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(54) Title: UNC13A ANTISENSE OLIGONUCLEOTIDES

(57) Abrégé/Abstract:
The present invention relates to UNC13A cryptic exon antisense oligonucleotides (ASOs), pharmaceutical compositions containing them, and methods for treating, inhibiting, suppressing, and preventing neurological diseases with them.

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(54) Title: UNC13A ANTISENSE OLIGONUCLEOTIDES

(57) Abstract: The present invention relates to UNC13A cryptic exon antisense oligonucleotides (ASOs), pharmaceutical compositions containing them, and methods for treating, inhibiting, suppressing, and preventing neurological diseases with them.

WO 2023/004049 A1

UNC13A ANTISENSE OLIGONUCLEOTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/203,409, filed July 21, 2021, and U.S. Provisional Application No. 63/364,830, filed May 17, 2022, each of which is hereby incorporated in its entirety by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to UNC13A cryptic exon antisense oligonucleotides (ASOs), pharmaceutical compositions containing them, and methods for treating, inhibiting, suppressing, and preventing neurological or neurodegenerative diseases with them.

BACKGROUND OF THE INVENTION

[0003] Many neurodegenerative disorders in patients are difficult to effectively treat, especially where the pathology of a neurodegenerative disorder in a particular patient is not completely understood.

[0004] UNC13A belongs to a family of genes originally discovered in *C. elegans* and was named based on the uncoordinated (unc) movements exhibited by animals with mutations in these genes, owing to deficits in neurotransmitter release. UNC13A encodes a large multidomain protein expressed in the nervous system, where it localizes to neuromuscular junctions and plays an essential role in the vesicle priming step, prior to synaptic vesicle fusion. Variants within the UNC13A gene increase the risk of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), two related neurodegenerative diseases defined by

mislocalization of the RNA-binding protein TDP-43. Rosa Ma *et al.*, https://doi.org/10.1101/2021.04.02.438213, *bioRxiv*, April 4, 2021.

[0005] Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are complex diseases that result from many diverse genetic etiologies. Although therapeutic strategies that target specific causal mutations (e.g. C9ORF72 antisense oligonucleotides (ASOs)) may prove effective against individual forms of ALS or FTD, these approaches cannot address the vast majority of cases that have unknown genetic etiology. Moreover, given the large number of different genes that likely contribute to ALS and FTD and the fact that each genetic form is relatively rare, this strategy may be difficult to implement for all cases. Thus, there is a pressing need for new therapeutic strategies that rescue multiple forms of ALS and FTD, particularly those with unknown genetic etiologies.

[0006] A recent analysis of 205 patients from the Mayo Clinic bank who had FTD with TDP-43 pathology, stratified the cases on the basis of UNC13A genotype, and showed a dose-dependent decrease in survival time in individuals carrying UNC13A risk alleles (Rosa Ma *et al.*, *Nature*, 603:124-130, 2022). Patients with two risk alleles had a median survival time 3 years less than those with the normal transcript. Similarly, variants of UNC13A increase the risk of ALS (Brown *et al.*, *Nature*, 603:131-137, 2022). TDP-43 depletion induces robust inclusion of a cryptic exon in UNC13A, resulting in nonsense-mediated decay and loss of UNC13A protein (*id.*).

[0007] International Publication No. WO 2022/122872 describes particular antisense oligonucleotides which are said to be capable of modulating splicing by preventing inclusion of an UNC13A cryptic exon into an UNC13A mature mRNA.

[0008] There remains a need for effective treatments for many neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS).

SUMMARY OF THE INVENTION

[0009] The present invention relates to UNC13A cryptic exon antisense oligonucleotides (ASOs or UNC13A ASOs), pharmaceutical compositions containing them, and their use in the treatment of neurodegenerative disorders. In particular, the ASOs described herein are to a cryptic exon between canonical exons 20 and 21 of UNC13A, and result in exclusion of the cryptic exon in the UNC13A transcript and increased UNC13A protein expression.

[0010] One embodiment is a single stranded ASO that suppresses the expression of UNC13A, wherein the ASO has a nucleobase sequence that comprises at least 12 or 15 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 1-1282. The ASO can also be any of SEQ ID Nos: 1-1282. In a preferred embodiment, the single stranded ASO has a nucleobase sequence that comprises at least 12 or 15 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 4-6, 9-11, 22-25, 53, 55, 359, or 360. In another preferred embodiment, the single stranded ASO has a nucleobase sequence comprising the consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 4-6, 9-11, 22-25, 53, 55, 359, or 360. In yet another preferred embodiment, the ASO is any one of SEQ ID NOs: 645-647, 650-652, 663-666, 694, 696, 1000, and 1001.

[0011] Another embodiment is an oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, at least 12,

at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 1-1282.

[0012] In certain embodiments, at least one internucleoside linkage in the ASO or oligonucleotide is a modified internucleoside linkage, and the modified internucleoside linkage may be a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. At least one of the nucleosides may also be a modified nucleobase.

[0013] In other embodiments, at least one nucleoside of the ASO may be a modified sugar moiety, where that modified sugar moiety can be a bicyclic sugar moiety, or the modified sugar moiety may comprise a 2'-O-methoxyethyl group. In certain aspects, the bicyclic sugar moiety comprises a 4'-CH(R)-O-2' bridge where the R group is, independently, H, C_{1-12} alkyl, or a protecting group.

[0014] In a preferred embodiment, the ASO is a steric blocking ASO. The steric blocking ASO binds to the target RNA and sterically denies other molecules access for base pairing to the RNA. In one embodiment, each nucleoside in the ASO has a 2'-modified sugar moiety, such as a sugar moiety with a 2'-O-methoxyethyl group, and each internucleoside linkage is a phosphorothioate linkage.

[0015] In certain other embodiments, the nucleobase sequence of the oligonucleotide is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to any one of SEQ ID NOs: 1-1282.

[0016] In other embodiments, the oligonucleotide consists of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, at least 12,

at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 1-1282.

[0017] In one embodiment, the ASO or oligonucleotide is 100% complementary to SEQ ID NO: 1207 (chr19:17641557 – 17642844).

[0018] Another embodiment is a pharmaceutical composition comprising an UNC13A ASO of the present invention and one or more pharmaceutically acceptable carriers, diluents, and/or excipients. In one embodiment, the pharmaceutical composition is suitable for parenteral administration, such as intracerebroventricular injection or intrathecal administration.

[0019] Yet another embodiment is a method of treating a subject having a neurological or neurodegenerative disease by administering to the subject a therapeutically effective amount of a UNC13A ASO or a pharmaceutical composition described herein. One embodiment is a method of treating amyotrophic lateral sclerosis (ALS) in a subject in need thereof by administering to the subject a therapeutically effective amount of a UNC13A ASO or a pharmaceutical composition described herein. Another embodiment is a method of treating frontotemporal dementia (FTD) in a subject in need thereof by administering to the subject a therapeutically effective amount of a UNC13A ASO or a pharmaceutical composition described herein.

[0020] Yet another embodiment is a method of treating a subject having an UNC13A disease or disorder by administering to the subject a therapeutically effective amount of an UNC13A ASO or a pharmaceutical composition described herein.

[0021] Yet another embodiment is a method of increasing UNC13A protein expression in a subject in need thereof by administering to the subject an effective amount of a UNC13A ASO or a pharmaceutical composition described herein.

[0022] In one embodiment of any of the methods described herein, the subject possesses a SNP variant associated with rs12973192 (C>G), rs12608932 (A>C), or both. Subjects having alleles with mutation on both SNPs may show a stronger response to treatment.

BRIEF DESCRIPTION OF THE FIGURES

[0023] A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying figures, wherein:

[0024] FIG. 1 is a chart showing mRNA levels of UNC13A cryptic exon (CE) after treatment with an ASO in a healthy control line with TDP-43 siRNA. For the screen, 14-day old Ngn2-induced neurons (Ngn2-iNs) were treated with TDP-43 siRNA (siTDP-43) for 7 days. qRT-PCR were performed with n=4 biological replicates. Mean +/- s.e.m. One-way ANOVA was used for statistical analysis. p-value *<0.05, **<0.01, ***<0.001,****<0.0001. "NCASO" refers to a negative control ASO.

[0025] FIG. 2 shows the locations of the ASOs with respect to the cryptic exon, TDP-43 binding site, and relative to the SNPs, rs12608932 and rs12973192.

[0026] FIG. 3A is a chart showing mRNA levels of UNC13A CE after treatment with an ASO in a sporadic ALS patient line carrying the risk haplotype on one allele (annotated as +/-). qRT-PCR were performed with n=4 biological replicates. Mean +/- s.e.m. One-way ANOVA was used for statistical analysis. p-value *<0.05, **<0.01, ***<0.001,****<0.0001.

[0027] FIG. 3B is a chart showing mRNA levels of UNC13A after treatment with an ASO in a sporadic ALS patient line carrying the risk haplotype on one allele (annotated as +/-). qRT-PCR

were performed with n=4 biological replicates. Mean +/- s.e.m. One-way ANOVA was used for statistical analysis. p-value *<0.05, **<0.01, ***<0.001,****<0.0001.

[0028] FIG. 3C is a chart showing mRNA levels of UNC13A CE after treatment with an ASO in one C9ALS patient line that does not carry the UNC13A SNPs (C9ALS-SNP-/-). qRT-PCR were performed with n=4 biological replicates. Mean +/- s.e.m. One-way ANOVA was used for statistical analysis. p-value *<0.05, **<0.01, ***<0.001,****<0.0001.

[0029] FIG. 3D is a chart showing mRNA levels of UNC13A after treatment with an ASO in one C9ALS patient line that does not carry the UNC13A SNPs (C9ALS-SNP-/-). qRT-PCR were performed with n=4 biological replicates. Mean +/- s.e.m. One-way ANOVA was used for statistical analysis. p-value *<0.05, **<0.01, ***<0.001,****<0.0001.

[0030] FIG. 3E is a western blot in which the protein expression of UNC13A was reduced by TDP-43 siRNA compared to negative control siRNA, where the effect can be rescued by UNC13A ASOs.

[0031] FIG. 3F is a chart showing that the level of TDP-43 protein was reduced significantly by TDP-43 siRNA (siTDP-43) compared to negative control siRNA. One-way ANOVA was performed for statistical significance.

[0032] FIG. 3G is a chart showing that the UNC13A protein level was significantly reduced by TDP-43 KD and was rescued by ASO treatment compared to negative control siRNA. One-way ANOVA was performed for statistical significance.

[0033] FIG. 4A is a chart showing that the efficiency in targeting the UNC 13A cryptic exon is highly sequence-dependent and does not demonstrate a consistent pattern. Ngn2-induced cortical excitatory neurons were treated with 10uM of ASOs for seven days before RNA collection. All

ASOs are with 2MOE modified bases and phosphorothioate linkages. The fold change is relative to the TDP-43 siRNA+ NCASO treatment group, which not shown in the chart.

[0034] FIG. 4B is another chart showing that the efficiency in targeting the UNC13A cryptic exon is highly sequence-dependent and does not demonstrate a consistent pattern, even among variations of the same ASO (ASO 55). Ngn2-induced cortical excitatory neurons were treated with 10uM of ASOs for seven days before RNA collection. All ASOs shown have 2MOE modified bases and phosphonothioate linkages.

[0035] FIG. 4C is a chart showing a one-way ANOVA analysis using Dunnett's multiple comparison test to compare all test groups to the siTDP43+NCASO group. All results have p value <0.0001. The chart shows that efficiency in targeting the UNC13A cryptic exon is highly sequence-dependent and does not demonstrate a consistent pattern among ASO 55 variations.

[0036] FIG. 4D is a chart showing one-way ANOVA analysis using Dunnett's multiple comparison test to compare all test groups to the siTDP43+NCASO group. Only the ASO 55_12-9 group show a significant difference ($p<0.0001$). However, if the ASO 55_12-9 and 55_12-8 groups are removed, ASO 37, 55, 55_12-1, 55_12-2, 55_12-4, and the siNC+NCASO showed significant differences relative to the reference group.

[0037] FIG. 4E is a chart showing one-way ANOVA analysis using Dunnett's multiple comparison test to compare all test groups to the siTDP43+NCASO group, demonstrating that efficiency in targeting the UNC13A cryptic exon is highly sequence-dependent and does not demonstrate a consistent pattern among ASO 55 variations.

