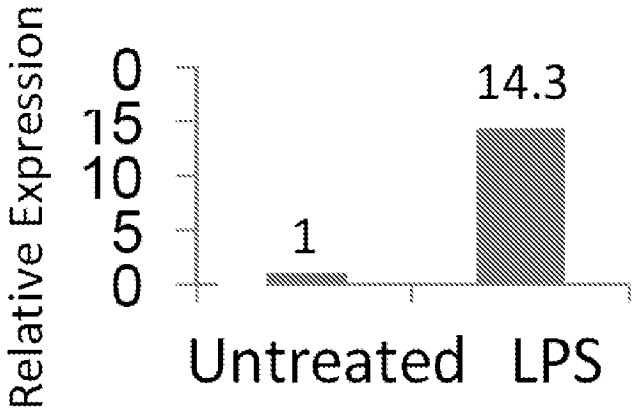

Relative Expression
0
15
10
5
0
1
14.3
Untreated
LPS

FIG. 1

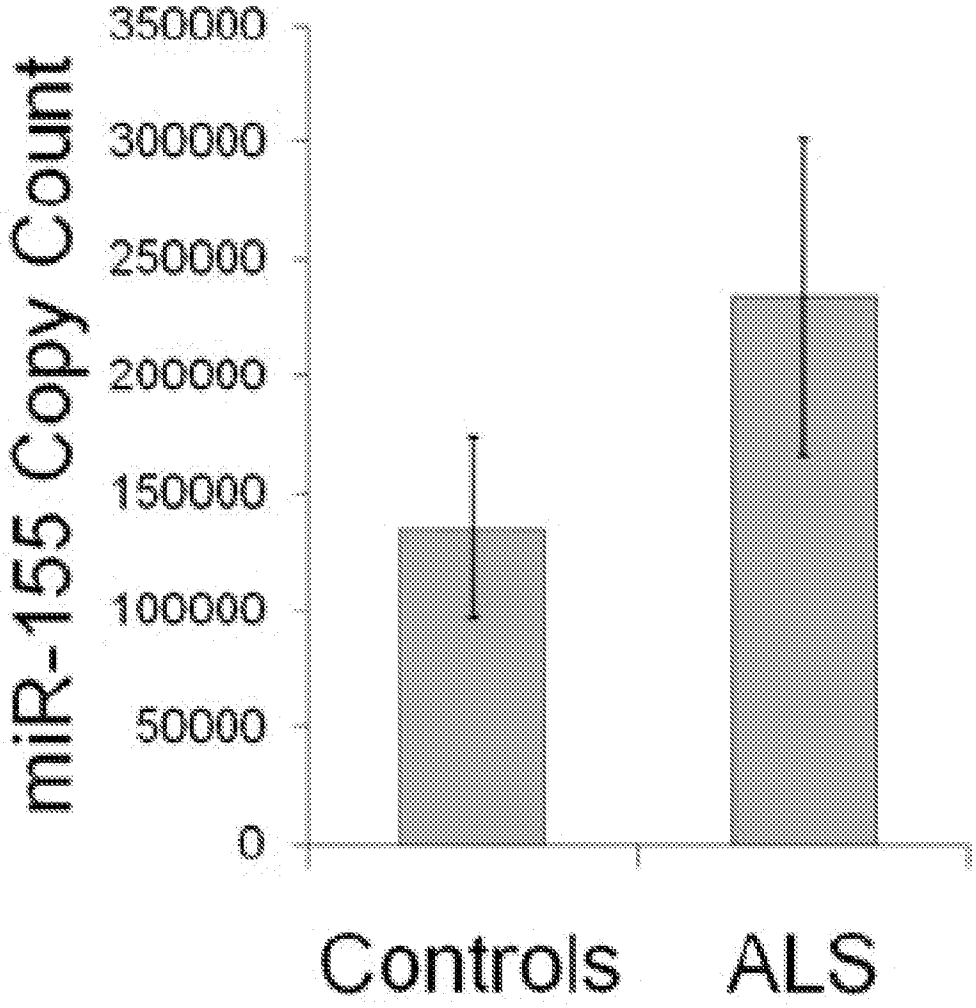

miR-155 Copy Count
350000
300000
250000
200000
150000
100000
50000
0
Controls
ALS

FIG. 2

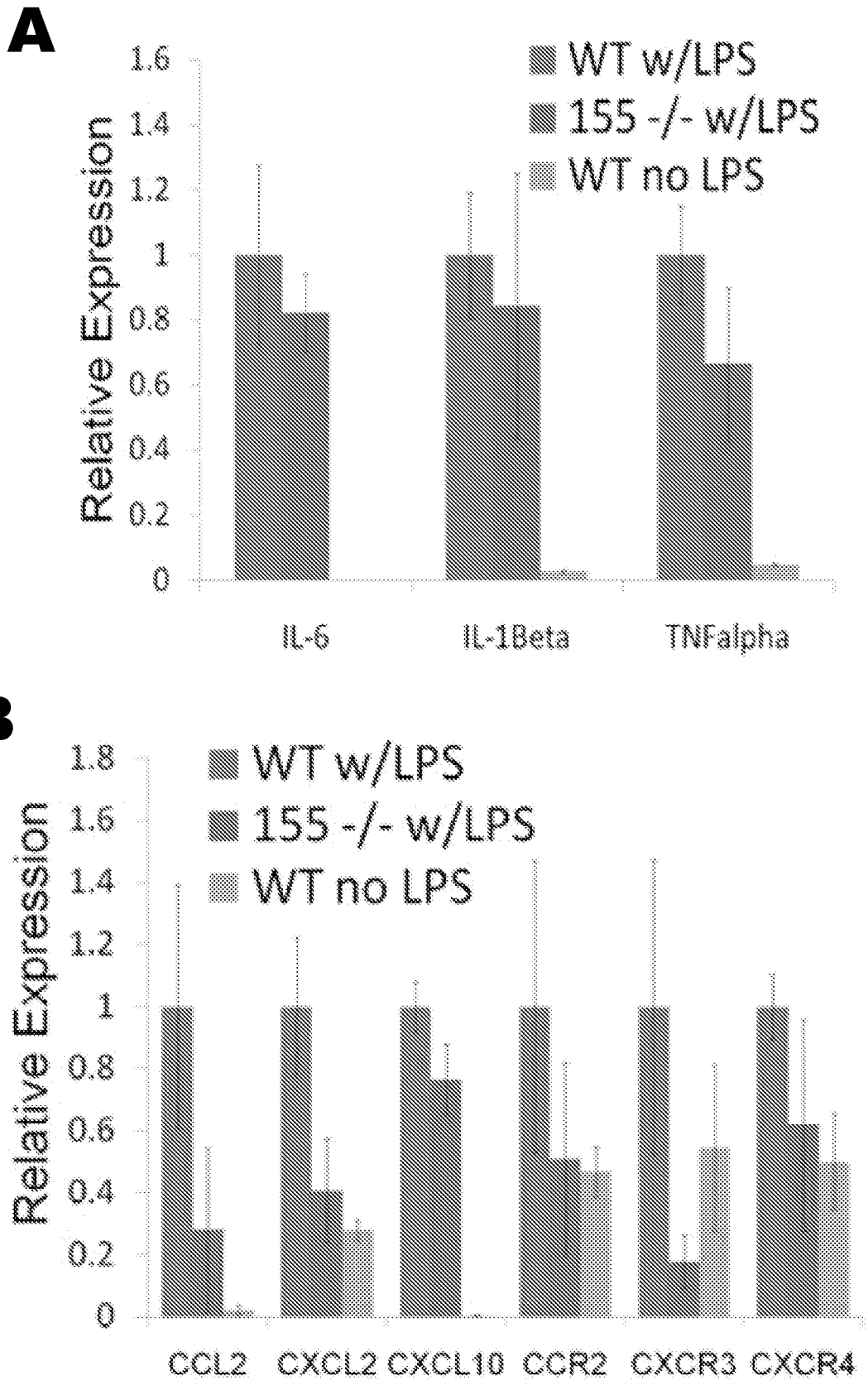
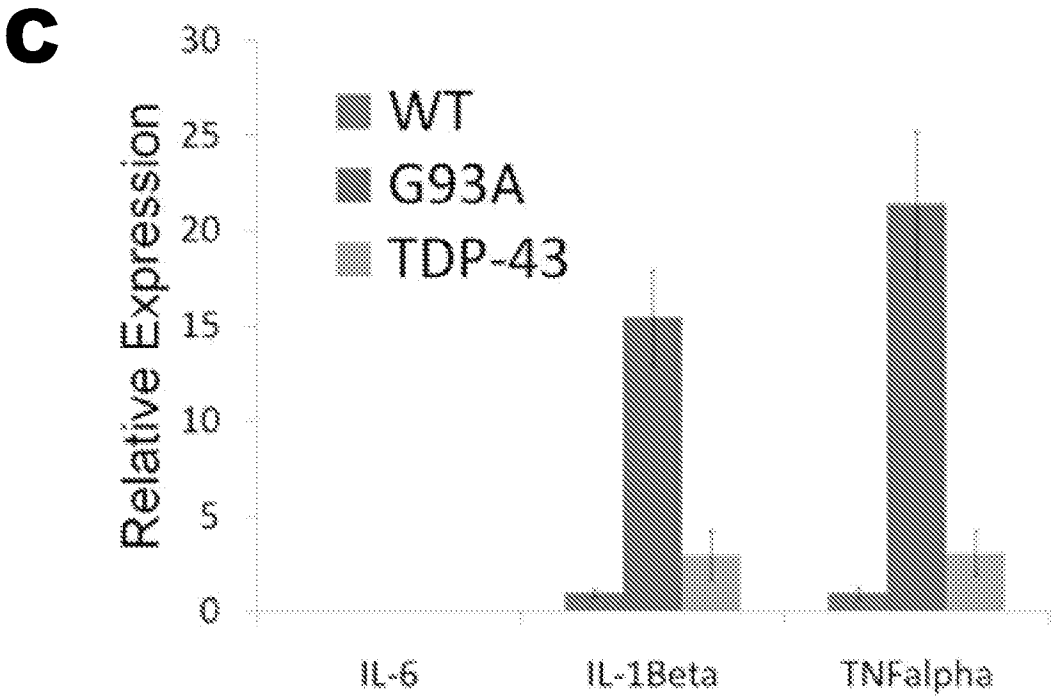

A
Relative Expression
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT w/LPS
155 -/- w/LPS
WT no LPS
IL-6
IL-1Beta
TNFalpha
B
Relative Expression
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT w/LPS
155 -/- w/LPS
WT no LPS
CCL2
CXCL2
CXCL10
CCR2
CXCR3
CXCR4

FIG. 3


C
Relative Expression
30
25
20
15
10
5
0
WT
G93A
TDP-43
IL-6
IL-1Beta
TNFalpha

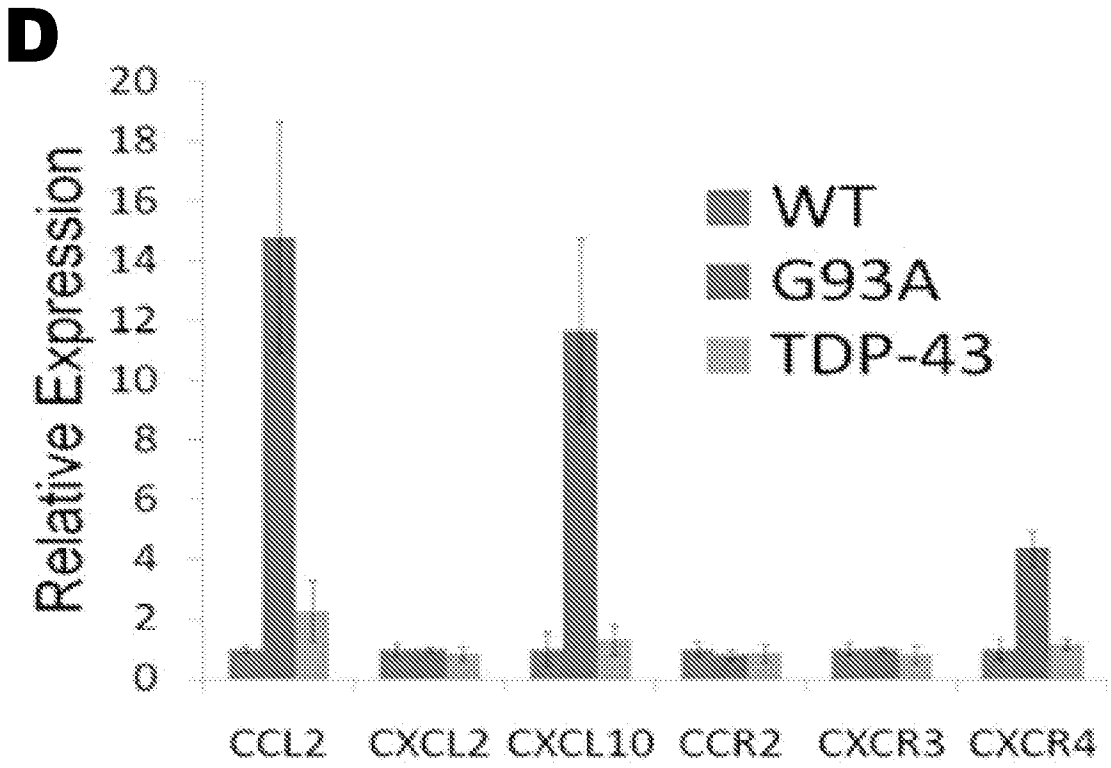

D
Relative Expression
20
18
16
14
12
10
8
6
4
2
0
WT
G93A
TDP-43
CCL2
CXCL2
CXCL10
CCR2
CXCR3
CXCR4