[0038] FIG. 4F is a chart showing that efficiency in targeting the UNC13A cryptic exon is highly sequence-dependent and does not demonstrate a consistent pattern among ASO 55 variations. As shown the table, the sequences in blue are identical in the 6 ASOs, and the

sequences in red in the 3 ASOs were tested. For the 12mer version of ASO-55, the composition of the sequences is highly similar, but the ability to skip the UNC13A CE is largely different. Ngn2-induced cortical excitatory neurons were treated with 10uM of ASOs for seven days before RNA collection. All ASOs are with 2MOE modified bases and phosphonothioate linkages.

DETAILED DESCRIPTION OF THE INVENTION

[0039] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0040] **Definitions**

[0041] The terms "comprise(s)," "include(s)," "having," "has," "can," "contain(s)," "may" and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures.

[0042] The singular forms "a," "an" and "the" include plural references unless the context clearly dictates otherwise.

[0043] The present disclosure also contemplates other embodiments "comprising," "consisting of" and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0044] As used herein, "2'-deoxynucleoside" means a nucleoside comprising a 2'-H(H) furanosyl sugar moiety, as found in naturally occurring deoxyribonucleic acids (DNA) and a nucleobase. In certain embodiments, a 2'-deoxynucleoside may comprise a modified nucleobase and a furanosyl sugar moiety or may comprise an RNA nucleobase (uracil) furanosyl sugar moiety.

[0045] As used herein, "2'-substituted nucleoside" means a nucleoside comprising a 2'-substituted sugar moiety. As used herein, "2'-substituted" in reference to a sugar moiety means a sugar moiety comprising at least one 2'-substituent group other than H or OH.

[0046] As used herein, "antisense molecule" means an oligomeric nucleic acid or oligomeric duplex capable of achieving at least one antisense activity.

[0047] The modifier "about" used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (for example, it includes at least the degree of error associated with the measurement of the particular quantity). The modifier "about" should also be considered as disclosing the range defined by the absolute values of the two endpoints. For example, the expression "from about 2 to about 4" also discloses the range "from 2 to 4." The term "about" may refer to plus or minus 10% of the indicated number. For example, "about 10%" may indicate a range of 9% to 11%, and "about 1" may mean from 0.9-1.1. Other meanings of "about" may be apparent from the context, such as rounding off, so, for example "about 1" may also mean from 0.5 to 1.4.

[0048] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0049] As used herein, "bicyclic sugar" or "bicyclic sugar moiety" means a modified sugar moiety comprising two rings, wherein the second ring is formed via a bridge connecting two of the atoms in the first ring thereby forming a bicyclic structure. In certain embodiments, the first ring of the bicyclic sugar moiety is a furanosyl moiety. In certain embodiments, the bicyclic sugar moiety does not comprise a furanosyl moiety. As used herein, "bicyclic nucleoside" or "BNA" means a nucleoside comprising a bicyclic sugar moiety.

[0050] As used herein, "chirally enriched population" means a plurality of molecules of identical molecular formula, wherein the number or percentage of molecules within the population that contain a particular stereochemical configuration at a particular chiral center is greater than the number or percentage of molecules expected to contain the same particular stereochemical configuration at the same particular chiral center within the population if the particular chiral center were stereorandom. Chirally enriched populations of molecules having multiple chiral centers within each molecule may contain one or more stereorandom chiral centers. In certain embodiments, the molecules are modified oligonucleotides. In certain embodiments, the molecules are compounds comprising modified oligonucleotides.

[0051] As used herein, "complementary" in reference to an oligonucleotide means that at least 70% of the nucleobases of the oligonucleotide or one or more regions thereof and the nucleobases of another nucleic acid or one or more regions thereof are capable of hydrogen bonding with one another when the nucleobase sequence of the oligonucleotide and the other

nucleic acid are aligned in opposing directions. Complementary nucleobases means nucleobases that are capable of forming hydrogen bonds with one another. Complementary nucleobase pairs include adenine (A) and thymine (T); adenine (A) and uracil (U); cytosine (C) and guanine (G); and 5-methylcytosine (mC) and guanine (G). Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside. Rather, some mismatches are tolerated. As used herein, "fully complementary" or "100% complementary" in reference to oligonucleotides means that oligonucleotides are complementary to another oligonucleotide or nucleic acid at each nucleoside of the oligonucleotide.

[0052] In certain embodiments, oligonucleotides comprise one or more type of modified sugar and/or unmodified sugar moiety arranged along the oligonucleotide or region thereof in a defined pattern or sugar motif. In certain instances, such sugar motifs include, but are not limited to, any of the sugar modifications discussed herein.

[0053] In certain embodiments, modified oligonucleotides comprise or consist of a region having a fully modified sugar motif. In such embodiments, each nucleoside of the fully modified region of the modified oligonucleotide comprises a modified sugar moiety. In certain embodiments, each nucleoside of the entire modified oligonucleotide comprises a modified sugar moiety. In certain embodiments, modified oligonucleotides comprise or consist of a region having a fully modified sugar motif, wherein each nucleoside within the fully modified region comprises the same modified sugar moiety, referred to herein as a uniformly modified sugar motif. In certain embodiments, a fully modified oligonucleotide is a uniformly modified oligonucleotide. In certain embodiments, each nucleoside of a uniformly modified oligonucleotide comprises the same 2'-modification.

[0054] "Inhibit" as used herein refers to the ability to substantially antagonize, prohibit, prevent, suppress, restrain, slow, disrupt, alter, eliminate, stop, or reverse the progression or severity of the activity of a particular agent (e.g., infectious agent) or disease.

[0055] As used herein, the term "internucleoside linkage" is the covalent linkage between adjacent nucleosides in an oligonucleotide. As used herein "modified internucleoside linkage" means any internucleoside linkage other than a phosphodiester internucleoside linkage. "Phosphorothioate linkage" is a modified internucleoside linkage in which one of the non-bridging oxygen atoms of a phosphodiester internucleoside linkage is replaced with a sulfur atom.

[0056] In certain embodiments, nucleosides of modified oligonucleotides may be linked together using any internucleoside linkage. The two main classes of internucleoside linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus-containing internucleoside linkages include, but are not limited to, phosphates, which contain a phosphodiester bond (also referred to as unmodified or naturally occurring linkages), phosphotriesters, methylphosphonates or other alkylphosphonates, phosphoramidates, and phosphorothioates, and phosphorodithioates. Representative non-phosphorus containing internucleoside linking groups include but are not limited to methylenemethylimino (-CH_2-$N(CH_3)$-O-CH_2-), thiodiester, thionocarbamate (-O-C(=O)(NH)-S-); siloxane (-O-SiH_2-O-); and N,N'-dimethylhydrazine (-CH_2-$N(CH_3)$-$N(CH_3)$-). Modified internucleoside linkages, compared to naturally occurring phosphate linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotide. Methods of preparation of phosphorous-containing and non-phosphorous-containing internucleoside linkages are well known to those skilled in the art.

[0057] Representative internucleoside linkages having a chiral center include, but are not limited to, alkylphosphonates and phosphorothioates. Modified oligonucleotides comprising internucleoside linkages having a chiral center can be prepared as populations of modified oligonucleotides comprising stereo-random internucleoside linkages, or as populations of modified oligonucleotides comprising phosphorothioate linkages in particular stereochemical configurations. In certain embodiments, populations of modified oligonucleotides comprise phosphorothioate internucleoside linkages wherein all of the phosphorothioate internucleoside linkages are stereo-random. Such modified oligonucleotides can be generated using synthetic methods that result in random selection of the stereochemical configuration of each phosphorothioate linkage. Nonetheless, as is well understood by those of skill in the art, each individual phosphorothioate of each individual oligonucleotide molecule has a defined stereoconfiguration. In certain embodiments, populations of modified oligonucleotides are enriched for modified oligonucleotides comprising one or more particular phosphorothioate internucleoside linkages in a particular, independently selected stereochemical configuration. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 65% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 70% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 80% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 90% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 99% of the molecules in the population. Such chirally enriched populations of modified oligonucleotides

can be generated using synthetic methods known in the art, e.g., methods described in Oka *et al.*, *JACS* 125, 8307 (2003); Wan *et al.*, *Nuc. Acid. Res.* 42, 13456 (2014); Chapter 10 of Locked Nucleic Acid Aptamers in Nucleic Acid and Peptide Aptamers: Methods and Protocols v 535, 2009 by Barciszewski et al., editor Gunter Mayerand; and WO 2017/015555. In certain embodiments, a population of modified oligonucleotides is enriched for modified oligonucleotides having at least one indicated phosphorothioate in the (Sp) configuration. In another embodiment, a population of modified oligonucleotides is enriched for modified oligonucleotides having at least one indicated phosphorothioate in the (Rp) configuration.

[0058] As used herein, "MOE" means methoxyethyl. "2'-MOE" means a $-OCH_2CH_2OCH_3$ group at the 2' position of a furanosyl ring.

[0059] A "neurological disease" is any disease that causes electrical, biochemical, or structural abnormalities in the brain, spine, or neurons. For example, a neurological disease may be a neurodegenerative disease. The neurodegenerative disease may result in motor neuron degeneration, for example. The neurological disease may be amyotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, or frontotemporal dementia, for example. Further examples of neurological diseases include, but are not limited to, Parkinson's disease, chronic traumatic encephalopathy, multiple sclerosis, peripheral myopathy, Rasmussen's encephalitis, attention deficit hyperactivity disorder, autism, central pain syndromes, anxiety, and/or depression, for example. In one embodiment, the patient suffers from a neurological disease in which TDP-43 depletion occurs.

[0060] The neurological disease may be associated with aberrant endosomal trafficking. For example, endosomal pathways and endosomes are necessary components for the recycling or breakdown of membrane-bound proteins, trafficking of Golgi-associated proteins, and the

extracellular release of proteins in exosomes. These processes aid neurotransmission and drive a balance between recycling and degradation of synaptic vesicles or neurotransmitter receptors, for example.

[0061] The neurological disease may be associated with aberrant lysosome degradation. Alterations in the lysosome degradation may be present in the neurological disease, such as a neurodegenerative disease. Cathepsin imbalance during aging and age-related diseases may provoke deleterious effects on central nervous system (CNS) neurons and lysosomes may be sites for the unfolding and partial degradation of membrane proteins or their precursors that subsequently become expelled from a cell, or are released from dead cells and accumulate as pathological entities.

[0062] A health care professional may diagnose a subject as having a disease associated with motor neuron degeneration by the assessment of one or more symptoms of motor neuron degeneration. To diagnose a neurological disease, a physical exam may be followed by a thorough neurological exam. The neurological exam may assess motor and sensory skills, nerve function, hearing and speech, vision, coordination and balance, mental status, and changes in mood or behavior. Non-limiting symptoms of a disease associated with a neurological disease may be weakness in the arms, legs, feet, or ankles; slurring of speech; difficulty lifting the front part of the foot and toes; hand weakness or clumsiness; muscle paralysis; rigid muscles; involuntary jerking or writing movements (chorea); involuntary, sustained contracture of muscles (dystonia); bradykinesia; loss of automatic movements; impaired posture and balance; lack of flexibility; tingling parts in the body; electric shock sensations that occur with movement of the head; twitching in arm, shoulders, and tongue; difficulty swallowing; difficulty breathing; difficulty chewing; partial or complete loss of vision; double vision; slow or abnormal eye

movements; tremor; unsteady gait; fatigue; loss of memory; dizziness; difficulty thinking or concentrating; difficulty reading or writing; misinterpretation of spatial relationships; disorientation; depression; anxiety; difficulty making decisions and judgments; loss of impulse control; difficulty in planning and performing familiar tasks; aggressiveness; irritability; social withdrawal; mood swings; dementia; change in sleeping habits; wandering; and change in appetite.

[0063] Tests may be performed to rule diseases and disorders that may have symptoms similar to those of neurological diseases, measure muscle involvement, assess neuron degeneration. Non-limiting examples of tests are electromyography (EMG); nerve conduction velocity study; laboratory tests of blood, urine, or other substances; magnetic resonance imaging (MRI); magnetic resonance spectroscopy; muscle or nerve biopsy; transcranial magnetic stimulation; genetic screening; x-rays; fluoroscopy; angiography; computed tomography (CT); positron emission tomography; cerebrospinal fluid analysis; intrathecal contrast-enhanced CT scan; electroencephalography; electronystagmography; evoked response; polysomnogram; thermography; and ultrasound. A health care professional may also assess the patient's family history of diseases associated with motor neuron degeneration and make a diagnosis in part based on a familial history of neurological diseases. A healthcare professional may diagnose a disease associated with neurological disease in a subject after the presentation of one or more symptoms.

[0064] Neurodegenerative diseases result in the progressive destruction of neurons that affects neuronal signaling. For example, a neurodegeneration may be amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's disease, Friedreich's ataxia, Lewy body disease, Parkinson's

disease, spinal muscle atrophy, primary lateral sclerosis, progressive muscle atrophy, progressive bulbar palsy, and pseudobulbar palsy.

[0065] Diseases associated with motor neuron degeneration may be a condition that results in the progressive destruction of motor neurons that interferes with neuronal signaling to the muscles, leading to muscle weakness and wasting. In healthy individuals, upper motor neurons transmit signals from the brain to lower motor neurons in the brain stem and spinal cord, which then transmit the signal to the muscles to result in voluntary muscle activity. The destruction of upper and lower motor neurons affects activity such as breathing, talking, swallowing, and walking, and overtime these functions can be lost. Examples of motor neuron diseases include, but are not limited to, amyotrophic lateral sclerosis, primary lateral sclerosis, progressive muscle atrophy, progressive bulbar palsy, and pseudobulbar palsy.

[0066] Neuronal hyperexcitability may occur when receptors for the excitatory neurotransmitter glutamate (glutamate receptors) such as the NMDA receptor and AMPA receptor are over-activated by excess glutamate or by other compounds or neurotransmitters acting on the glutamate receptors. Excitotoxicity may result from neuronal hyperexcitability. Excitotoxicity is the pathological process by which nerve cells are damaged or killed by excessive stimulation. The excessive stimulation allows high levels of calcium ions (Ca^{2+}) to enter the cell. Ca^{2+} influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases such as calpain. These enzymes can damage cell structures such as components of the cytoskeleton, membrane, and DNA.

[0067] Neuronal hyperexcitability may be involved in spinal cord injury, stroke, traumatic brain injury, hearing loss (through noise overexposure or ototoxicity), epilepsy, painful neuropathies, attention deficit hyperactivity disorder, autism, central pain syndromes,

neurodegenerative diseases, multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, frontotemporal dementia, schizophrenia, Rasmussen's encephalitis, Huntington's disease, alcoholism or alcohol withdrawal and especially over-rapid benzodiazepine withdrawal, and also Huntington's disease. Other common conditions that cause excessive glutamate concentrations around neurons are hypoglycemia. Blood sugars are the primary glutamate removal method from inter-synaptic spaces at the NMDA and AMPA receptor site.

[0068] As used herein, "non-bicyclic modified sugar moiety" means a modified sugar moiety that comprises a modification, such as a substituent, that does not form a bridge between two atoms of the sugar to form a second ring.

[0069] As used herein, "nucleobase" means an unmodified nucleobase or a modified nucleobase. As used herein, an "unmodified nucleobase" is adenine (A), thymine (T), cytosine (C), uracil (U), or guanine (G). As used herein, a "modified nucleobase" is a group of atoms other than unmodified A, T, C, U, or G capable of pairing with at least one unmodified nucleobase or modified nucleobase. A "5-methylcytosine" or "mC" is a modified nucleobase. A universal base is a modified nucleobase that can pair with any one of the five unmodified nucleobases. As used herein, "nucleobase sequence" means the order of contiguous nucleobases in a nucleic acid or oligonucleotide independent of any sugar or internucleoside linkage modification.

[0070] In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising an unmodified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising a modified nucleobase. In certain embodiments,

modified oligonucleotides comprise one or more nucleoside that does not comprise a nucleobase, referred to as an abasic nucleoside.

[0071] In certain embodiments, modified nucleobases are selected from: 5-substituted pyrimidines, 6-azapyrimidines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6 substituted purines. In certain embodiments, modified nucleobases are selected from: 2-aminopropyladenine, 5-hydroxymethylcytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-Nmethylguanine, 6-N-methyladenine, 2-propyladenine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl (-C≡C-CH_3) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly 5-bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N-benzoyladenine, 2-N-isobutyrylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-benzoylcytosine, 5-methyl 4-N-benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. Further modified nucleobases include tricyclic pyrimidines, such as 1,3-diazaphenoxazine-2-one, 1,3-diazaphenothiazine-2-one and 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2-one (G-clamp). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in U.S. Patent No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, Kroschwitz, J. I., Ed., John Wiley & Sons, 1990, 858-859; Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613; Sanghvi, Y. S., Chapter 15, Antisense Research and Applications,

Crooke, S. T. and Lebleu, B., Eds., CRC Press, 1993, 273-288; and those disclosed in Chapters 6 and 15, Antisense Drug Technology, Crooke S. T., Ed., CRC Press, 2008, 163-166 and 442-443.

[0072] As used herein, "nucleoside" means a compound comprising a nucleobase and a sugar moiety. The nucleobase and sugar moiety are each, independently, unmodified or modified. As used herein, "modified nucleoside" means a nucleoside comprising a modified nucleobase and/or a modified sugar moiety. Modified nucleosides include abasic nucleosides, which lack a nucleobase. "Linked nucleosides" are nucleosides that are connected in a continuous sequence (i.e., no additional nucleosides are presented between those that are linked).

[0073] As used herein, "oligomeric compound" means an oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group. An oligomeric compound may be paired with a second oligomeric compound that is complementary to the first oligomeric compound or may be unpaired. A "singled stranded oligomeric compound" is an unpaired oligomeric compound. The term "oligomeric duplex" means a duplex formed by two oligomeric compounds having complementary nucleobase sequences. Each oligomeric compound of an oligomeric duplex may be referred to as a "duplexed oligomeric compound."

[0074] As used herein, "oligonucleotide" means a strand of linked nucleosides connected via internucleoside linkages, wherein each nucleoside and internucleoside linkage may be modified or unmodified. The internucleoside linkages may be any described herein. Unless otherwise indicated, oligonucleotides consist of 8-50 linked nucleosides. As used herein, "modified oligonucleotide" means an oligonucleotide, wherein at least one nucleoside or internucleoside linkage is modified. As used herein, "unmodified oligonucleotide" means an oligonucleotide that does not comprise any nucleoside modifications or internucleoside modifications.

[0075] "*UNC13A*," belongs to a family of genes originally discovered in C. elegans and was named based on the uncoordinated (unc) movements exhibited by animals with mutations in these genes, owing to deficits in neurotransmitter release. *UNC13A* encodes a large multidomain protein expressed in the nervous system, where it localizes to neuromuscular junctions and plays an essential role in the vesicle priming step, prior to synaptic vesicle fusion. Variants within the *UNC13A* gene have been known to increase risk of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), two related neurodegenerative diseases defined by mislocalization of the RNA-binding protein TDP-43.

[0076] As used herein, "sugar moiety" means an unmodified sugar moiety or a modified sugar moiety. The superscript prime symbol (') is used to describe the numbering of a sugar in a nucleoside or nucleotide (the nucleobase positions are numbered without the prime). When describing the sugar only, the prime symbol is not used. As used herein, "unmodified sugar moiety" means a 2-OH(H) furanosyl moiety, as found in RNA (an "unmodified RNA sugar moiety"), or a 2-H(H) moiety, as found in DNA (an "unmodified DNA sugar moiety"). Unmodified sugar moieties have one hydrogen at each of the 1, 3, and 4 positions, an oxygen at the 3 position, and two hydrogens at the 5 position. As used herein, "modified sugar moiety" or "modified sugar" means a modified furanosyl sugar moiety or a sugar surrogate. As used herein, modified furanosyl sugar moiety means a furanosyl sugar comprising a non-hydrogen substituent in place of at least one hydrogen of an unmodified sugar moiety. In certain embodiments, a modified furanosyl sugar moiety is a 2-substituted sugar moiety. Such modified furanosyl sugar moieties include bicyclic sugars and nonbicyclic sugars.

[0077] In certain embodiments, modified sugar moieties are nonbicyclic modified sugar moieties comprising a furanosyl ring with one or more substituent groups none of which bridges

two atoms of the furanosyl ring to form a bicyclic structure. Such non bridging substituents may be at any position of the furanosyl, including but not limited to substituents at the 2, 4, and/or 5 positions. In certain embodiments one or more non-bridging substituent of nonbicyclic modified sugar moieties is branched. Examples of 2-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to: 2-F, 2-OCH_3 ("OMe" or "O-methyl"), and 2-$O(CH_2)_2OCH_3$ ("MOE"). In certain embodiments, 2-substituent groups are selected from among: halo, allyl, amino, azido, SH, CN, OCN, CF_3, OCF_3, O-C_{1-10} alkoxy, O-C_{1-10} substituted alkoxy, O-C_{1-10} alkyl, O-C_{1-10} substituted alkyl, S-alkyl, N(R_m)-alkyl, O-alkenyl, S-alkenyl, N(R_m)-alkenyl, O-alkynyl, S-alkynyl, N(Rm)-alkynyl, O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$ or $OCH_2C(=O)$-$N(R_m)(R_n)$, where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_{1-10} alkyl, and the 2-substituent groups can be further substituted with one or more substituent groups independently selected from among: hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO_2), thiol, thioalkoxy, thioalkyl, halogen, alkyl, aryl, alkenyl and alkynyl. Examples of 4'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to alkoxy (e.g., methoxy), and alkyl. Examples of 5-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to: 5-methyl (R or S), 5-vinyl, and 5-methoxy. In certain embodiments, non-bicyclic modified sugar moieties comprise more than one non-bridging sugar substituent, for example, 2-F-5-methyl sugar moieties and the like.

[0078] In certain embodiments, a 2'-substituted non-bicyclic modified nucleoside comprises a sugar moiety comprising a nonbridging 2'-substituent group selected from: F, NH_2, N_3, OCF_3, OCH_3, $O(CH_2)_3NH_2$, $CH_2CH{=}CH_2$, $OCH_2CH{=}CH_2$, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$,

$O(CH_2)_2ON(R_m)(R_n)$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and N-substituted acetamide ($OCH_2C(=O)$-$N(R_m)(R_n)$), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_{1-10} alkyl.

[0079] In certain embodiments, a 2'-substituted nucleoside nonbicyclic modified nucleoside comprises a sugar moiety comprising a non-bridging 2'-substituent group selected from: F, OCF_3, OCH_3, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(CH_3)_2$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and $OCH_2C(=O)$-$N(H)CH_3$ ("NMA").

[0080] In certain embodiments, a 2'-substituted non-bicyclic modified nucleoside comprises a sugar moiety comprising a nonbridging 2'-substituent group selected from: F, OCH_3, and $OCH_2CH_2OCH_3$.

[0081] Certain modified sugar moieties comprise a substituent that bridges two atoms of the furanosyl ring to form a second ring, resulting in a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety comprises a bridge between the 4 and the 2 furanose ring atoms. Examples of such 4 to 2 bridging sugar substituents include but are not limited to: 4-CH_2-2, 4-$(CH_2)_2$-2, 4-$(CH_2)_3$-2, 4-CH_2-O-2 ("LNA"), 4-CH_2-S-2, 4-(CH_2)2-O-2 ("ENA"), 4-$CH(CH_3)$-O-2 (referred to as "constrained ethyl" or "cEt"), 4-CH_2-O-CH_2-2, 4-CH_2-N(R)-2, 4-$CH(CH_2OCH_3)$-O-2 ("constrained MOE" or "cMOE") and analogs thereof, 4-$C(CH_3)(CH_3)$-O-2 and analogs thereof, 4-CH_2-$N(OCH_3)$-2 and analogs thereof, 4-CH_2-O-$N(CH_3)$-2, 4-CH_2-$C(H)(CH_3)$-2, 4-CH2-$C(=CH_2)$-2 and analogs thereof, 4-$C(R_aR_b)$-N(R)-O-2, 4-$C(R_aR_b)$-O-N(R)-2, 4-CH_2-O-N(R)-2, and 4-CH_2-N(R)-O-2, wherein each R, R_a, and R_b, is, independently, H, a protecting group, or C_{1-12} alkyl.