FIG. 3

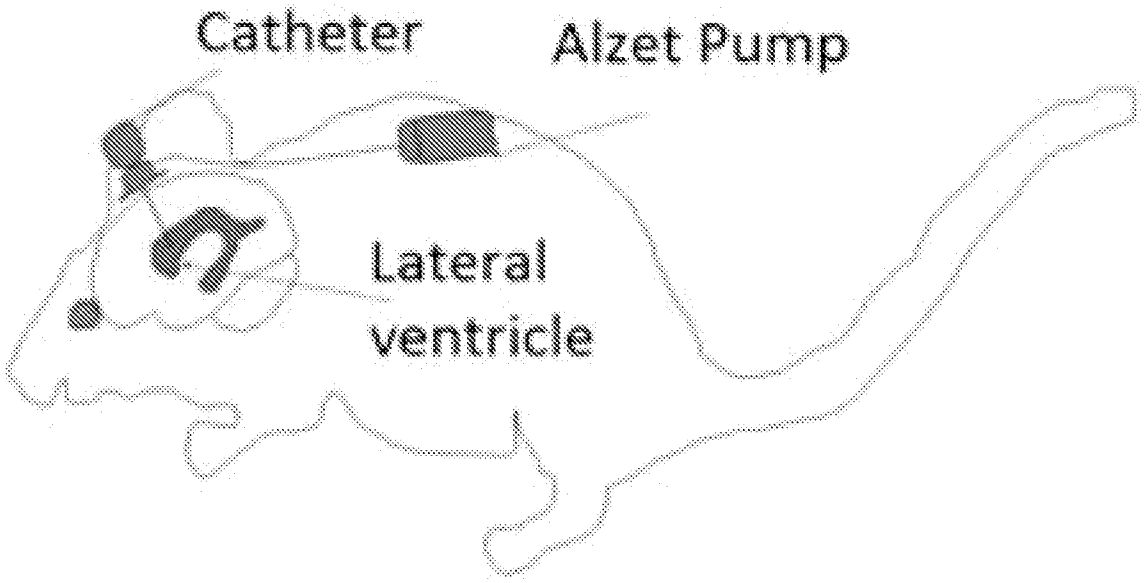

Catheter
Alzet Pump
Lateral
ventricle

FIG. 4

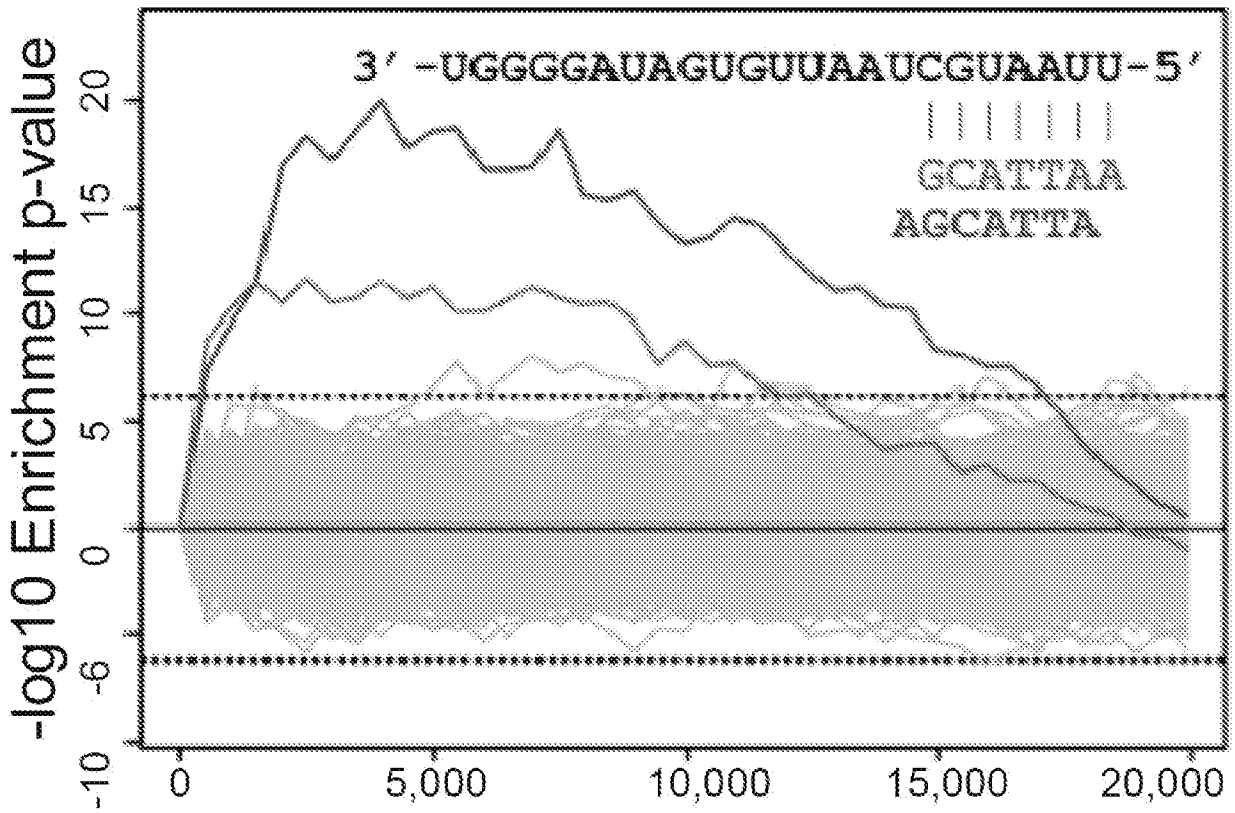

3′ -UGGGGAUAGUGUUAAUCGUAAUU-5′
GCATTAA
AGCATTA
-log10 Enrichment p-value
20
15
10
5
0
-6
-10
0
5,000
10,000
15,000
20,000

FIG. 5

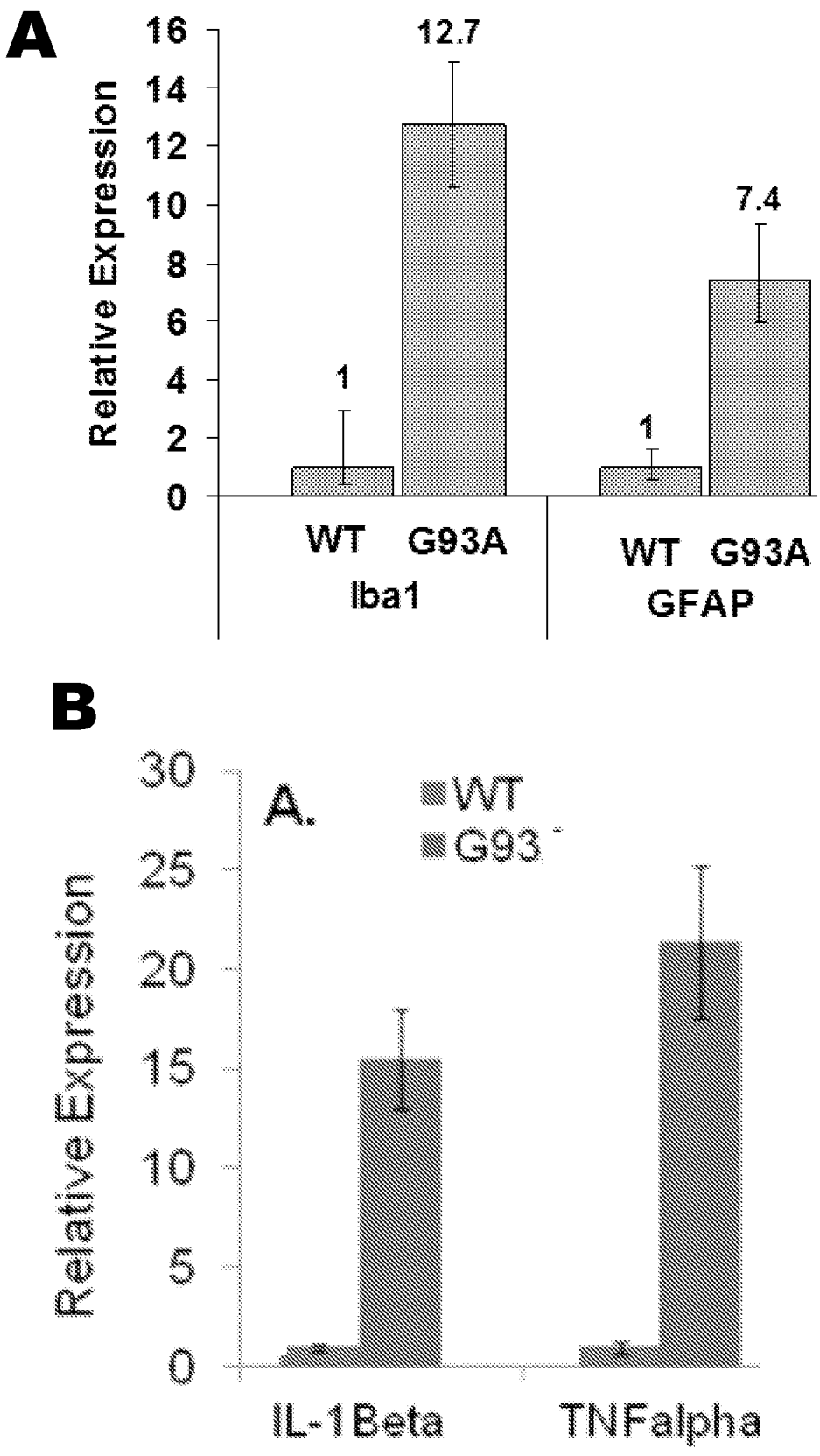

A
Relative Expression
16
14
12
10
8
6
4
2
0
12.7
7.4
1
1
WT
G93A
Iba1
WT
G93A
GFAP
B
Relative Expression
30
25
20
15
10
5
0
A.
WT
G93
IL-1Beta
TNFalpha

FIG. 6

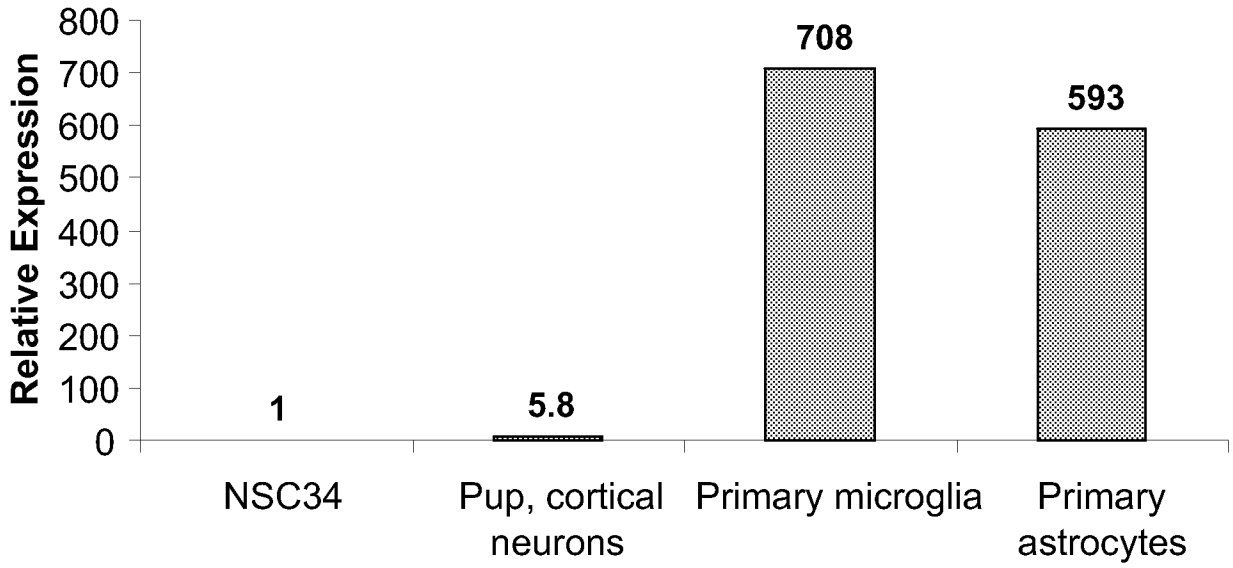

Relative Expression
800
700
600
500
400
300
200
100
0
1
5.8
708
593
NSC34
Pup, cortical neurons
Primary microglia
Primary astrocytes

FIG. 7

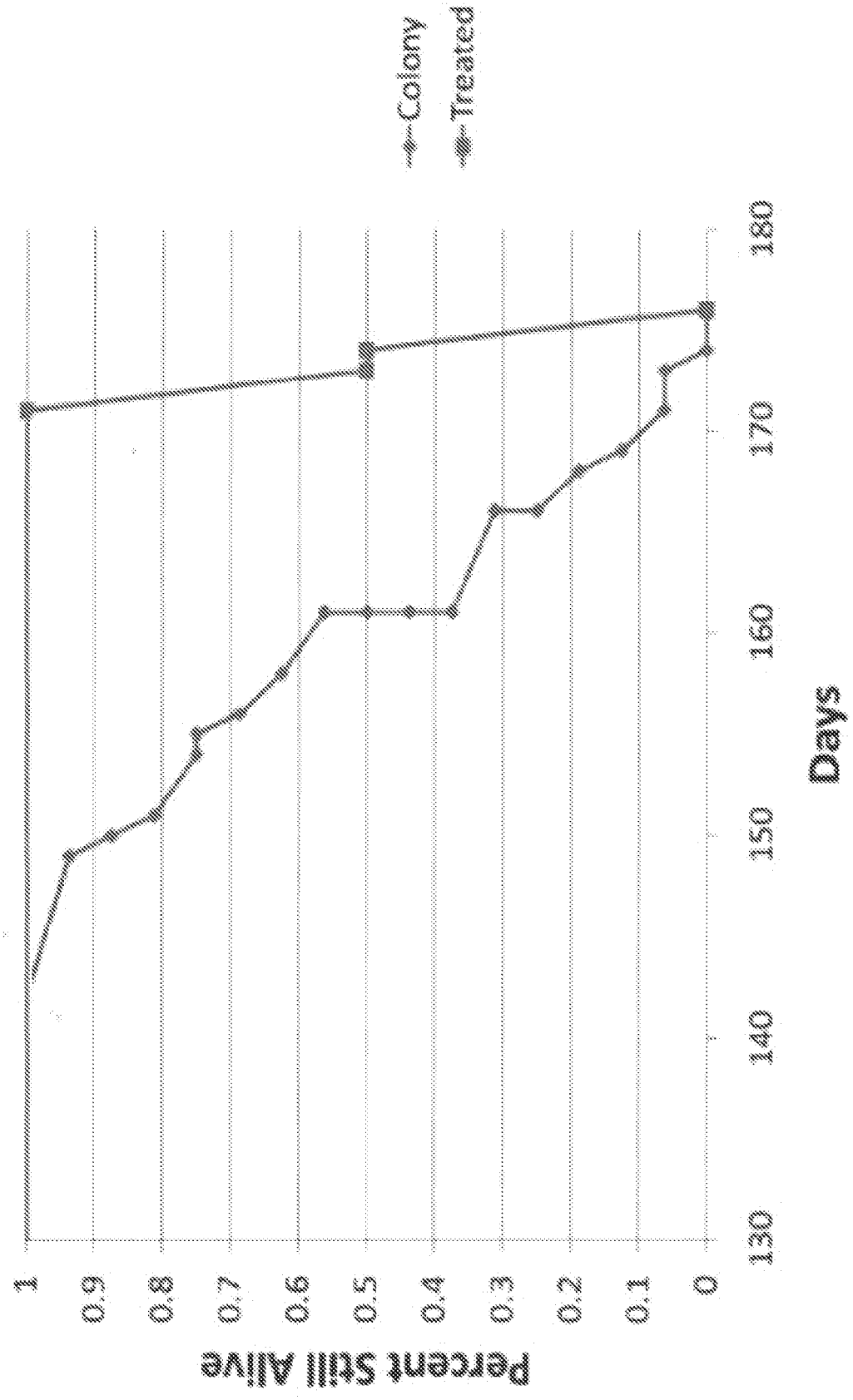

Colony
Treated
Percent Still Alive
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
130
140
150
160
170
180
Days