[0082] In certain embodiments, such 4 to 2 bridges independently comprise from 1 to 4 linked groups independently selected from: -$[C(R_a)(R_b)]_n$-, -$[C(R_a)(R_b)]_n$-O-, -$C(R_a)=C(R_b)$-, -$C(R_a)=N$-,

-C(=NRa)-, -C(=O)-, -C(=S)-, -O-, -Si(Ra)2-, -S(=O)x-, and - N(Ra)-; wherein: x is 0, 1, or 2; n is 1, 2, 3, or 4; each Ra and Rb is, independently, H, a protecting group, hydroxyl, C1-12 alkyl, substituted C1-12 alkyl, C1-12 alkenyl, substituted C2-12 alkenyl, C2-12 alkynyl, substituted C2-12 alkynyl, C5-20 aryl, substituted C5-20 aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C5-7 alicyclic radical, substituted C5-7 alicyclic radical, halogen, OJ1, NJ1J2, SJ1, N3, COOJ1, acyl (C(=O)-H), substituted acyl, CN, sulfonyl (S(=O)2-J1), or sulfoxyl (S(=O)-J1); and each J1 and J2 is, independently, H, C1-12 alkyl, substituted C1-12 alkyl, C2-12 alkenyl, substituted C2-12 alkenyl, C2-12 alkynyl, substituted C2-12 alkynyl, C5-20 aryl, substituted C5-20 aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C1-12 aminoalkyl, substituted C1-12 aminoalkyl, or a protecting group.

[0083] Additional bicyclic sugar moieties are known in the art, see, for example: Freier *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4429-4443, Albaek *et al.*, *J. Org. Chem.*, 2006, 71, 7731-7740, Singh *et al.*, *Chem. Commun.*, 1998, 4, 455-456; Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630; Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222; Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039; Srivastava *et al.*, *J. Am. Chem. Soc.*, 20017, 129, 8362-8379; Wengel et a., U.S. Pat. No. 7,053,207; Imanishi et al., U.S. Pat. No. 6,268,490; Imanishi et al. U.S. Pat. No. 6,770,748; Imanishi et al., U.S. RE44,779; Wengel et al., U.S. Pat. No. 6,794,499; Wengel et al., U.S. Pat. No. 6,670,461; Wengel et al., U.S. Pat. No. 7,034,133; Wengel et al., U.S. Pat. No. 8,080,644; Wengel et al., U.S. Pat. No. 8,034,909; Wengel et al., U.S. Pat. No. 8,153,365; Wengel et al., U.S. Pat. No. 7,572,582; and Ramasamy et al., U.S. Pat. No. 6,525,191; Torsten et al., WO 2004/106356; Wengel et al., WO 1999/014226; Seth et al., WO 2007/134181; Seth et al., U.S. Pat. No. 7,547,684; Seth et al., U.S. Pat. No. 7,666,854; Seth et al., U.S. Pat. No. 8,088,746; Seth et al., U.S. Pat. No. 7,750,131; Seth et al., U.S. Pat. No.

8,030,467; Seth et al., U.S. Pat. No. 8,268,980; Seth et al., U.S. Pat. No. 8,546,556; Seth et al., U.S. Pat. No. 8,530,640; Migawa et al., U.S. Pat. No. 9,012,421; Seth et al., U.S. Pat. No. 8,501,805; and U.S. Patent Publication Nos. Allerson et al., US2008/0039618 and Migawa et al., US2015/0191727.

[0084] "Subject" and "patient" as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (e.g., cow, pig, camel, llama, horse, goat, rabbit, sheep, hamsters, guinea pig, cat, dog, rat, and mouse, a non-human primate (for example, a monkey, such as a cynomolgous or rhesus monkey, chimpanzee, etc.) and a human). In some embodiments, the subject may be a human or a non-human. In a preferred embodiment, the subject or patient is a human. The subject or patient may be undergoing other forms of treatment.

[0085] A "therapeutically effective amount," or "effective dosage" or "effective amount" as used interchangeably herein unless otherwise defined, means a dosage of a drug effective for periods of time necessary, to achieve the desired therapeutic result. An effective dosage may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the drug to elicit a desired response in the individual. This term as used herein may also refer to an amount effective at bringing about a desired in vivo effect in an animal, mammal, or human. A therapeutically effective amount may be administered in one or more administrations (e.g., the agent may be given as a preventative treatment or therapeutically at any stage of disease progression, before or after symptoms, and the like), applications or dosages and is not intended to be limited to a particular formulation, combination or administration route. It is within the scope of the present disclosure that the drug may be administered at various times during the course of treatment of

the subject. The times of administration and dosages used will depend on several factors, such as the goal of treatment (e.g., treating v. preventing), condition of the subject, etc. and can be readily determined by one skilled in the art.

[0086] As used herein, the term "treat" or "treating" a subject, refers to administering a composition or agent described herein to the subject, such that at least one symptom of a disease or disorder is healed, alleviated, relieved, altered, remedied, reduced, ameliorated, or improved. Treating includes administering an amount effective to alleviate, relieve, alter, remedy, reduce, ameliorate, and/or improve one or more symptoms associated with a disease or disorder. The treatment may inhibit deterioration or worsening of a symptom associated with the disease or disorder.

[0087] A hallmark pathological feature of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is the depletion of RNA-binding protein TDP-43 from the nucleus of neurons in the brain and spinal cord to the cytoplasm where it aggregates into insoluble inclusion bodies in more than 95% of ALS cases and about 45% of FTD cases postmortem (Brown, *supra*). A major function of TDP-43 is as a repressor of cryptic exon (between canonical exons 20 and 21) inclusion during RNA splicing. Single nucleotide polymorphisms (SNPs) in UNC13A are among the strongest genome-wide association study (GWAS) hits associated with FTD/ALS in humans (Diekstra *et al.*, *Ann. Neurol.* 76, 120–133, 2014). Evidence has shown that TDP-43 represses a cryptic exon splicing event in UNC13A. (Unlike normal conserved exons, these cryptic exons lurk in introns and are normally excluded from mature mRNAs.) Loss of TDP-43 from the nucleus in the human brain, neuronal cell lines, and iPSC-derived motor neurons resulted in the inclusion of the cryptic exon in UNC13A mRNA and reduced UNC13A protein expression. Rosa Ma et al., "TDP-43 represses cryptic exon

inclusion in FTD/ALS gene *UNC13A*.", https://doi.org/10.1101/2021.04.02.438213, *bioRxiv* (posted Apr. 4, 2021). The top variants associated with FTD/ALS risk in humans are located in the cryptic exon harboring intron itself and it is shown that they increase UNC13A cryptic exon splicing in the face of TDP-43 dysfunction. Data shows that there is a direct functional link between one of the strongest genetic risk factors for FTD/ALS (UNC13A genetic variants) and loss of TDP-43 function.

[0088] According to Rosa Ma, the most significant genetic variants associated with FTD/ALS disease risk are located within the intron harboring the cryptic exon itself. Brain samples from Frontotemporal lobar degeneration (FTLD) with TDP-43 inclusions (FTLD-TDP) patients carrying these SNPs exhibited more UNC13A cryptic exon inclusion than those from FTLD-TDP patients lacking the risk alleles. These risk alleles, according to Rosa Ma, are insufficient to cause cryptic exon inclusion because the cryptic exon is not detected in RNA sequence data from healthy control samples (GTEx) and functional studies indicate that TDP-43 dysfunction is required for UNC13A cryptic exon inclusion. Instead, the UNC13A risk alleles exert a TDP-43 loss-of-function-dependent disease modifying effect. Without being bound by any particular theory, the inventors theorize that increase of UNC13A protein expression is an effective treatment for neurodegenerative diseases, such as ALS and FTD.

[0089] The cryptic exon occurs in two forms distinguishable by their size, between exons 20 and 21 after TDP-43 knockdown. One risk SNP, rs12973192, lies 16 base pairs inside the cryptic exon, and another is located 534 base pairs downstream of the donor splice site of the cryptic exon within the same intron. The risk SNPs increase the amount of cryptic exon inclusion in cortex from ALS and FTD cases in an independent and additive fashion. A recent analysis of UNC13A CE inclusion in bulk RNA-seq data from brain and spinal cord tissues of 377

individuals including ALS, FTD and controls, showed that the UNC13A CE was detected in post-mortem tissues from ALS or FTD patients with TDP-43 pathology (Brown *et al.*, *Nature* 603:131–137, 2022), and not UNC13A risk SNP carriers. Cryptic exon expression mirrored the known tissue distribution of TDP-43 aggregation and clearance: it was specific to ALS spinal cord and motor cortex as well as FTD frontal and temporal cortices, but was absent from the cerebellum in both disease and control.

[0090] The herein described methods of treatment comprises administering to a subject in need thereof a composition comprising an effective amount of one or more antisense oligonucleotides that treats neurological diseases by suppressing, preventing, or inhibiting transcription of the cryptic exon of UNC13A. The one or more antisense oligonucleotides may decrease or inhibit neurodegeneration.

[0091] Restoring UNC13A levels in ALS patients who have TDP-43 pathology may result in extending survival in patients with neurological and neurodegenerative diseases, such as ALS or FTD, for instance by several years. The presently claimed antisense oligonucleotides (ASOs) can be injected directly into the spinal cord and achieve sustained target engagement throughout the central nervous system with minimal peripheral toxicity.

[0092] The disclosure provides oligonucleotides (modified or unmodified) that can be used to modulate UNC13A expression. Table 1 provides (5' to 3') generic sequence of bases for the human UNC13A antisense oligonucleotides or inhibitory nucleic acids of the disclosure.

[0093] UNC13A risk haplotype associated with ALS/FTD susceptibility potentiates cryptic exon inclusion when TDP-43 is dysfunctional. SNPs associated with ALS/FTD in UNC13A include rs12608932 and rs12973192. rs12608932 (A>C) and rs12973192 (C>G), are both located in the same intron that was found to harbor the cryptic exon. FIG. 2 shows the locations

of the ASOs with respect to the cryptic exon, TDP-43 binding site, and relative to the SNPs, rs12608932 and rs12973192.

TABLE 1

SEQ ID NO.	Sequence
1	GAATCTACCCACCAACTCAT
2	AATCTACCCACCAACTCATC
3	ATCTACCCACCAACTCATCC
4	TCTACCCACCAACTCATCCA
5	CTACCCACCAACTCATCCAT
6	TACCCACCAACTCATCCATC
7	ACCCACCAACTCATCCATCT
8	CCCACCAACTCATCCATCTA
9	CCACCAACTCATCCATCTAT
10	CACCAACTCATCCATCTATC
11	ACCAACTCATCCATCTATCC
12	CCAACTCATCCATCTATCCA
13	CAACTCATCCATCTATCCAT
14	AACTCATCCATCTATCCATC
15	ACTCATCCATCTATCCATCC
16	CTCATCCATCTATCCATCCA
17	TCATCCATCTATCCATCCAT
18	CATCCATCTATCCATCCATG
19	ATCCATCTATCCATCCATGT
20	TCCATCTATCCATCCATGTA
21	CCATCTATCCATCCATGTAC
22	CATCTATCCATCCATGTACT
23	ATCTATCCATCCATGTACTC
24	TCTATCCATCCATGTACTCA
25	CTATCCATCCATGTACTCAC
26	TATCCATCCATGTACTCACC
27	ATCCATCCATGTACTCACCC
28	TCCATCCATGTACTCACCCA
29	CCATCCATGTACTCACCCAT
30	CATCCATGTACTCACCCATC

31	ATCCATGTACTCACCCATCT
32	TCCATGTACTCACCCATCTC
33	CCATGTACTCACCCATCTCT
34	CATGTACTCACCCATCTCTC
35	ATGTACTCACCCATCTCTCC
36	TGTACTCACCCATCTCTCCA
37	GTACTCACCCATCTCTCCAT
38	TACTCACCCATCTCTCCATC
39	ACTCACCCATCTCTCCATCC
40	CTCACCCATCTCTCCATCCA
41	TCACCCATCTCTCCATCCAT
42	CACCCATCTCTCCATCCATC
43	ACCCATCTCTCCATCCATCC
44	CCCATCTCTCCATCCATCCT
45	CCATCTCTCCATCCATCCTT
46	CATCTCTCCATCCATCCTTT
47	ATCTCTCCATCCATCCTTTT
48	TCTCTCCATCCATCCTTTTA
49	CTCTCCATCCATCCTTTTAT
50	TCTCCATCCATCCTTTTATC
51	CTCCATCCATCCTTTTATCT
52	CCATCCATCCTTTTATCTAC
53	CTACTCATCACTCATTCATC
54	ACTCATCACTCATTCATCTG
55	CTCATCACTCATTCATCTGT
56	CATCACTCATTCATCTGTTC
57	TCATTCATTCATTCACCAGC
58	CATTCATTCATTCACCAGCA
59	GGATAAGAGTTCTTTCCAGG
60	GATAAGAGTTCTTTCCAGGA
61	TTCCAGGAAACCCAGGCAGC
62	TCCAGGAAACCCAGGCAGCT
63	AGCTGGAAGAGACATACCCA
64	GCTGGAAGAGACATACCCAG
65	CTGGAAGAGACATACCCAGA
66	TGGAAGAGACATACCCAGAC

67	GGAAGAGACATACCCAGACA
68	GAAGAGACATACCCAGACAC
69	AAGAGACATACCCAGACACA
70	AGAGACATACCCAGACACAA
71	GAGACATACCCAGACACAAA
72	AGACATACCCAGACACAAAC
73	GCCCAATCCTGAGTGGTTAG
74	CCCAATCCTGAGTGGTTAGG
75	GGCTGGAATAGAAGGAAGAA
76	GCTGGAATAGAAGGAAGAAC
77	CTGGAATAGAAGGAAGAACC
78	TGGAATAGAAGGAAGAACCT
79	GGAATAGAAGGAAGAACCTG
80	GAATAGAAGGAAGAACCTGA
81	ATAGAAGGAAGAACCTGATG
82	TAGAAGGAAGAACCTGATGA
83	AGAAGGAAGAACCTGATGAT
84	GAAGGAAGAACCTGATGATG
85	AAGGAAGAACCTGATGATGA
86	AGGAAGAACCTGATGATGAG
87	GGAAGAACCTGATGATGAGT
88	GAAGAACCTGATGATGAGTA
89	AAGAACCTGATGATGAGTAG
90	AGAACCTGATGATGAGTAGT
91	GAACCTGATGATGAGTAGTG
92	AACCTGATGATGAGTAGTGA
93	ACCTGATGATGAGTAGTGAG
94	CCTGATGATGAGTAGTGAGA
95	CTGATGATGAGTAGTGAGAG
96	TGATGATGAGTAGTGAGAGT
97	GATGATGAGTAGTGAGAGTC
98	ATGATGAGTAGTGAGAGTCA
99	TGATGAGTAGTGAGAGTCAA
100	GATGAGTAGTGAGAGTCAAC
101	ATGAGTAGTGAGAGTCAACC
102	TGAGTAGTGAGAGTCAACCT

103	GAGTAGTGAGAGTCAACCTG
104	AGTAGTGAGAGTCAACCTGG
105	GTAGTGAGAGTCAACCTGGA
106	TAGTGAGAGTCAACCTGGAG
107	AGTGAGAGTCAACCTGGAGG
108	GTGAGAGTCAACCTGGAGGC
109	TTCCCAGAGGAGGTGACCCT
110	CCAGAGGAGGTGACCCTGAA
111	CAGAGGAGGTGACCCTGAAT
112	AGAGGAGGTGACCCTGAATC
113	GAGGAGGTGACCCTGAATCT
114	AGGAGGTGACCCTGAATCTG
115	GGAGGTGACCCTGAATCTGG
116	GAGGTGACCCTGAATCTGGA
117	AGGTGACCCTGAATCTGGAC
118	GGTGACCCTGAATCTGGACT
119	GTGACCCTGAATCTGGACTT
120	TGACCCTGAATCTGGACTTT
121	GACCCTGAATCTGGACTTTG
122	ACCCTGAATCTGGACTTTGA
123	CCCTGAATCTGGACTTTGAT
124	CCTGAATCTGGACTTTGATG
125	CTGAATCTGGACTTTGATGG
126	TGAATCTGGACTTTGATGGA
127	GAATCTGGACTTTGATGGAT
128	ATCTGGACTTTGATGGATAG
129	TCTGGACTTTGATGGATAGG
130	GGAGGAGTTTTCCAGGTAAA
131	GAGGAGTTTTCCAGGTAAAG
132	AGGAGTTTTCCAGGTAAAGG
133	GCCAGGAGAGTGTGGATGGT
134	CCAGGAGAGTGTGGATGGTG
135	CAGGAGAGTGTGGATGGTGT
136	AGGAGAGTGTGGATGGTGTG
137	GGAGAGTGTGGATGGTGTGG
138	GAGAGTGTGGATGGTGTGGC

139	AATTACCCCCAAATTCACCC
140	ATTACCCCCAAATTCACCCA
141	TTACCCCCAAATTCACCCAT
142	TACCCCCAAATTCACCCATC
143	ACCCCCAAATTCACCCATCC
144	CCCCCAAATTCACCCATCCA
145	CCCCAAATTCACCCATCCAT
146	CCCAAATTCACCCATCCATA
147	CCAAATTCACCCATCCATAC
148	CAAATTCACCCATCCATACA
149	AATTCACCCATCCATACATC
150	ATTCACCCATCCATACATCT
151	TTCACCCATCCATACATCTA
152	TCACCCATCCATACATCTAT
153	CACCCATCCATACATCTATA
154	ACCCATCCATACATCTATAC
155	CCCATCCATACATCTATACT
156	CATATATCCATCCATCTGTC
157	ATATATCCATCCATCTGTCC
158	TATATCCATCCATCTGTCCA
159	ATATCCATCCATCTGTCCAT
160	TATCCATCCATCTGTCCATC
161	ATCCATCCATCTGTCCATCC
162	TCCATCCATCTGTCCATCCA
163	CCATCCATCTGTCCATCCAT
164	CATCCATCTGTCCATCCATC
165	ATCCATCTGTCCATCCATCC
166	TCCATCTGTCCATCCATCCA
167	CCATCTGTCCATCCATCCAT
168	CATCTGTCCATCCATCCATC
169	ATCTGTCCATCCATCCATCA
170	TCTGTCCATCCATCCATCAT
171	CTGTCCATCCATCCATCATC
172	TGTCCATCCATCCATCATCC
173	GTCCATCCATCCATCATCCA
174	TCCATCCATCCATCATCCAT

175	CCATCCATCCATCATCCATC
176	CATCCATCCATCATCCATCT
177	ATCCATCCATCATCCATCTA
178	TCCATCCATCATCCATCTAG
179	CCATCCATCATCCATCTAGC
180	CATCCATCATCCATCTAGCC
181	ATCCATCATCCATCTAGCCA
182	TCCATCATCCATCTAGCCAC
183	GGAGAGAAAGTGTCATGGAG
184	GAGAGAAAGTGTCATGGAGA
185	AGAGAAAGTGTCATGGAGAG
186	GAGAAAGTGTCATGGAGAGT
187	AGAAAGTGTCATGGAGAGTG
188	GAAAGTGTCATGGAGAGTGC
189	GGCAGCTTACATCATCCATC
190	GCAGCTTACATCATCCATCT
191	CAGCTTACATCATCCATCTG
192	AGCTTACATCATCCATCTGC
193	GCTTACATCATCCATCTGCC
194	CTTACATCATCCATCTGCCT
195	TTACATCATCCATCTGCCTG
196	TACATCATCCATCTGCCTGT
197	ACATCATCCATCTGCCTGTT
198	CATCATCCATCTGCCTGTTT
199	ATCATCCATCTGCCTGTTTA
200	TCATCCATCTGCCTGTTTAT
201	CATCCATCTGCCTGTTTATT
202	ATCCATCTGCCTGTTTATTC
203	TCCATCTGCCTGTTTATTCA
204	CCATCTGCCTGTTTATTCAT
205	CTACTCTTTTATCCATCCAC
206	ACTCTTTTATCCATCCACAC
207	CTCTTTTATCCATCCACACA
208	TCTTTTATCCATCCACACAC
209	CTTTTATCCATCCACACACC
210	TTTTATCCATCCACACACCC

211	TTTATCCATCCACACACCCA
212	TTATCCATCCACACACCCAC
213	TATCCATCCACACACCCACC
214	ATCCATCCACACACCCACCC
215	TCCATCCACACACCCACCCA
216	CCATCCACACACCCACCCAT
217	CATCCACACACCCACCCATC
218	ATCCACACACCCACCCATCT
219	TCCACACACCCACCCATCTA
220	CCACACACCCACCCATCTAA
221	CACACACCCACCCATCTAAC
222	ACACACCCACCCATCTAACT
223	CACACCCACCCATCTAACTA
224	ACACCCACCCATCTAACTAC
225	CACCCACCCATCTAACTACC
226	ACCCACCCATCTAACTACCC
227	CCCACCCATCTAACTACCCC
228	CCACCCATCTAACTACCCCA
229	CACCCATCTAACTACCCCAA
230	ACCCATCTAACTACCCCAAA
231	CCCATCTAACTACCCCAAAT
232	CCATCTAACTACCCCAAATT
233	AATTTCACCCATCCACTCTT
234	ATTTCACCCATCCACTCTTC
235	TTTCACCCATCCACTCTTCC
236	TTCACCCATCCACTCTTCCA
237	TCACCCATCCACTCTTCCAA
238	CACCCATCCACTCTTCCAAC
239	ACCCATCCACTCTTCCAACC
240	CCCATCCACTCTTCCAACCT
241	CCATCCACTCTTCCAACCTT
242	CATCCACTCTTCCAACCTTT
243	ATCCACTCTTCCAACCTTTC
244	TCCACTCTTCCAACCTTTCA
245	CCACTCTTCCAACCTTTCAG
246	CACTCTTCCAACCTTTCAGT

247	ACTCTTCCAACCTTTCAGTA
248	CTCTTCCAACCTTTCAGTAA
249	CCTTTCAGTAATTCAACCAC
250	CAGTAATTCAACCACACATC
251	AGTAATTCAACCACACATCC
252	GTAATTCAACCACACATCCA
253	AATTCAACCACACATCCATC
254	ATTCAACCACACATCCATCC
255	TTCAACCACACATCCATCCA
256	TCAACCACACATCCATCCAT
257	CAACCACACATCCATCCATC
258	AACCACACATCCATCCATCC
259	ACCACACATCCATCCATCCA
260	CCACACATCCATCCATCCAT
261	CACACATCCATCCATCCATC
262	ACACATCCATCCATCCATCC
263	CACATCCATCCATCCATCCA
264	ACATCCATCCATCCATCCAT
265	CATCCATCCATCCATCCATT
266	ATCCATCCATCCATCCATTC
267	TCCATCCATCCATCCATTCA
268	CCATCCATCCATCCATTCAT
269	CATCCATCCATCCATTCATC
270	ATCCATCCATCCATTCATCC
271	TCCATCCATCCATTCATCCA
272	CCATCCATCCATTCATCCAT
273	CATCCATCCATTCATCCATC
274	ATCCATCCATTCATCCATCC
275	TCCATCCATTCATCCATCCC
276	CCATCCATTCATCCATCCCA
277	CATCCATTCATCCATCCCAT
278	ATCCATTCATCCATCCCATA
279	TCCATTCATCCATCCCATAC
280	CCATTCATCCATCCCATACA
281	CATTCATCCATCCCATACAT
282	TTCATCCATCCCATACATTG

283	TCATCCATCCCATACATTGA
284	CATCCATCCCATACATTGAT
285	ATCCATCCCATACATTGATC
286	TCCATCCCATACATTGATCC
287	GCAACTTAATCCACCTACCC
288	CAACTTAATCCACCTACCCA
289	AACTTAATCCACCTACCCAA
290	ACTTAATCCACCTACCCAAT
291	CTTAATCCACCTACCCAATC
292	TTAATCCACCTACCCAATCA
293	TAATCCACCTACCCAATCAT
294	AATCCACCTACCCAATCATT
295	ATCCACCTACCCAATCATTC
296	TCCACCTACCCAATCATTCA
297	CCACCTACCCAATCATTCAT
298	CACCTACCCAATCATTCATT
299	ACCTACCCAATCATTCATTC
300	CCTACCCAATCATTCATTCT
301	CTTTCATACAACCAACCATC
302	TTTCATACAACCAACCATCC
303	TTCATACAACCAACCATCCA
304	TCATACAACCAACCATCCAT
305	CATACAACCAACCATCCATC
306	ATACAACCAACCATCCATCC
307	TACAACCAACCATCCATCCA
308	ACAACCAACCATCCATCCAC
309	CAACCAACCATCCATCCACC
310	AACCAACCATCCATCCACCC
311	ACCAACCATCCATCCACCCA
312	CCAACCATCCATCCACCCAT
313	CAACCATCCATCCACCCATC
314	AACCATCCATCCACCCATCA
315	ACCATCCATCCACCCATCAA
316	CCATCCATCCACCCATCAAT
317	CATCCATCCACCCATCAATT
318	ATCCATCCACCCATCAATTT