FIG. 8A

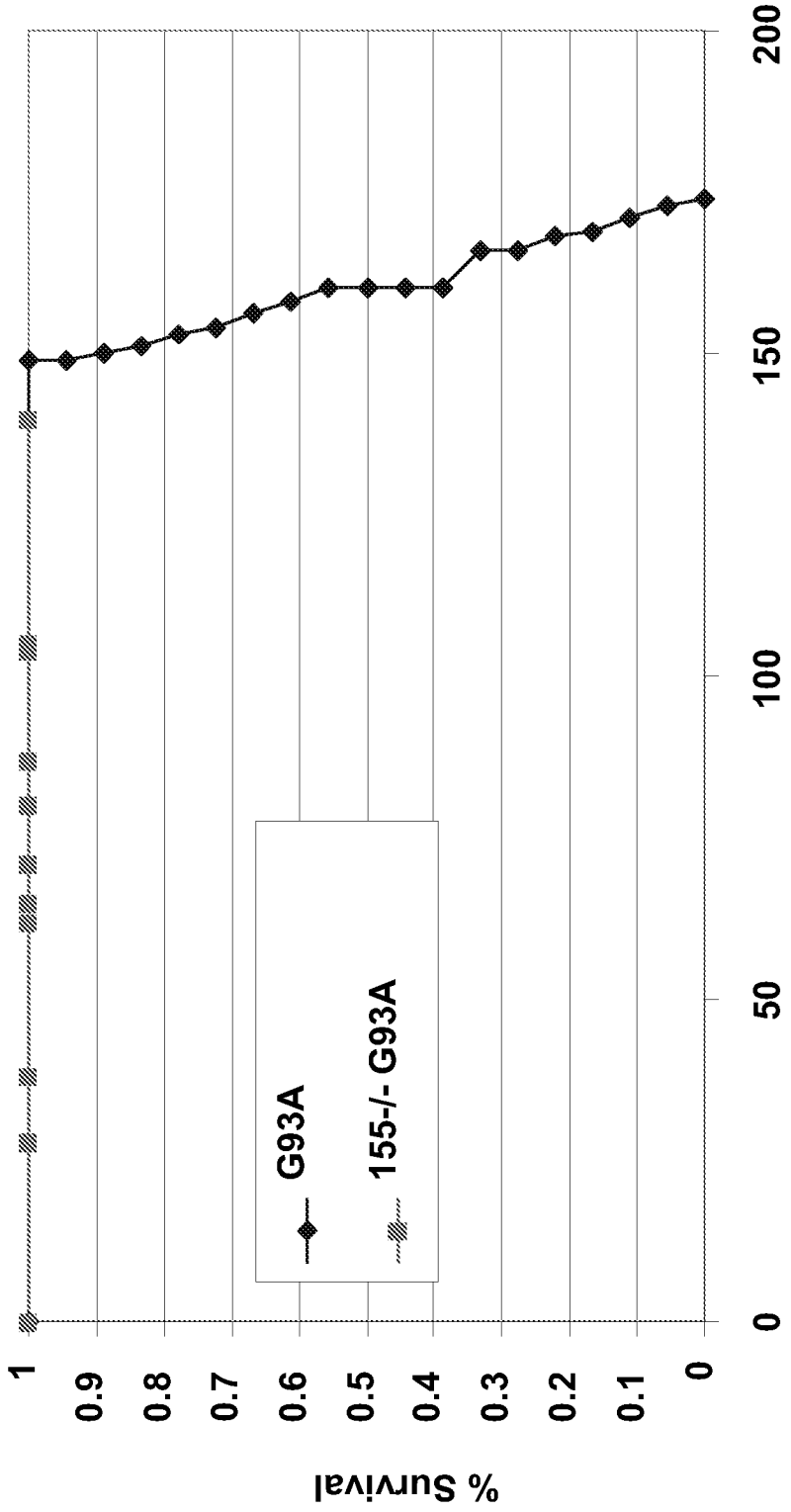

% Survival
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
Age (days)
G93A
155-/- G93A

FIG. 8B

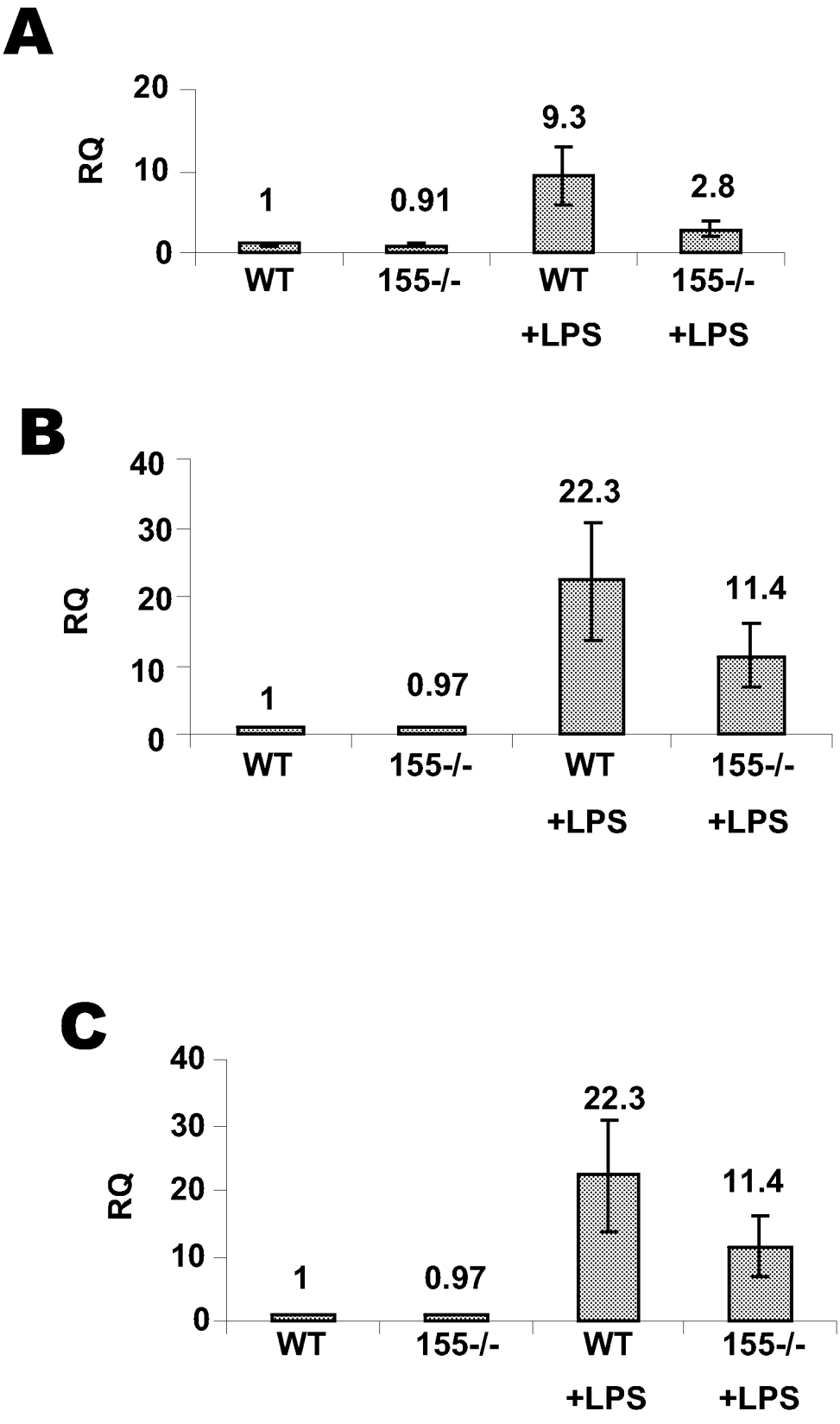
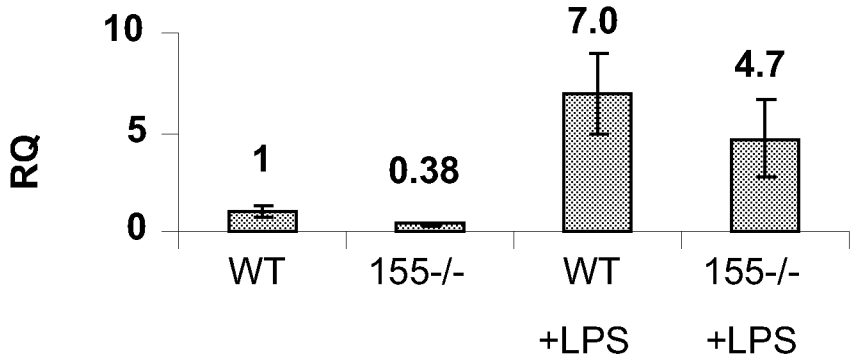

A
RQ
20
10
0
1
0.91
9.3
2.8
WT
155-/-
WT
+LPS
155-/-
+LPS
B
RQ
40
30
20
10
0
1
0.97
22.3
11.4
WT
155-/-
WT
+LPS
155-/-
+LPS
C
RQ
40
30
20
10
0
1
0.97
22.3
11.4
WT
155-/-
WT
+LPS
155-/-
+LPS

FIG. 9

D


10
5
0
RQ
1
0.38
7.0
4.7
WT
155-/-
WT
+LPS
155-/-
+LPS

E

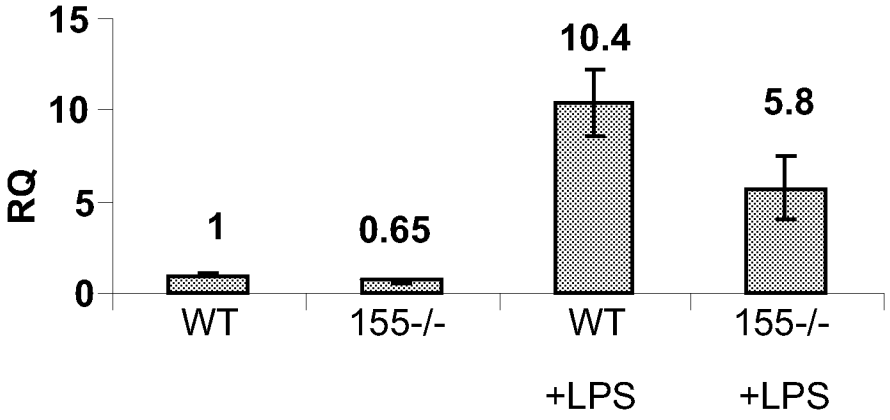

15
10
5
0
RQ
1
0.65
10.4
5.8
WT
155-/-
WT
+LPS
155-/-
+LPS

FIG. 9

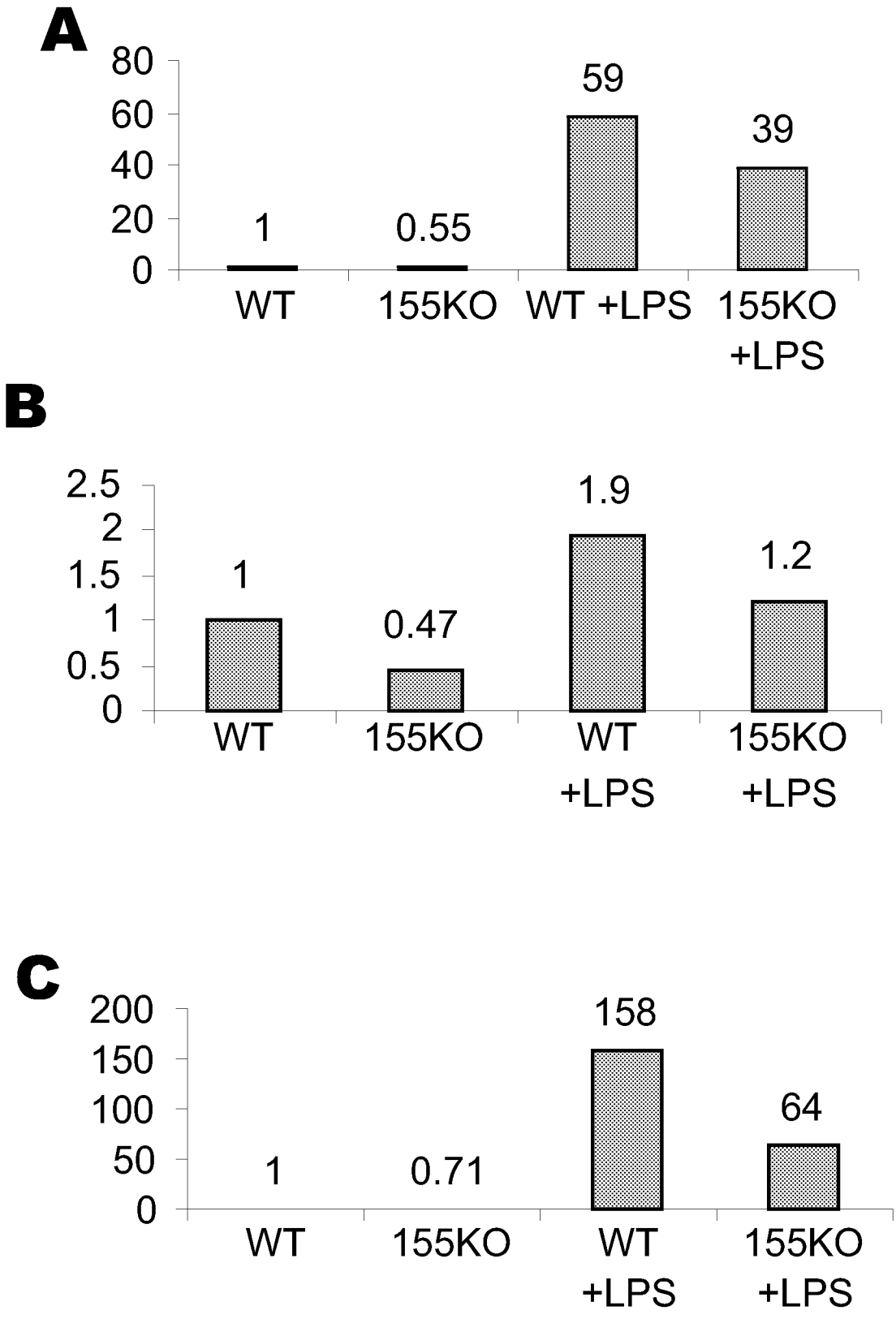

A
80
60
40
20
0
1
0.55
59
39
WT
155KO
WT +LPS
155KO +LPS
B
2.5
2
1.5
1
0.5
0
1
0.47
1.9
1.2
WT
155KO
WT +LPS
155KO +LPS
C
200
150
100
50
0
1
0.71
158
64
WT
155KO
WT +LPS
155KO +LPS