319	TCCATCCACCCATCAATTTA
320	CCATCCACCCATCAATTTAT
321	CATCCACCCATCAATTTATC
322	ATCCACCCATCAATTTATCC
323	TCCACCCATCAATTTATCCA
324	CCACCCATCAATTTATCCAA
325	CACCCATCAATTTATCCAAC
326	ACCCATCAATTTATCCAACC
327	CCCATCAATTTATCCAACCA
328	ATCCAACCATCCATTTTTCG
329	TCCAACCATCCATTTTTCGT
330	CCAACCATCCATTTTTCGTC
331	CAACCATCCATTTTTCGTCT
332	AACCATCCATTTTTCGTCTG
333	ACCATCCATTTTTCGTCTGT
334	CCATCCATTTTTCGTCTGTC
335	CATCCATTTTTCGTCTGTCC
336	ATCCATTTTTCGTCTGTCCA
337	TCCATTTTTCGTCTGTCCAC
338	CCATTTTTCGTCTGTCCACC
339	CATTTTTCGTCTGTCCACCA
340	ATTTTTCGTCTGTCCACCAG
341	TTTTTCGTCTGTCCACCAGC
342	TTTTCGTCTGTCCACCAGCC
343	TTTCGTCTGTCCACCAGCCA
344	TTCGTCTGTCCACCAGCCAC
345	TCGTCTGTCCACCAGCCACT
346	GTCTGTCCACCAGCCACTCA
347	TCTGTCCACCAGCCACTCAC
348	CTGTCCACCAGCCACTCACA
349	TGTCCACCAGCCACTCACAA
350	GTCCACCAGCCACTCACAAC
351	TCCACCAGCCACTCACAACC
352	CCACCAGCCACTCACAACCA
353	CACCAGCCACTCACAACCAT
354	ACCAGCCACTCACAACCATC

355	CCAGCCACTCACAACCATCC
356	CAGCCACTCACAACCATCCA
357	AGCCACTCACAACCATCCAT
358	GCCACTCACAACCATCCATC
359	CCACTCACAACCATCCATCT
360	CACTCACAACCATCCATCTA
361	ACTCACAACCATCCATCTAA
362	CTCACAACCATCCATCTAAA
363	GCAATAGTTCAACCACACAT
364	CAATAGTTCAACCACACATC
365	AATAGTTCAACCACACATCC
366	ATAGTTCAACCACACATCCT
367	TAGTTCAACCACACATCCTT
368	AGTTCAACCACACATCCTTC
369	GTTCAACCACACATCCTTCC
370	TTCAACCACACATCCTTCCA
371	TCAACCACACATCCTTCCAT
372	CAACCACACATCCTTCCATT
373	AACCACACATCCTTCCATTC
374	ACCACACATCCTTCCATTCA
375	CCACACATCCTTCCATTCAT
376	CACACATCCTTCCATTCATC
377	ACACATCCTTCCATTCATCC
378	CACATCCTTCCATTCATCCA
379	ACATCCTTCCATTCATCCAC
380	CATCCTTCCATTCATCCACC
381	ATCCTTCCATTCATCCACCC
382	TCCTTCCATTCATCCACCCA
383	CCTTCCATTCATCCACCCAC
384	CTTCCATTCATCCACCCACC
385	TTCCATTCATCCACCCACCC
386	TCCATTCATCCACCCACCCA
387	CATTCATCCACCCACCCATT
388	ATTCATCCACCCACCCATTC
389	TTCATCCACCCACCCATTCA
390	TCATCCACCCACCCATTCAT

391	CATCCACCCACCCATTCATC
392	ATCCACCCACCCATTCATCC
393	TCCACCCACCCATTCATCCA
394	CCACCCACCCATTCATCCAT
395	CACCCACCCATTCATCCATT
396	ACCCACCCATTCATCCATTT
397	CCCACCCATTCATCCATTTG
398	CCACCCATTCATCCATTTGT
399	CACCCATTCATCCATTTGTC
400	ACCCATTCATCCATTTGTCC
401	CCATTCATCCATTTGTCCAT
402	CATTCATCCATTTGTCCATC
403	TTCATCCATTTGTCCATCTG
404	TCATCCATTTGTCCATCTGC
405	CATCCATTTGTCCATCTGCC
406	ATCCATTTGTCCATCTGCCT
407	TCCATTTGTCCATCTGCCTA
408	CCATTTGTCCATCTGCCTAT
409	CATTTGTCCATCTGCCTATA
410	ATTTGTCCATCTGCCTATAC
411	TTTGTCCATCTGCCTATACA
412	TTGTCCATCTGCCTATACAT
413	TGTCCATCTGCCTATACATC
414	GTCCATCTGCCTATACATCC
415	TCCATCTGCCTATACATCCA
416	CCATCTGCCTATACATCCAT
417	CATCTGCCTATACATCCATC
418	ATCTGCCTATACATCCATCC
419	TCTGCCTATACATCCATCCA
420	CTGCCTATACATCCATCCAT
421	TGCCTATACATCCATCCATC
422	GCCTATACATCCATCCATCC
423	CCTATACATCCATCCATCCA
424	CTATACATCCATCCATCCAT
425	TATACATCCATCCATCCATC
426	ATACATCCATCCATCCATCC

427	TACATCCATCCATCCATCCA
428	ACATCCATCCATCCATCCAT
429	CATCCATCCATCCATCCATC
430	ATCCATCCATCCATCCATCC
431	TCCATCCATCCATCCATCCA
432	CCATCCATCCATCCATCCAT
433	CATCCATCCATCCATCCATC
434	ATCCATCCATCCATCCATCT
435	TCCATCCATCCATCCATCTA
436	CCATCCATCCATCCATCTAC
437	CATCCATCCATCCATCTACC
438	ATCCATCCATCCATCTACCT
439	TCCATCCATCCATCTACCTA
440	CCATCCATCCATCTACCTAT
441	CATCCATCCATCTACCTATC
442	ATCCATCCATCTACCTATCT
443	TCCATCCATCTACCTATCTA
444	CCATCCATCTACCTATCTAC
445	CATCCATCTACCTATCTACC
446	ATCCATCTACCTATCTACCC
447	TCCATCTACCTATCTACCCA
448	CCATCTACCTATCTACCCAT
449	CATCTACCTATCTACCCATC
450	ATCTACCTATCTACCCATCT
451	TCTACCTATCTACCCATCTG
452	CTACCTATCTACCCATCTGA
453	TACCTATCTACCCATCTGAC
454	ACCTATCTACCCATCTGACT
455	CCTATCTACCCATCTGACTA
456	CTATCTACCCATCTGACTAT
457	TATCTACCCATCTGACTATC
458	ATCTACCCATCTGACTATCA
459	TCTACCCATCTGACTATCAA
460	CTACCCATCTGACTATCAAC
461	TACCCATCTGACTATCAACA
462	ACCCATCTGACTATCAACAA

463	CCCATCTGACTATCAACAAA
464	CACCTATCTACTCAATCTTC
465	ACCTATCTACTCAATCTTCC
466	CCTATCTACTCAATCTTCCT
467	CCTTCTAATAACTCAACCAC
468	AATAACTCAACCACACTTCC
469	ATAACTCAACCACACTTCCA
470	TAACTCAACCACACTTCCAT
471	AACTCAACCACACTTCCATC
472	ACTCAACCACACTTCCATCC
473	CTCAACCACACTTCCATCCA
474	TCAACCACACTTCCATCCAT
475	CAACCACACTTCCATCCATC
476	AACCACACTTCCATCCATCC
477	ACCACACTTCCATCCATCCC
478	CCACACTTCCATCCATCCCA
479	CACACTTCCATCCATCCCAT
480	ACACTTCCATCCATCCCATC
481	CACTTCCATCCATCCCATCC
482	ACTTCCATCCATCCCATCCA
483	CTTCCATCCATCCCATCCAA
484	TTCCATCCATCCCATCCAAT
485	TCCATCCATCCCATCCAATA
486	CCATCCATCCCATCCAATAC
487	CATCCATCCCATCCAATACA
488	ATCCATCCCATCCAATACAA
489	TCCATCCCATCCAATACAAC
490	CCATCCCATCCAATACAACT
491	CATCCCATCCAATACAACTT
492	ACAACTTAATCTGCTCATCC
493	CAACTTAATCTGCTCATCCA
494	ACTTAATCTGCTCATCCAAC
495	CTTAATCTGCTCATCCAACA
496	ATCTGCTCATCCAACATTTC
497	TCTGCTCATCCAACATTTCA
498	CTGCTCATCCAACATTTCAT

499	TGCTCATCCAACATTTCATC
500	GCTCATCCAACATTTCATCT
501	CCAACATTTCATCTATCCAC
502	CAACATTTCATCTATCCACC
503	AACATTTCATCTATCCACCC
504	ACATTTCATCTATCCACCCA
505	CATTTCATCTATCCACCCAG
506	ATTTCATCTATCCACCCAGT
507	TTTCATCTATCCACCCAGTC
508	TTCATCTATCCACCCAGTCA
509	TCATCTATCCACCCAGTCAA
510	CATCTATCCACCCAGTCAAT
511	ATCTATCCACCCAGTCAATC
512	TCTATCCACCCAGTCAATCA
513	CTATCCACCCAGTCAATCAT
514	TATCCACCCAGTCAATCATC
515	ATCCACCCAGTCAATCATCT
516	TCCACCCAGTCAATCATCTA
517	CCACCCAGTCAATCATCTAT
518	CACCCAGTCAATCATCTATC
519	ACCCAGTCAATCATCTATCC
520	CCCAGTCAATCATCTATCCA
521	CCAGTCAATCATCTATCCAG
522	CAGTCAATCATCTATCCAGC
523	AGTCAATCATCTATCCAGCA
524	GTCAATCATCTATCCAGCAA
525	CAATCATCTATCCAGCAATC
526	CATCTATCCAGCAATCTATC
527	ATCCAGCAATCTATCTATCC
528	TCCAGCAATCTATCTATCCA
529	CCAGCAATCTATCTATCCAC
530	CAGCAATCTATCTATCCACT
531	AGCAATCTATCTATCCACTC
532	GCAATCTATCTATCCACTCA
533	CTATCTATCCACTCATCAAG
534	ATCCACTCATCAAGTTATCC

535	TCCACTCATCAAGTTATCCA
536	CCACTCATCAAGTTATCCAT
537	CACTCATCAAGTTATCCATC
538	ACTCATCAAGTTATCCATCC
539	CTCATCAAGTTATCCATCCA
540	CATCAAGTTATCCATCCATC
541	CCATCATCTAACAATTACCC
542	CATCATCTAACAATTACCCC
543	ATCATCTAACAATTACCCCC
544	TCATCTAACAATTACCCCCA
545	CATCTAACAATTACCCCCAA
546	ACAATTACCCCCAAATTCAC
547	CAATTACCCCCAAATTCACC
548	CCATCCCATACATTGATCCG
549	CATCCCATACATTGATCCGC
550	ATCCCATACATTGATCCGCA
551	TCCCATACATTGATCCGCAA
552	CCCATACATTGATCCGCAAC
553	CCATACATTGATCCGCAACT
554	CATACATTGATCCGCAACTT
555	CATTGATCCGCAACTTAATC
556	ATTGATCCGCAACTTAATCC
557	TTGATCCGCAACTTAATCCA
558	TGATCCGCAACTTAATCCAC
559	GATCCGCAACTTAATCCACC
560	ATCCGCAACTTAATCCACCT
561	TCCGCAACTTAATCCACCTA
562	CCGCAACTTAATCCACCTAC
563	CGCAACTTAATCCACCTACC
564	CCATTCATCCACCCACCCAT
565	CCCATTCATCCATTTGTCCA
566	CCATCATCCATCTAGCCACG
567	CATCATCCATCTAGCCACGA
568	ATCATCCATCTAGCCACGAA
569	TCATCCATCTAGCCACGAAT
570	CATCCATCTAGCCACGAATC