FIG. 10

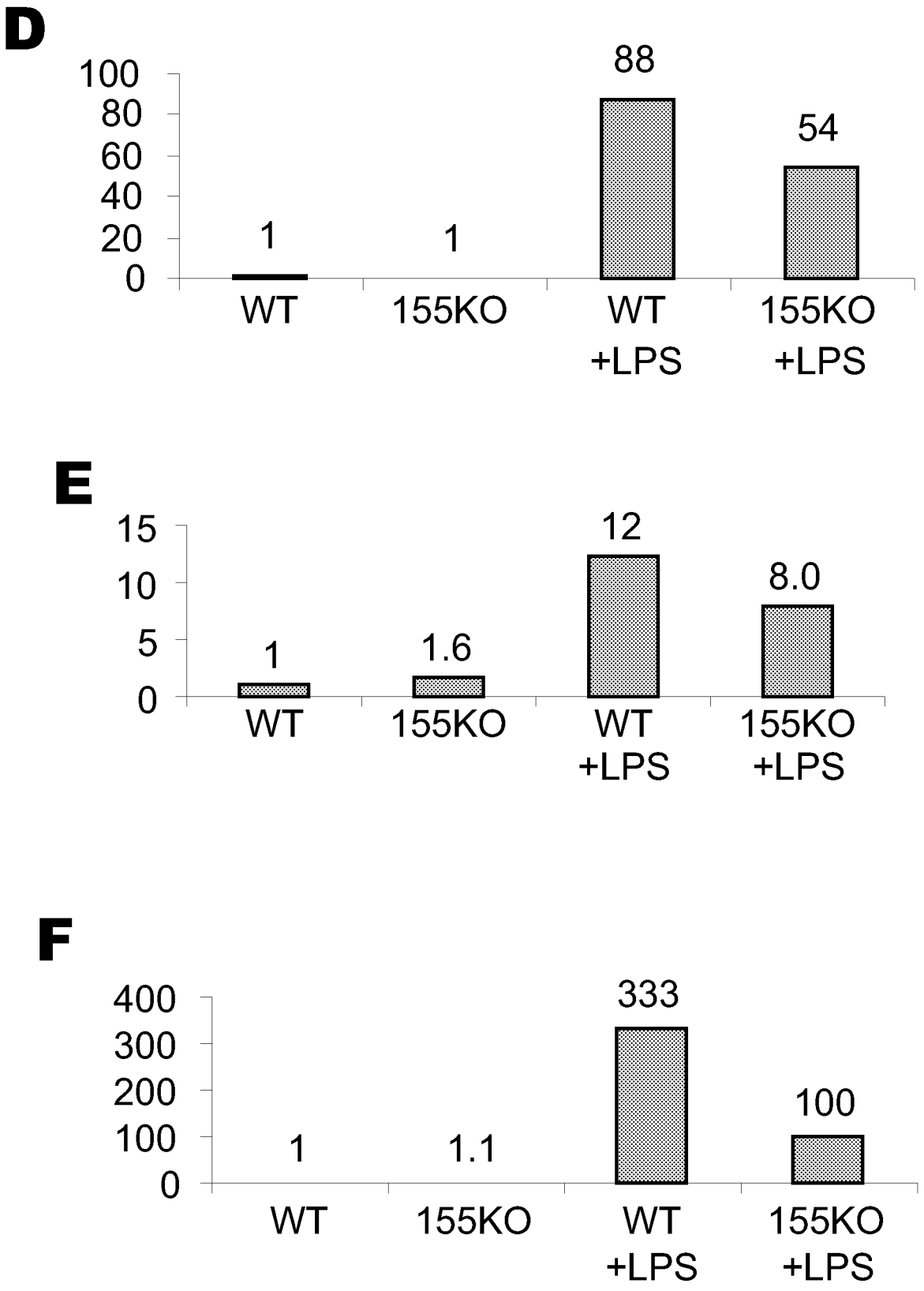

D
100
80
60
40
20
0
1
1
88
54
WT
155KO
WT +LPS
155KO +LPS
E
15
10
5
0
1
1.6
12
8.0
WT
155KO
WT +LPS
155KO +LPS
F
400
300
200
100
0
1
1.1
333
100
WT
155KO
WT +LPS
155KO +LPS

FIG. 10

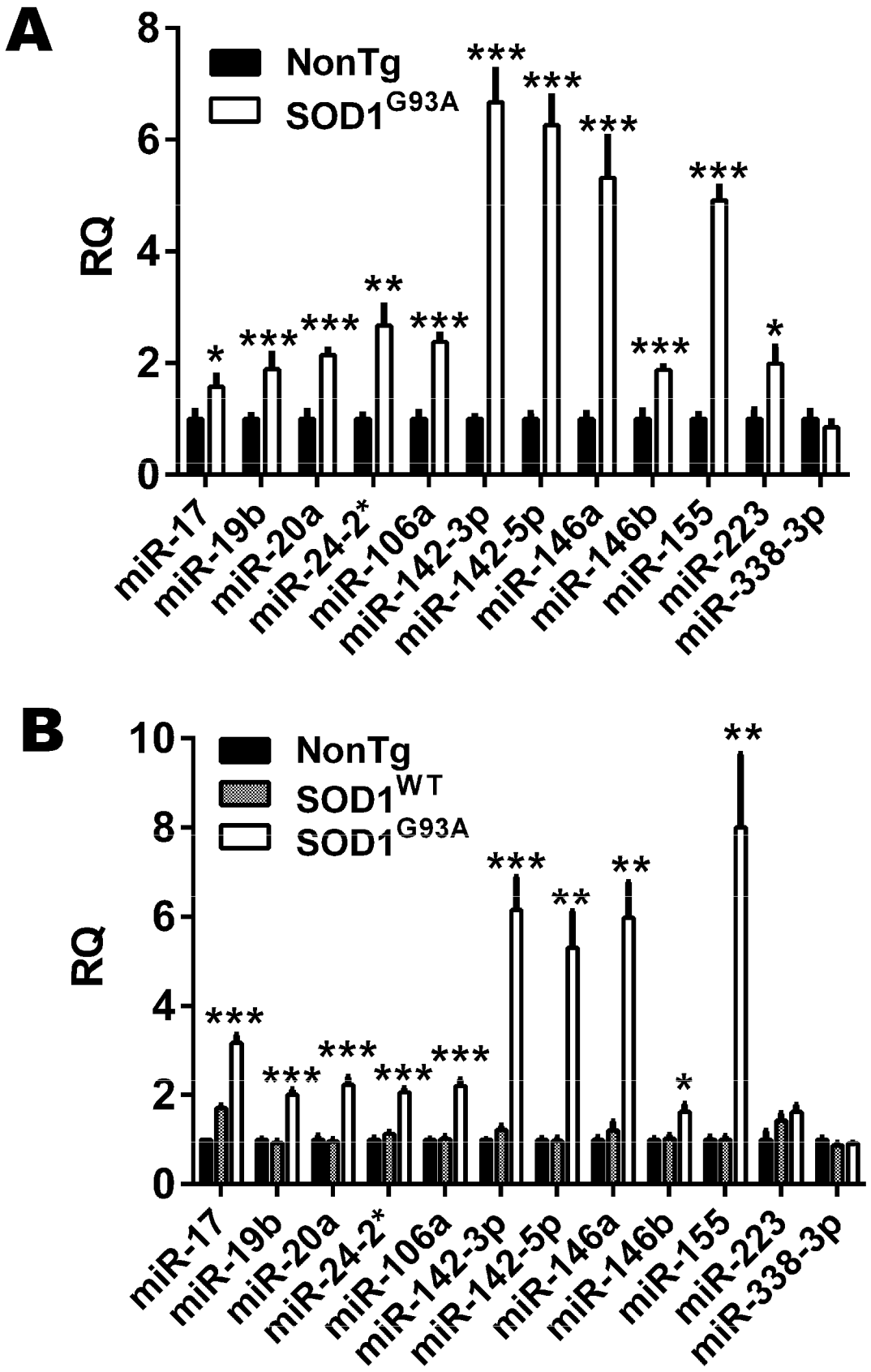

A
RQ
NonTg
SOD1G93A
miR-17
miR-19b
miR-20a
miR-24-2*
miR-106a
miR-142-3p
miR-142-5p
miR-146a
miR-146b
miR-155
miR-223
miR-338-3p
B
RQ
NonTg
SOD1WT
SOD1G93A
miR-17
miR-19b
miR-20a
miR-24-2*
miR-106a
miR-142-3p
miR-142-5p
miR-146a
miR-146b
miR-155
miR-223
miR-338-3p

FIG. 11

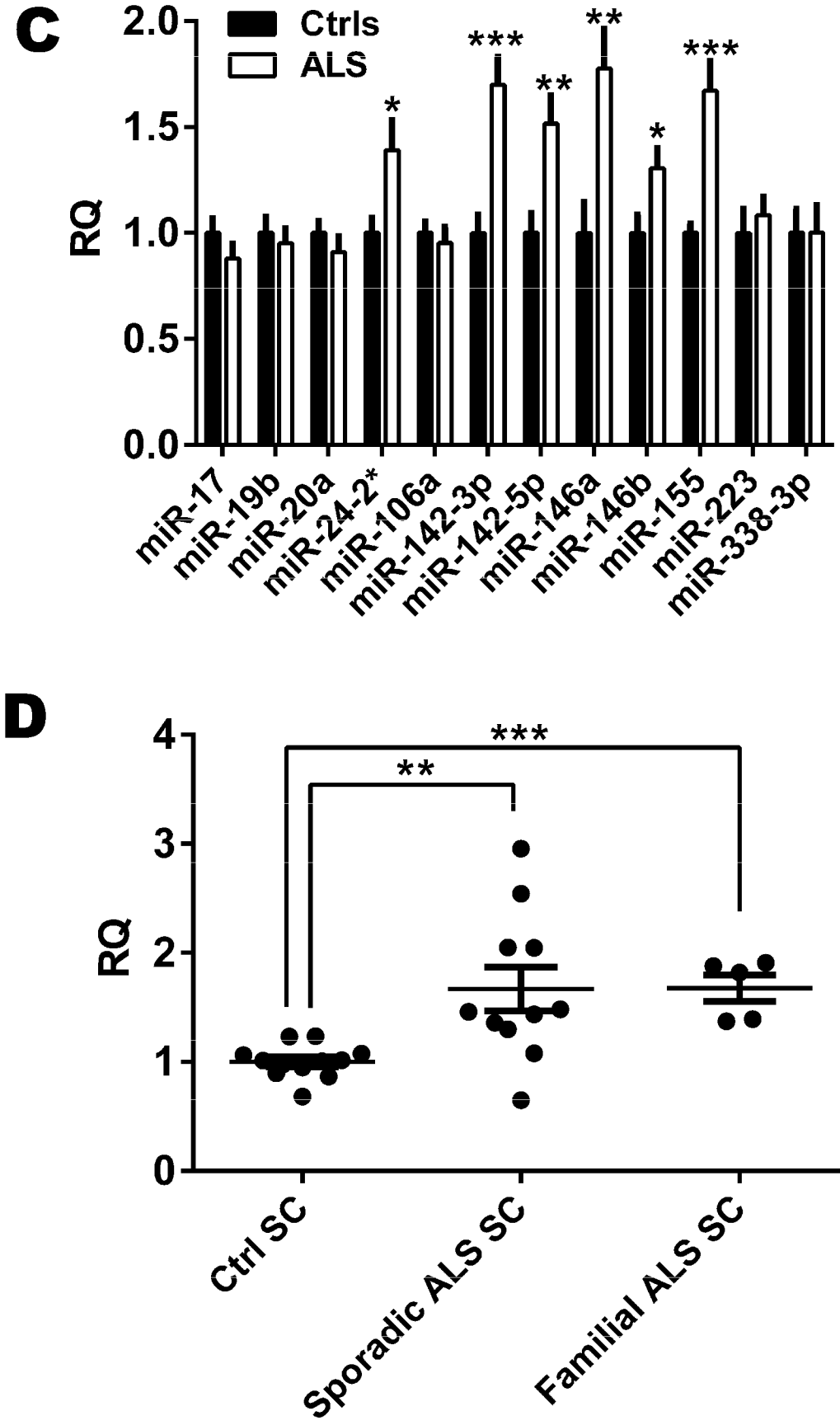
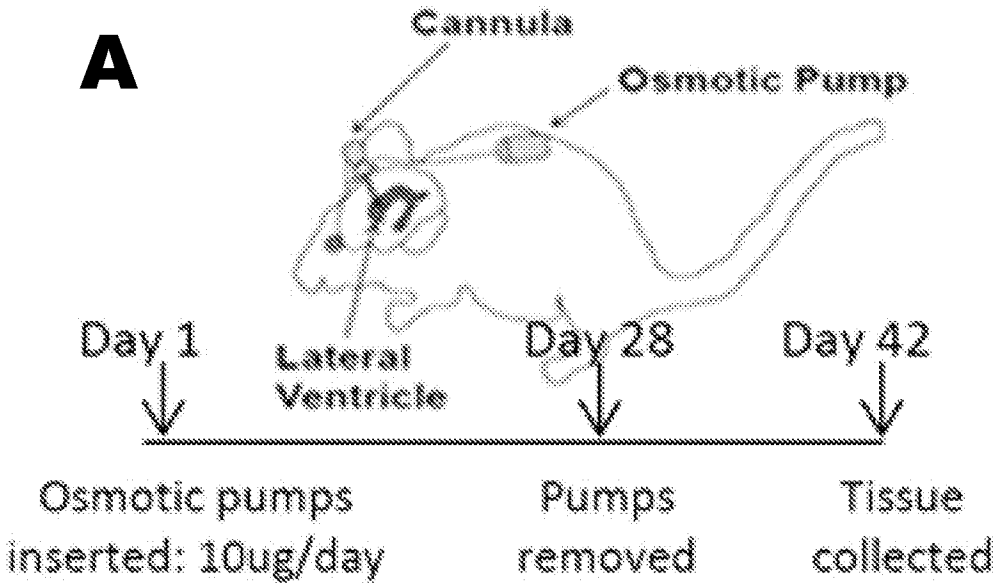

C
Ctrls
ALS
RQ
2.0
1.5
1.0
0.5
0.0
*
***
**
**
*
***
miR-17
miR-19b
miR-20a
miR-24-2*
miR-106a
miR-142-3p
miR-142-5p
miR-146a
miR-146b
miR-155
miR-223
miR-338-3p
D
RQ
4
3
2
1
0
***
**
Ctrl SC
Sporadic ALS SC
Familial ALS SC

FIG. 11


A
Cannula
Osmotic Pump
Day 1
Lateral Ventricle
Day 28
Day 42
Osmotic pumps inserted: 10ug/day
Pumps removed
Tissue collected

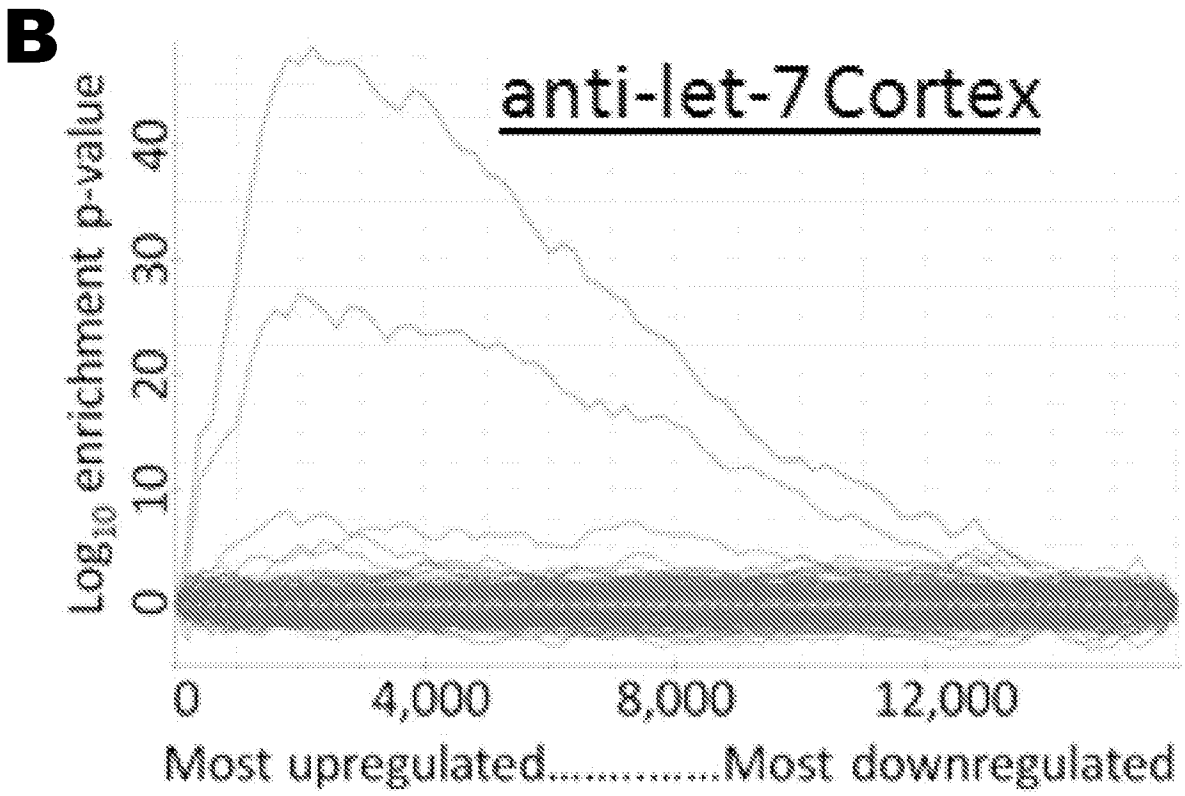

B
anti-let-7 Cortex
Log10 enrichment p-value
0
10
20
30
40
0
4,000
8,000
12,000
Most upregulated.....Most downregulated

FIG. 12

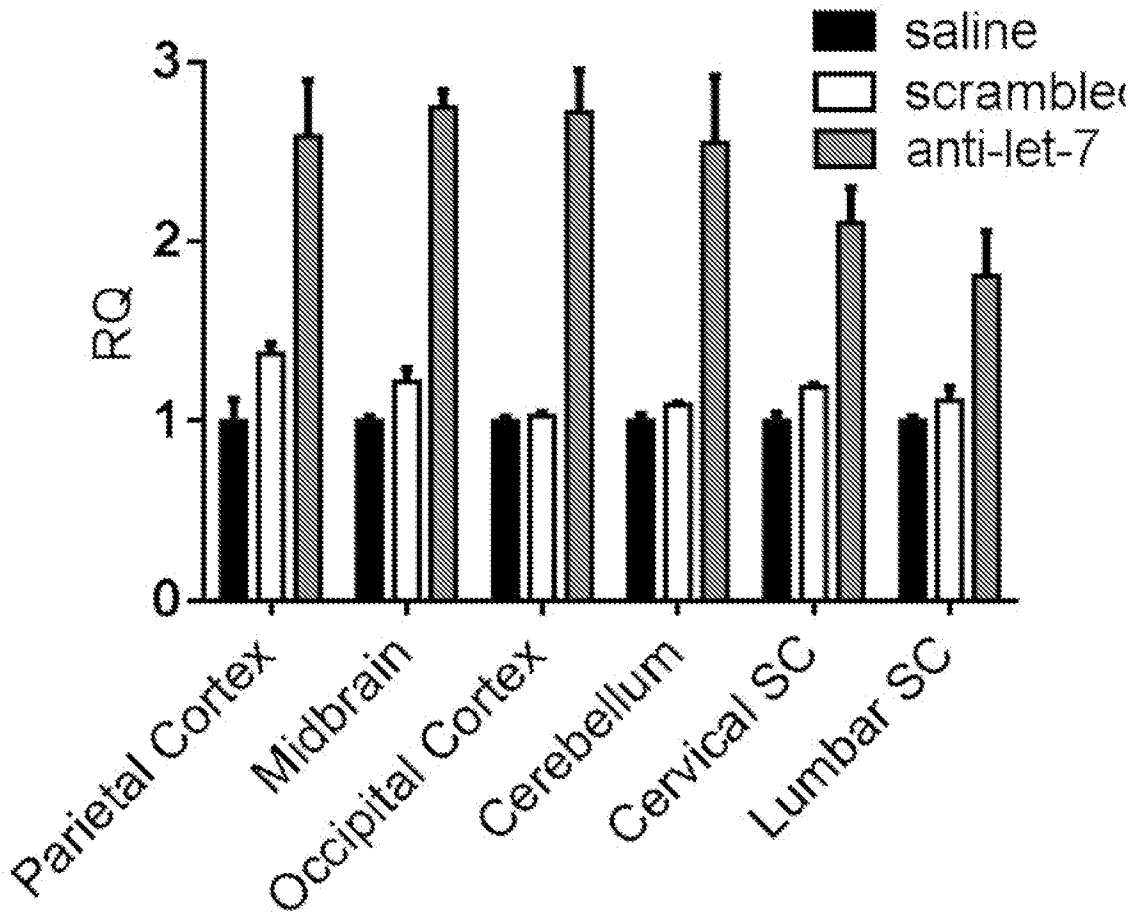

saline
scramble
anti-let-7
RQ
0
1
2
3
Parietal Cortex
Midbrain
Occipital Cortex
Cerebellum
Cervical SC
Lumbar SC

FIG. 12C

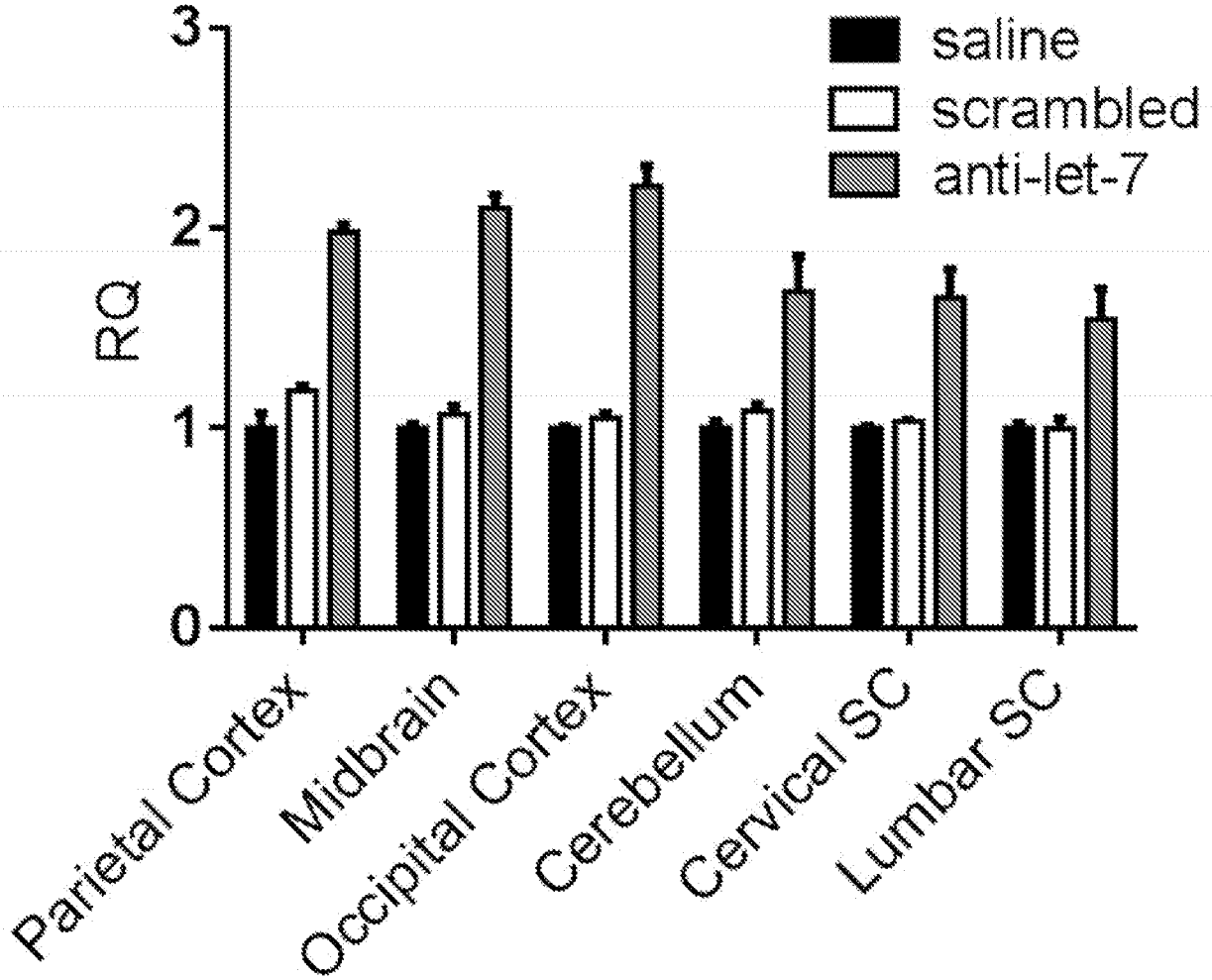
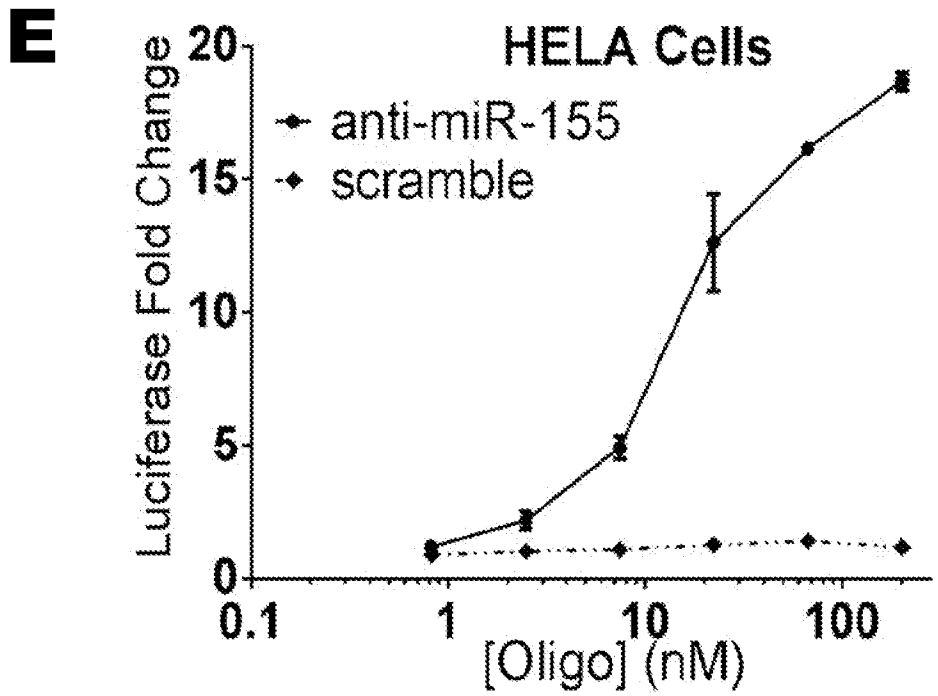

saline
scrambled
anti-let-7
RQ
0
1
2
3
Parietal Cortex
Midbrain
Occipital Cortex
Cerebellum
Cervical SC
Lumbar SC