571	ATCCATCTAGCCACGAATCT
572	TCCATCTAGCCACGAATCTA
573	CCATCTAGCCACGAATCTAC
574	CATCTAGCCACGAATCTACC
575	ATCTAGCCACGAATCTACCC
576	TCTAGCCACGAATCTACCCA
577	CTAGCCACGAATCTACCCAC
578	TAGCCACGAATCTACCCACC
579	AGCCACGAATCTACCCACCA
580	GCCACGAATCTACCCACCAA
581	CCACGAATCTACCCACCAAC
582	CACGAATCTACCCACCAACT
583	ACGAATCTACCCACCAACTC
584	CGAATCTACCCACCAACTCA
585	GACATACCCAGACACAAACG
586	ACATACCCAGACACAAACGG
587	CATACCCAGACACAAACGGC
588	GCCAGAAAGAGGAAGAGCTG
589	CCAGAAAGAGGAAGAGCTGG
590	GGCAGGCAGGAATGGTGAGT
591	GCAGGCAGGAATGGTGAGTG
592	CAGGCAGGAATGGTGAGTGG
593	AGGCAGGAATGGTGAGTGGA
594	GGCAGGAATGGTGAGTGGAA
595	GCAGGAATGGTGAGTGGAAG
596	CAGGAATGGTGAGTGGAAGT
597	AGGAATGGTGAGTGGAAGTG
598	GGAATGGTGAGTGGAAGTGG
599	GAATGGTGAGTGGAAGTGGC
600	AATGGTGAGTGGAAGTGGCA
601	ATGGTGAGTGGAAGTGGCAT
602	TGGTGAGTGGAAGTGGCATG
603	GGTGAGTGGAAGTGGCATGG
604	TCATTCATCTGT
605	CTCATTCATCTG
606	ACTCATTCATCT

607	CACTCATTCATC
608	TCACTCATTCAT
609	ATCACTCATTCA
610	CATCACTCATTC
611	TCATCACTCATT
612	CTCATCACTCAT
613	CACTCATTCATCTGT
614	TCACTCATTCATCTG
615	ATCACTCATTCATCT
616	CATCACTCATTCATC
617	TCATCACTCATTCAT
618	CTCATCACTCATTCA
619	TCACTCATTCATCTGT
620	ATCACTCATTCATCTG
621	CATCACTCATTCATCT
622	TCATCACTCATTCATC
623	CTCATCACTCATTCAT
624	ATCACTCATTCATCTGT
625	CATCACTCATTCATCTG
626	TCATCACTCATTCATCT
627	CTCATCACTCATTCATC
628	CATCACTCATTCATCTGT
629	TCATCACTCATTCATCTG
630	CTCATCACTCATTCATCT
631	TCATCACTCATTCATCTGT
632	CTCATCACTCATTCATCTG
633	CTCATCACTCATTCATCTGTT
634	ACTCATCACTCATTCATCTGT
635	CTCATCACTCATTCATCTGTTC
636	ACTCATCACTCATTCATCTGTT
637	TACTCATCACTCATTCATCTGT
638	CTCATCACTCATTCATCTGTTCA
639	ACTCATCACTCATTCATCTGTTC
640	TACTCATCACTCATTCATCTGTT
641	CTACTCATCACTCATTCATCTGT

[0094] As shown in FIGs. 4A-4F, ASO 55 (SEQ ID. NO. 55) variations listed above are alternately labeled as below, to be more clearly identifiable as variations of ASO 55:

SEQ ID NO.	Alternate Identifier
604	hUNC13A-ASO55_12-1
605	hUNC13A-ASO55_12-2
606	hUNC13A-ASO55_12-3
607	hUNC13A-ASO55_12-4
608	hUNC13A-ASO55_12-5
609	hUNC13A-ASO55_12-6
610	hUNC13A-ASO55_12-7
611	hUNC13A-ASO55_12-8
612	hUNC13A-ASO55_12-9
613	hUNC13A-ASO55_15-1
614	hUNC13A-ASO55_15-2
615	hUNC13A-ASO55_15-3
616	hUNC13A-ASO55_15-4
617	hUNC13A-ASO55_15-5
618	hUNC13A-ASO55_15-6
619	hUNC13A-ASO55_16-1
620	hUNC13A-ASO55_16-2
621	hUNC13A-ASO55_16-3
622	hUNC13A-ASO55_16-4
623	hUNC13A-ASO55_16-5
624	hUNC13A-ASO55_17-1
625	hUNC13A-ASO55_17-2
626	hUNC13A-ASO55_17-3
627	hUNC13A-ASO55_17-4
628	hUNC13A-ASO55_18-1
629	hUNC13A-ASO55_18-2
630	hUNC13A-ASO55_18-3
631	hUNC13A-ASO55_19-1
632	hUNC13A-ASO55_19-2
633	hUNC13A-ASO55_21-1
634	hUNC13A-ASO55_21-2
635	hUNC13A-ASO55_22-1

636	hUNC13A-ASO55_22-2
637	hUNC13A-ASO55_22-3
638	hUNC13A-ASO55_23-1
639	hUNC13A-ASO55_23-2
640	hUNC13A-ASO55_23-3
641	hUNC13A-ASO55_23-4

[0095] In one embodiment, the disclosure provides modified oligonucleotides consisting of 12-30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11 at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19 or at least 20 consecutive nucleotide bases of any of the nucleobase sequences of SEQ ID NO:1-641 in Table 1. In some embodiments, the modified oligonucleotide is at least 80% to 100% (i.e., 80%, 82%, 84%, 86%, 88%, 90%, 92%, 94%, 96%, 98% or 100%; or any numerical range or value between any of the foregoing values) identical to any of the sequences comprising or consisting of SEQ ID NO:1-641. The sequences provided in Table 1 can be used to design antisense molecules for inhibition of UNC13A cryptic exon expression.

[0096] In some embodiments, the oligonucleotide is single stranded. In some embodiments the oligonucleotide comprises or is complexed with a moiety that neutralizes charge on the oligonucleotide to promote uptake and transfer across a cell membrane.

[0097] In another embodiment, each of the ASOs in Table 1 has a motif where each nucleobase has a 2'-OCH_2CH_2-OCH_3 group (i.e., 2'-MOE) and each internucleoside linkage is a phosphorothioate linkage. This would be the motif: 2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE*2MOE where (i) 2MOE is a nucleobase with a 2'-OCH_2CH_2-OCH_3 group (i.e., 2'-MOE), and (ii) the asterisk (*) refers to a phosphorothioate linkage. Table 2 below shows this motif.

[0098] Table 2: The Sequence of Bases in UNC13A Antisense Oligonucleotides (ASOs). Capital letters are 2'-methoxyethylribose nucleosides; lower case are DNA nucleosides; asterisks (*) are phosphorothioate linkages; linkages which do not have an asterisk are phosphodiester linkages)

TABLE 2

SEQ ID NO.	Sequence
642	G*A*A*T*C*T*A*C*C*C*A*C*C*A*A*C*T*C*A*T
643	A*A*T*C*T*A*C*C*C*A*C*C*A*A*C*T*C*A*T*C
644	A*T*C*T*A*C*C*C*A*C*C*A*A*C*T*C*A*T*C*C
645	T*C*T*A*C*C*C*A*C*C*A*A*C*T*C*A*T*C*C*A
646	C*T*A*C*C*C*A*C*C*A*A*C*T*C*A*T*C*C*A*T
647	T*A*C*C*C*A*C*C*A*A*C*T*C*A*T*C*C*A*T*C
648	A*C*C*C*A*C*C*A*A*C*T*C*A*T*C*C*A*T*C*T
649	C*C*C*A*C*C*A*A*C*T*C*A*T*C*C*A*T*C*T*A
650	C*C*A*C*C*A*A*C*T*C*A*T*C*C*A*T*C*T*A*T
651	C*A*C*C*A*A*C*T*C*A*T*C*C*A*T*C*T*A*T*C
652	A*C*C*A*A*C*T*C*A*T*C*C*A*T*C*T*A*T*C*C
653	C*C*A*A*C*T*C*A*T*C*C*A*T*C*T*A*T*C*C*A
654	C*A*A*C*T*C*A*T*C*C*A*T*C*T*A*T*C*C*A*T
655	A*A*C*T*C*A*T*C*C*A*T*C*T*A*T*C*C*A*T*C
656	A*C*T*C*A*T*C*C*A*T*C*T*A*T*C*C*A*T*C*C
657	C*T*C*A*T*C*C*A*T*C*T*A*T*C*C*A*T*C*C*A
658	T*C*A*T*C*C*A*T*C*T*A*T*C*C*A*T*C*C*A*T
659	C*A*T*C*C*A*T*C*T*A*T*C*C*A*T*C*C*A*T*G
660	A*T*C*C*A*T*C*T*A*T*C*C*A*T*C*C*A*T*G*T
661	T*C*C*A*T*C*T*A*T*C*C*A*T*C*C*A*T*G*T*A
662	C*C*A*T*C*T*A*T*C*C*A*T*C*C*A*T*G*T*A*C
663	C*A*T*C*T*A*T*C*C*A*T*C*C*A*T*G*T*A*C*T
664	A*T*C*T*A*T*C*C*A*T*C*C*A*T*G*T*A*C*T*C
665	T*C*T*A*T*C*C*A*T*C*C*A*T*G*T*A*C*T*C*A
666	C*T*A*T*C*C*A*T*C*C*A*T*G*T*A*C*T*C*A*C
667	T*A*T*C*C*A*T*C*C*A*T*G*T*A*C*T*C*A*C*C
668	A*T*C*C*A*T*C*C*A*T*G*T*A*C*T*C*A*C*C*C

669	T*C*C*A*T*C*C*A*T*G*T*A*C*T*C*A*C*C*C*A
670	C*C*A*T*C*C*A*T*G*T*A*C*T*C*A*C*C*C*A*T
671	C*A*T*C*C*A*T*G*T*A*C*T*C*A*C*C*C*A*T*C
672	A*T*C*C*A*T*G*T*A*C*T*C*A*C*C*C*A*T*C*T
673	T*C*C*A*T*G*T*A*C*T*C*A*C*C*C*A*T*C*T*C
674	C*C*A*T*G*T*A*C*T*C*A*C*C*C*A*T*C*T*C*T
675	C*A*T*G*T*A*C*T*C*A*C*C*C*A*T*C*T*C*T*C
676	A*T*G*T*A*C*T*C*A*C*C*C*A*T*C*T*C*T*C*C
677	T*G*T*A*C*T*C*A*C*C*C*A*T*C*T*C*T*C*C*A
678	G*T*A*C*T*C*A*C*C*C*A*T*C*T*C*T*C*C*A*T
679	T*A*C*T*C*A*C*C*C*A*T*C*T*C*T*C*C*A*T*C
680	A*C*T*C*A*C*C*C*A*T*C*T*C*T*C*C*A*T*C*C
681	C*T*C*A*C*C*C*A*T*C*T*C*T*C*C*A*T*C*C*A
682	T*C*A*C*C*C*A*T*C*T*C*T*C*C*A*T*C*C*A*T
683	C*A*C*C*C*A*T*C*T*C*T*C*C*A*T*C*C*A*T*C
684	A*C*C*C*A*T*C*T*C*T*C*C*A*T*C*C*A*T*C*C
685	C*C*C*A*T*C*T*C*T*C*C*A*T*C*C*A*T*C*C*T
686	C*C*A*T*C*T*C*T*C*C*A*T*C*C*A*T*C*C*T*T
687	C*A*T*C*T*C*T*C*C*A*T*C*C*A*T*C*C*T*T*T
688	A*T*C*T*C*T*C*C*A*T*C*C*A*T*C*C*T*T*T*T
689	T*C*T*C*T*C*C*A*T*C*C*A*T*C*C*T*T*T*T*A
690	C*T*C*T*C*C*A*T*C*C*A*T*C*C*T*T*T*T*A*T
691	T*C*T*C*C*A*T*C*C*A*T*C*C*T*T*T*T*A*T*C
692	C*T*C*C*A*T*C*C*A*T*C*C*T*T*T*T*A*T*C*T
693	C*C*A*T*C*C*A*T*C*C*T*T*T*T*A*T*C*T*A*C
694	C*T*A*C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C
695	A*C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G
696	C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T
697	C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T*T*C
698	T*C*A*T*T*C*A*T*T*C*A*T*T*C*A*C*C*A*G*C
699	C*A*T*T*C*A*T*T*C*A*T*T*C*A*C*C*A*G*C*A
700	G*G*A*T*A*A*G*A*G*T*T*C*T*T*T*C*C*A*G*G
701	G*A*T*A*A*G*A*G*T*T*C*T*T*T*C*C*A*G*G*A
702	T*T*C*C*A*G*G*A*A*A*C*C*C*A*G*G*C*A*G*C
703	T*C*C*A*G*G*A*A*A*C*C*C*A*G*G*C*A*G*C*T
704	A*G*C*T*G*G*A*A*G*A*G*A*C*A*T*A*C*C*C*A