FIG. 12D


E
HELA Cells
anti-miR-155
scramble
Luciferase Fold Change
20
15
10
5
0
0.1
1
10
100
[Oligo] (nM)

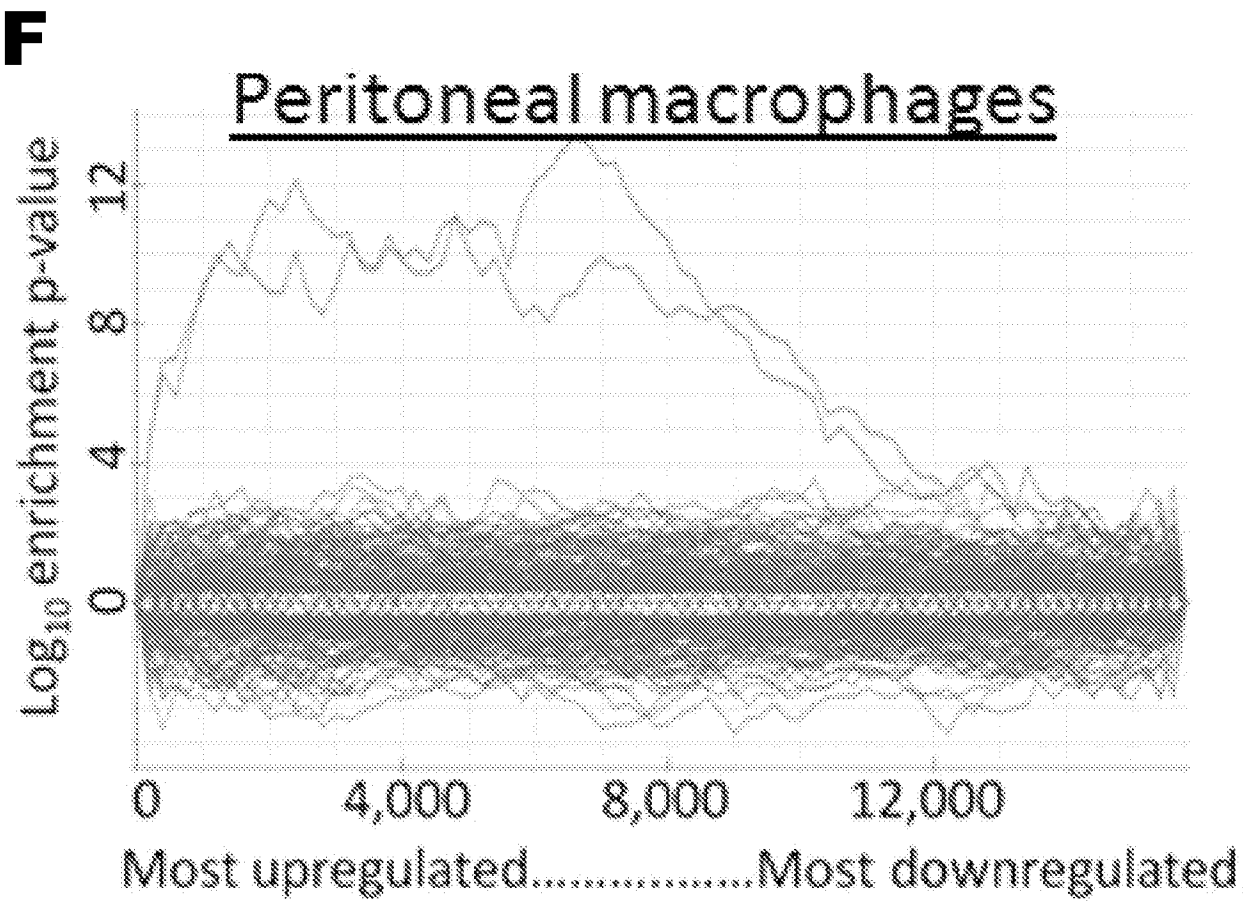

F
Peritoneal macrophages
Log10 enrichment p-value
0
4
8
12
0
4,000
8,000
12,000
Most upregulated.....Most downregulated

FIG. 12

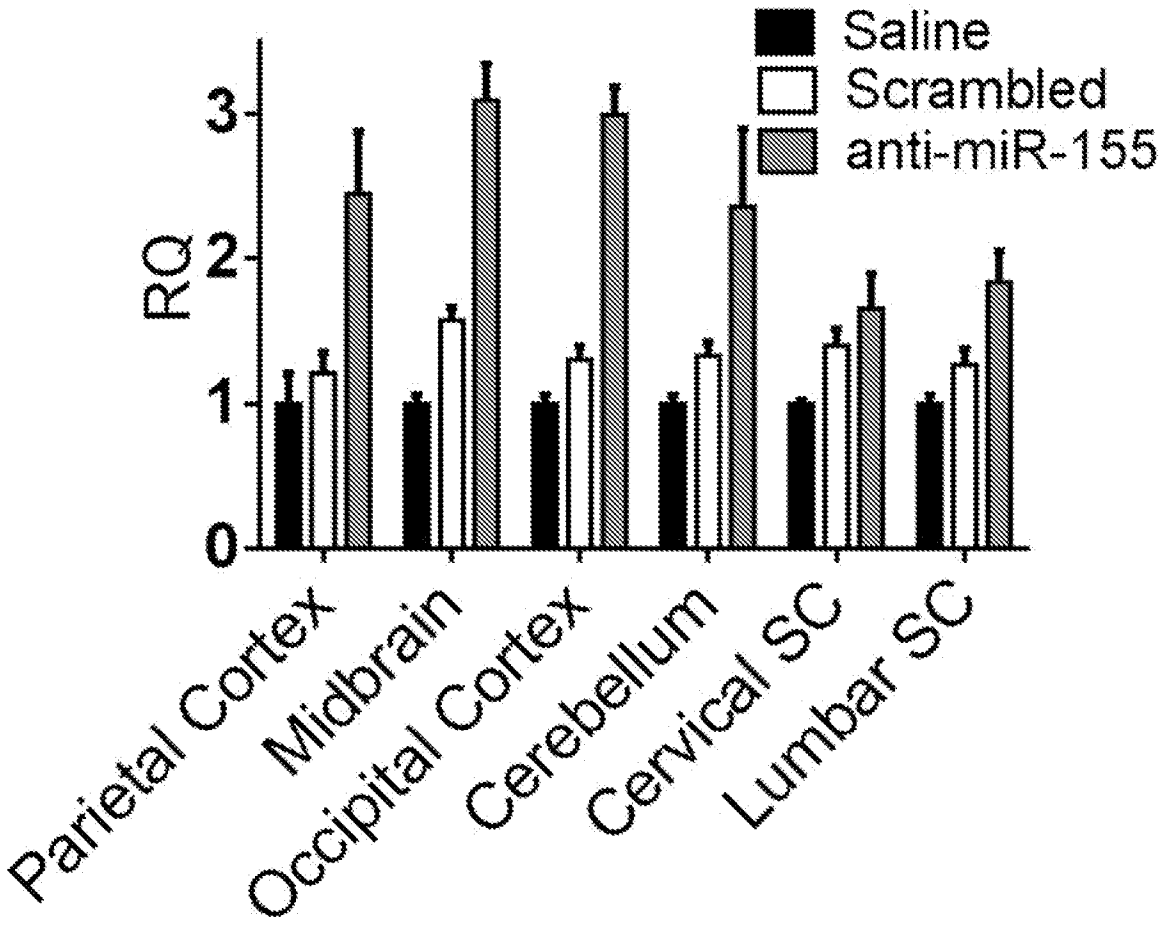

Saline
Scrambled
anti-miR-155
RQ
0
1
2
3
Parietal Cortex
Midbrain
Occipital Cortex
Cerebellum
Cervical SC
Lumbar SC

FIG. 12G

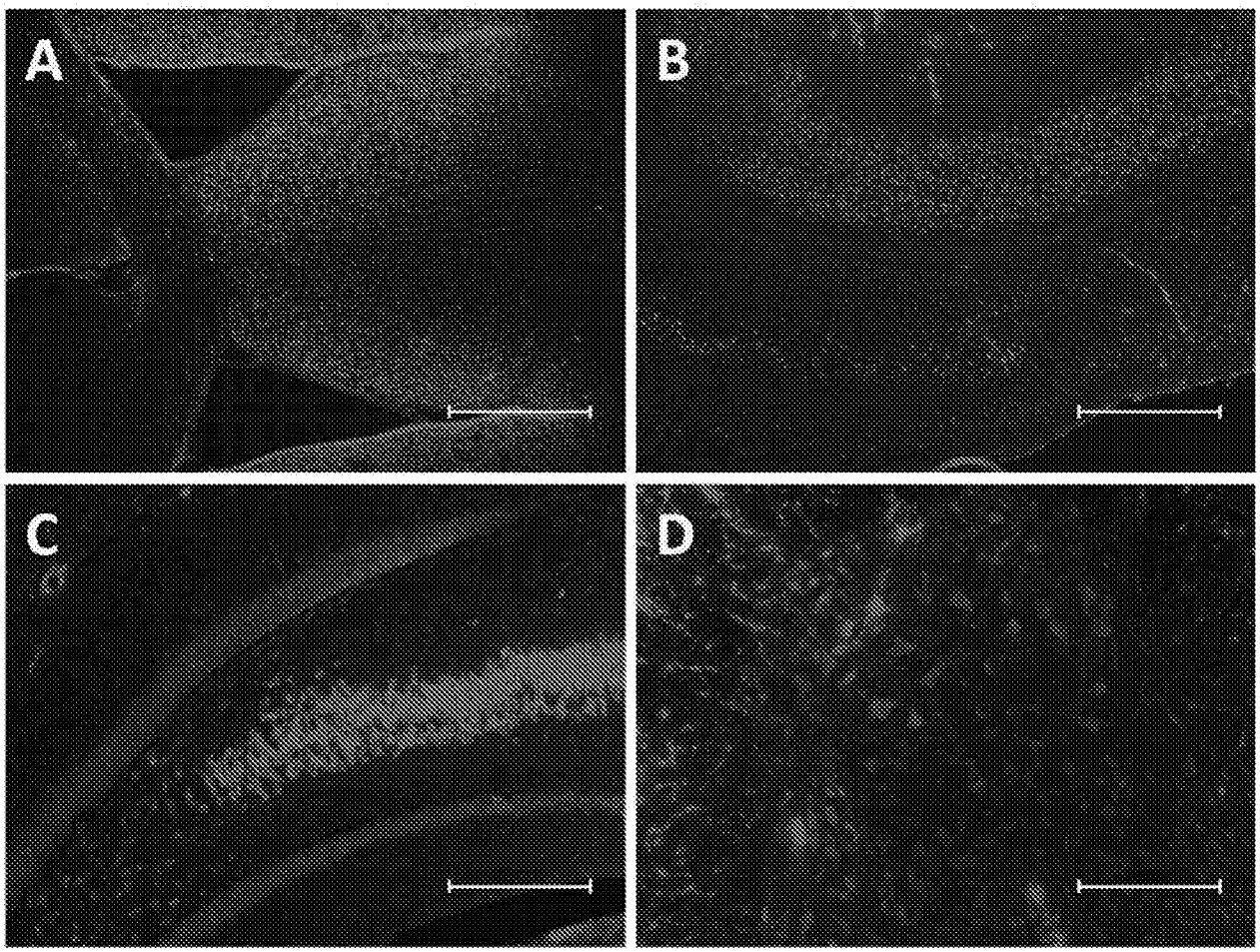


FIG. 13

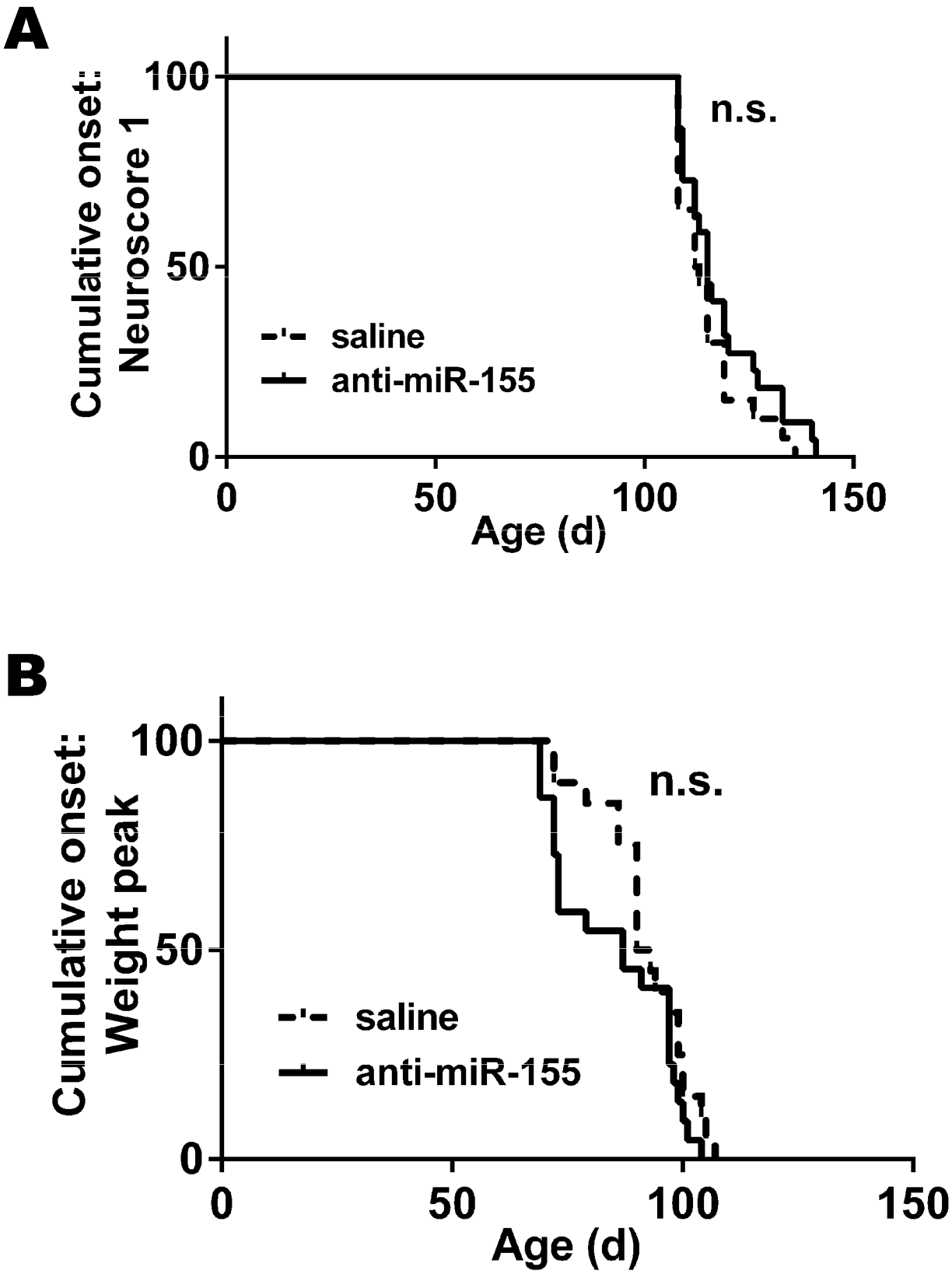
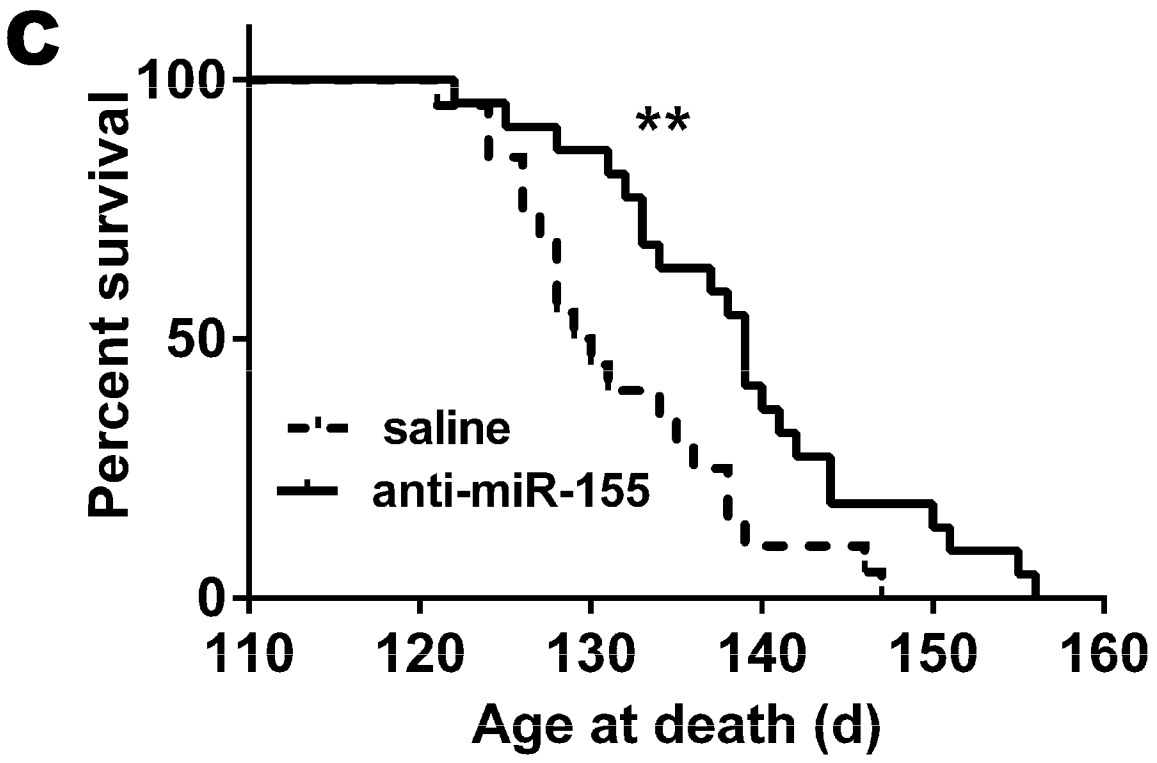

A
Cumulative onset:
Neuroscore 1
100
50
0
n.s.
saline
anti-miR-155
0
50
100
150
Age (d)
B
Cumulative onset:
Weight peak
100
50
0
n.s.
saline
anti-miR-155
0
50
100
150
Age (d)

FIG. 14


C
100
50
0
Percent survival
**
saline
anti-miR-155
110
120
130
140
150
160
Age at death (d)

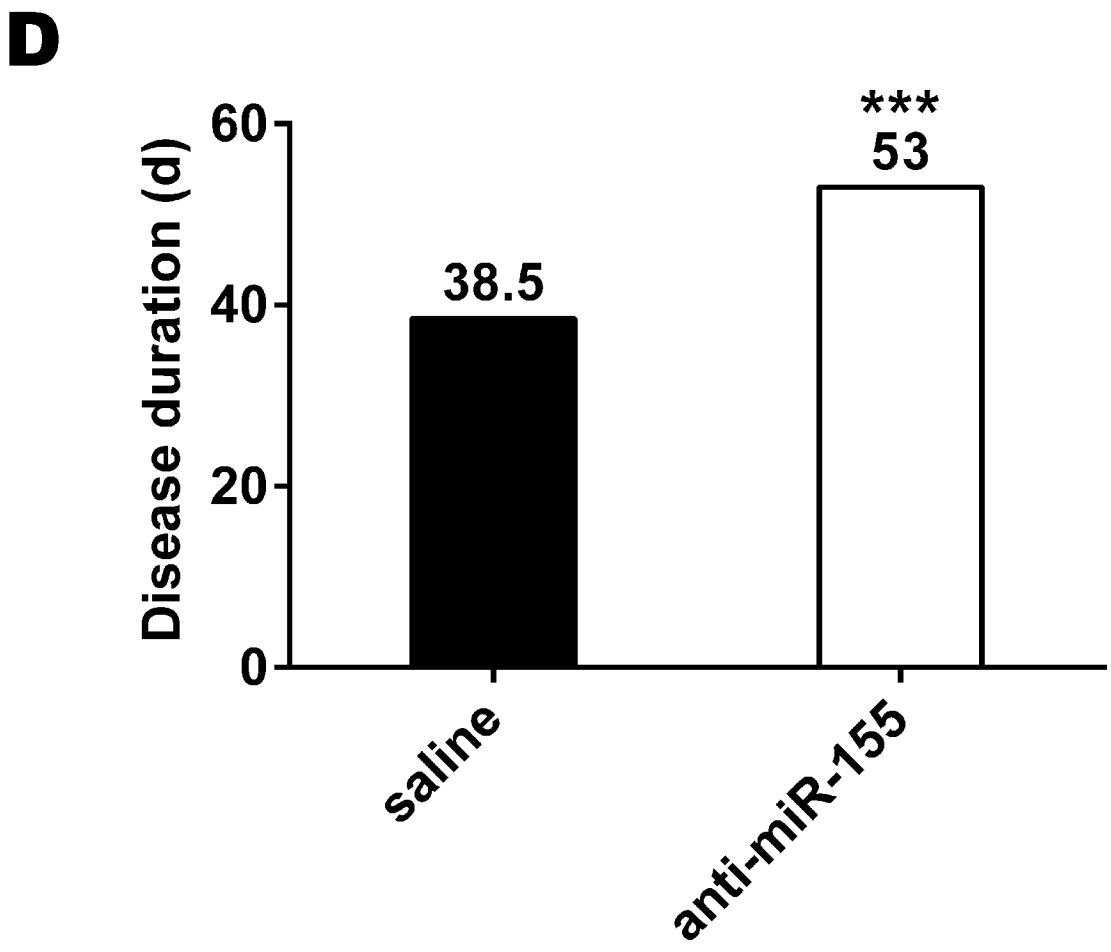

D
60
40
20
0
Disease duration (d)
38.5
***
53
saline
anti-miR-155

FIG. 14

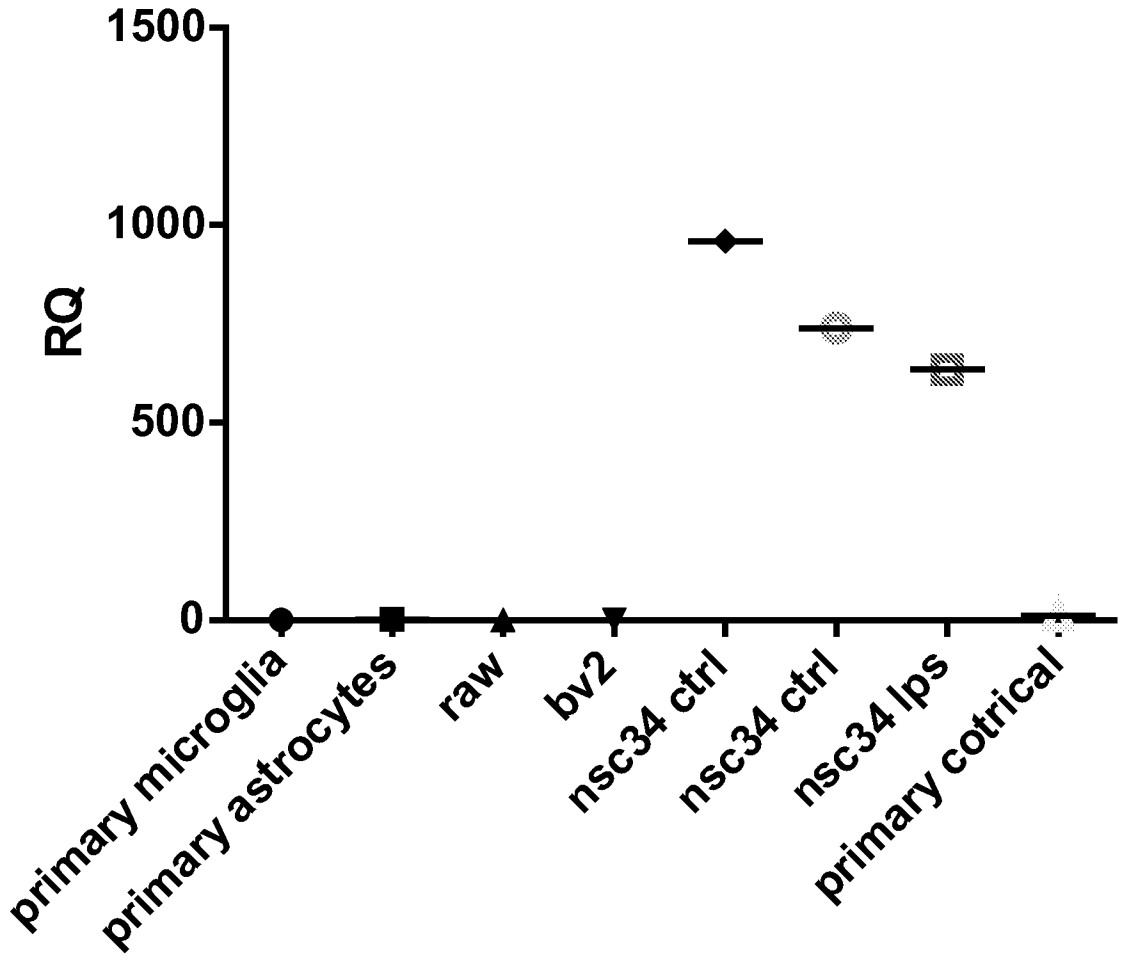

1500
1000
500
0
RQ
primary microglia
primary astrocytes
raw
bv2
nsc34 ctrl
nsc34 ctrl
nsc34 lps
primary cotrical

FIG. 15A

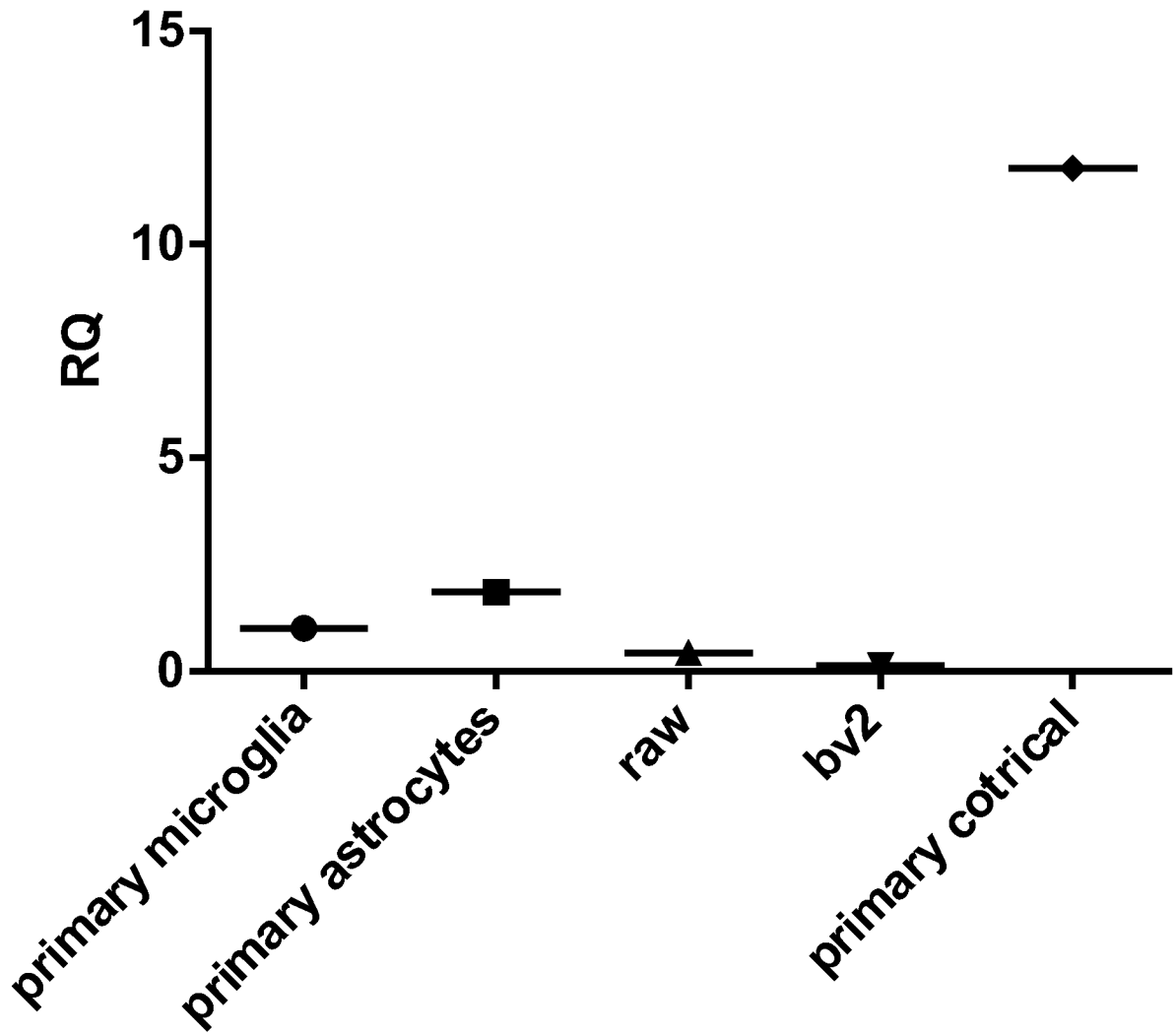

15
10
5
0
RQ
primary microglia
primary astrocytes
raw
bv2
primary cotrical

FIG. 15B

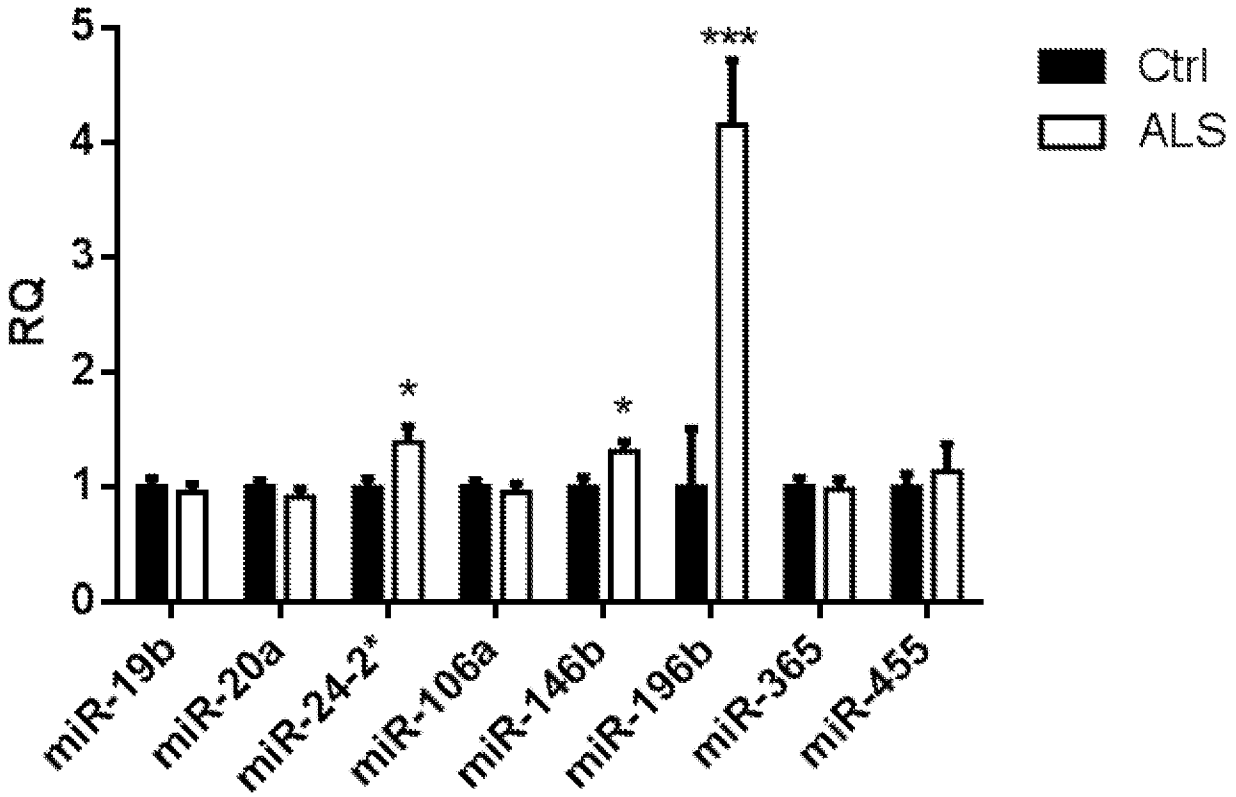

RQ
5
4
3
2
1
0
*
*
***
Ctrl
ALS
miR-19b
miR-20a
miR-24-2*
miR-106a
miR-146b
miR-196b
miR-365
miR-455

FIG. 16

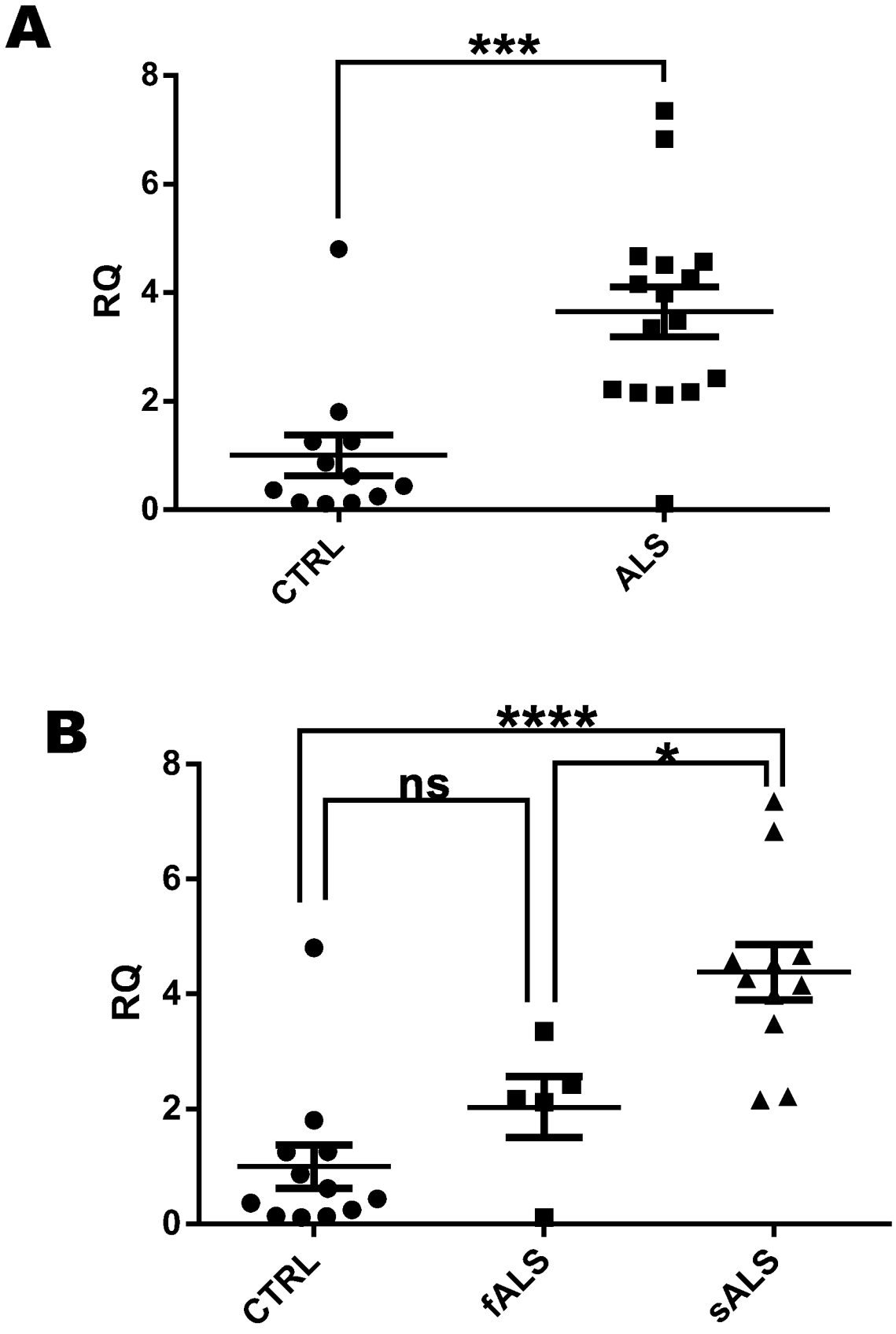


FIG. 17

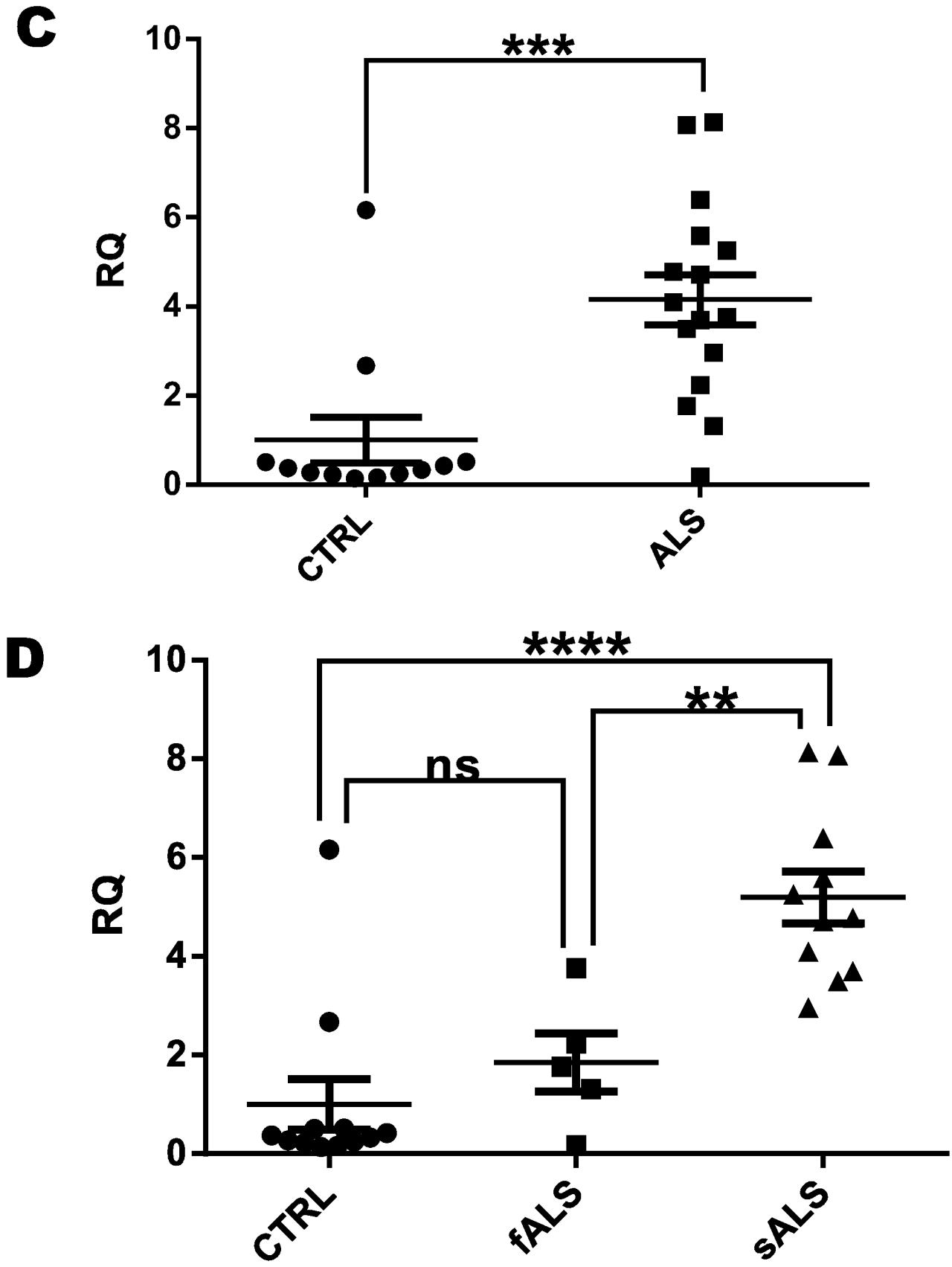


FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2013/029398

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/113 *(2010.01)*
A61K 31/712 *(2006.01)*
A61K 31/7115 *(2006.01)*
A61K 31/7105 *(2006.01)*
A61P 25/28 *(2006.01)*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 15/113, A61K 31/712, 31/7115, 31/7105, A61P 25/28

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSearch (RUPTO internal), USPTO, PAJ, Esp@cenet, Information Retrieval System of FIPS (http://www.fips.ru)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/043353 A2 (SANTARIS PHARMA A/S) 09.04.2009, claim 28	1-30
Y	ZHEN Liu et al. MicroRNA: an Emerging Therapeutic Target and Intervention Tool. Int. J. Mol. Sci. 2008, 9, 978-999, abstract	1-30
Y	SCHIPPER Hyman M. et al. MicroRNA Expression in Alzheimer Blood Mononuclear Cells. Gene Regulation and Systems Biology 2007:1 , 263-274, abstract, p. 265, tabl. 1	1, 2, 4-14, 16-22
Y	MAHMOUD Kiaei. Peroxisome Proliferator-Activated Receptor-γ in Amyotrophic Lateral Sclerosis and Huntington's Disease. Hindawi Publishing Corporation. PPAR Research, Volume 2008, Article ID 418765, 8 pages, abstract	3, 15, 23-30

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 April 2013 (29.04.2013)	06 June 2013 (06.06.2013)
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer E. Tereshkina Telephone No. (495) 531-65-38

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