705	G*C*T*G*G*A*A*G*A*G*A*C*A*T*A*C*C*C*A*G
706	C*T*G*G*A*A*G*A*G*A*C*A*T*A*C*C*C*A*G*A
707	T*G*G*A*A*G*A*G*A*C*A*T*A*C*C*C*A*G*A*C
708	G*G*A*A*G*A*G*A*C*A*T*A*C*C*C*A*G*A*C*A
709	G*A*A*G*A*G*A*C*A*T*A*C*C*C*A*G*A*C*A*C
710	A*A*G*A*G*A*C*A*T*A*C*C*C*A*G*A*C*A*C*A
711	A*G*A*G*A*C*A*T*A*C*C*C*A*G*A*C*A*C*A*A
712	G*A*G*A*C*A*T*A*C*C*C*A*G*A*C*A*C*A*A*A
713	A*G*A*C*A*T*A*C*C*C*A*G*A*C*A*C*A*A*A*C
714	G*C*C*C*A*A*T*C*C*T*G*A*G*T*G*G*T*T*A*G
715	C*C*C*A*A*T*C*C*T*G*A*G*T*G*G*T*T*A*G*G
716	G*G*C*T*G*G*A*A*T*A*G*A*A*G*G*A*A*G*A*A
717	G*C*T*G*G*A*A*T*A*G*A*A*G*G*A*A*G*A*A*C
718	C*T*G*G*A*A*T*A*G*A*A*G*G*A*A*G*A*A*C*C
719	T*G*G*A*A*T*A*G*A*A*G*G*A*A*G*A*A*C*C*T
720	G*G*A*A*T*A*G*A*A*G*G*A*A*G*A*A*C*C*T*G
721	G*A*A*T*A*G*A*A*G*G*A*A*G*A*A*C*C*T*G*A
722	A*T*A*G*A*A*G*G*A*A*G*A*A*C*C*T*G*A*T*G
723	T*A*G*A*A*G*G*A*A*G*A*A*C*C*T*G*A*T*G*A
724	A*G*A*A*G*G*A*A*G*A*A*C*C*T*G*A*T*G*A*T
725	G*A*A*G*G*A*A*G*A*A*C*C*T*G*A*T*G*A*T*G
726	A*A*G*G*A*A*G*A*A*C*C*T*G*A*T*G*A*T*G*A
727	A*G*G*A*A*G*A*A*C*C*T*G*A*T*G*A*T*G*A*G
728	G*G*A*A*G*A*A*C*C*T*G*A*T*G*A*T*G*A*G*T
729	G*A*A*G*A*A*C*C*T*G*A*T*G*A*T*G*A*G*T*A
730	A*A*G*A*A*C*C*T*G*A*T*G*A*T*G*A*G*T*A*G
731	A*G*A*A*C*C*T*G*A*T*G*A*T*G*A*G*T*A*G*T
732	G*A*A*C*C*T*G*A*T*G*A*T*G*A*G*T*A*G*T*G
733	A*A*C*C*T*G*A*T*G*A*T*G*A*G*T*A*G*T*G*A
734	A*C*C*T*G*A*T*G*A*T*G*A*G*T*A*G*T*G*A*G
735	C*C*T*G*A*T*G*A*T*G*A*G*T*A*G*T*G*A*G*A
736	C*T*G*A*T*G*A*T*G*A*G*T*A*G*T*G*A*G*A*G
737	T*G*A*T*G*A*T*G*A*G*T*A*G*T*G*A*G*A*G*T
738	G*A*T*G*A*T*G*A*G*T*A*G*T*G*A*G*A*G*T*C
739	A*T*G*A*T*G*A*G*T*A*G*T*G*A*G*A*G*T*C*A
740	T*G*A*T*G*A*G*T*A*G*T*G*A*G*A*G*T*C*A*A

741	G*A*T*G*A*G*T*A*G*T*G*A*G*A*G*T*C*A*A*C
742	A*T*G*A*G*T*A*G*T*G*A*G*A*G*T*C*A*A*C*C
743	T*G*A*G*T*A*G*T*G*A*G*A*G*T*C*A*A*C*C*T
744	G*A*G*T*A*G*T*G*A*G*A*G*T*C*A*A*C*C*T*G
745	A*G*T*A*G*T*G*A*G*A*G*T*C*A*A*C*C*T*G*G
746	G*T*A*G*T*G*A*G*A*G*T*C*A*A*C*C*T*G*G*A
747	T*A*G*T*G*A*G*A*G*T*C*A*A*C*C*T*G*G*A*G
748	A*G*T*G*A*G*A*G*T*C*A*A*C*C*T*G*G*A*G*G
749	G*T*G*A*G*A*G*T*C*A*A*C*C*T*G*G*A*G*G*C
750	T*T*C*C*C*A*G*A*G*G*A*G*G*T*G*A*C*C*C*T
751	C*C*A*G*A*G*G*A*G*G*T*G*A*C*C*C*T*G*A*A
752	C*A*G*A*G*G*A*G*G*T*G*A*C*C*C*T*G*A*A*T
753	A*G*A*G*G*A*G*G*T*G*A*C*C*C*T*G*A*A*T*C
754	G*A*G*G*A*G*G*T*G*A*C*C*C*T*G*A*A*T*C*T
755	A*G*G*A*G*G*T*G*A*C*C*C*T*G*A*A*T*C*T*G
756	G*G*A*G*G*T*G*A*C*C*C*T*G*A*A*T*C*T*G*G
757	G*A*G*G*T*G*A*C*C*C*T*G*A*A*T*C*T*G*G*A
758	A*G*G*T*G*A*C*C*C*T*G*A*A*T*C*T*G*G*A*C
759	G*G*T*G*A*C*C*C*T*G*A*A*T*C*T*G*G*A*C*T
760	G*T*G*A*C*C*C*T*G*A*A*T*C*T*G*G*A*C*T*T
761	T*G*A*C*C*C*T*G*A*A*T*C*T*G*G*A*C*T*T*T
762	G*A*C*C*C*T*G*A*A*T*C*T*G*G*A*C*T*T*T*G
763	A*C*C*C*T*G*A*A*T*C*T*G*G*A*C*T*T*T*G*A
764	C*C*C*T*G*A*A*T*C*T*G*G*A*C*T*T*T*G*A*T
765	C*C*T*G*A*A*T*C*T*G*G*A*C*T*T*T*G*A*T*G
766	C*T*G*A*A*T*C*T*G*G*A*C*T*T*T*G*A*T*G*G
767	T*G*A*A*T*C*T*G*G*A*C*T*T*T*G*A*T*G*G*A
768	G*A*A*T*C*T*G*G*A*C*T*T*T*G*A*T*G*G*A*T
769	A*T*C*T*G*G*A*C*T*T*T*G*A*T*G*G*A*T*A*G
770	T*C*T*G*G*A*C*T*T*T*G*A*T*G*G*A*T*A*G*G
771	G*G*A*G*G*A*G*T*T*T*T*C*C*A*G*G*T*A*A*A
772	G*A*G*G*A*G*T*T*T*T*C*C*A*G*G*T*A*A*A*G
773	A*G*G*A*G*T*T*T*T*C*C*A*G*G*T*A*A*A*G*G
774	G*C*C*A*G*G*A*G*A*G*T*G*T*G*G*A*T*G*G*T
775	C*C*A*G*G*A*G*A*G*T*G*T*G*G*A*T*G*G*T*G
776	C*A*G*G*A*G*A*G*T*G*T*G*G*A*T*G*G*T*G*T

777	A*G*G*A*G*A*G*T*G*T*G*G*A*T*G*G*T*G*T*G
778	G*G*A*G*A*G*T*G*T*G*G*A*T*G*G*T*G*T*G*G
779	G*A*G*A*G*T*G*T*G*G*A*T*G*G*T*G*T*G*G*C
780	A*A*T*T*A*C*C*C*C*C*A*A*A*T*T*C*A*C*C*C
781	A*T*T*A*C*C*C*C*C*A*A*A*T*T*C*A*C*C*C*A
782	T*T*A*C*C*C*C*C*A*A*A*T*T*C*A*C*C*C*A*T
783	T*A*C*C*C*C*C*A*A*A*T*T*C*A*C*C*C*C*A*T*C
784	A*C*C*C*C*C*A*A*A*T*T*C*A*C*C*C*C*A*T*C*C
785	C*C*C*C*C*A*A*A*T*T*C*A*C*C*C*C*A*T*C*C*A
786	C*C*C*C*A*A*A*T*T*C*A*C*C*C*C*A*T*C*C*A*T
787	C*C*C*A*A*A*T*T*C*A*C*C*C*C*A*T*C*C*A*T*A
788	C*C*A*A*A*T*T*C*A*C*C*C*C*A*T*C*C*C*A*T*A*C
789	C*A*A*A*T*T*C*A*C*C*C*C*A*T*C*C*C*A*T*A*C*A
790	A*A*T*T*C*A*C*C*C*C*A*T*C*C*C*A*T*A*C*C*A*T*C
791	A*T*T*C*A*C*C*C*C*A*T*C*C*C*A*T*A*C*C*A*T*C*T
792	T*T*C*A*C*C*C*C*A*T*C*C*C*A*T*A*C*C*A*T*C*T*A
793	T*C*A*C*C*C*C*A*T*C*C*C*A*T*A*C*C*A*T*C*C*T*A*T
794	C*A*C*C*C*C*A*T*C*C*C*A*T*A*C*C*A*T*C*C*T*A*T*A
795	A*C*C*C*C*A*T*C*C*C*A*T*C*A*T*C*C*A*T*C*T*A*T*A*C
796	C*C*C*C*A*T*C*C*C*A*T*C*A*T*C*C*A*T*C*C*T*A*T*A*C*T
797	C*A*T*A*T*A*T*C*C*C*A*T*C*C*C*A*T*C*C*T*G*T*C
798	A*T*A*T*A*T*C*C*C*A*T*C*C*C*A*T*C*C*T*G*T*C*C
799	T*A*T*A*T*C*C*C*A*T*C*C*C*A*T*C*C*T*G*T*C*C*C*A
800	A*T*A*T*C*C*C*A*T*C*C*C*A*T*C*C*T*G*T*C*C*C*A*T
801	T*A*T*C*C*C*A*T*C*C*C*A*T*C*C*T*G*T*C*C*C*A*T*C
802	A*T*C*C*C*A*T*C*C*C*A*T*C*C*T*G*T*C*C*C*A*T*C*C
803	T*C*C*C*A*T*C*C*C*A*T*C*C*T*G*T*C*C*C*A*T*C*C*C*A
804	C*C*C*A*T*C*C*C*A*T*C*C*T*G*T*C*C*C*A*T*C*C*C*A*T
805	C*A*T*C*C*C*A*T*C*C*T*G*T*C*C*C*A*T*C*C*C*A*T*C
806	A*T*C*C*C*A*T*C*C*T*G*T*C*C*C*A*T*C*C*C*A*T*C*C
807	T*C*C*C*A*T*C*C*T*G*T*C*C*C*A*T*C*C*C*A*T*C*C*C*A
808	C*C*C*A*T*C*C*T*G*T*C*C*C*A*T*C*C*C*A*T*C*C*C*A*T
809	C*C*A*T*C*C*T*G*T*C*C*C*A*T*C*C*C*A*T*C*C*C*A*T*C
810	A*T*C*C*T*G*T*C*C*C*A*T*C*C*C*A*T*C*C*C*A*T*C*C*A
811	T*C*C*T*G*T*C*C*C*A*T*C*C*C*A*T*C*C*C*A*T*C*C*A*T
812	C*C*T*G*T*C*C*C*A*T*C*C*C*A*T*C*C*C*A*T*C*C*A*T*C

813	T*G*T*C*C*A*T*C*C*A*T*C*C*A*T*C*A*T*C*C
814	G*T*C*C*A*T*C*C*A*T*C*C*A*T*C*A*T*C*C*A
815	T*C*C*A*T*C*C*A*T*C*C*A*T*C*A*T*C*C*A*T
816	C*C*A*T*C*C*A*T*C*C*A*T*C*A*T*C*C*A*T*C
817	C*A*T*C*C*A*T*C*C*A*T*C*A*T*C*C*A*T*C*T
818	A*T*C*C*A*T*C*C*A*T*C*A*T*C*C*A*T*C*T*A
819	T*C*C*A*T*C*C*A*T*C*A*T*C*C*A*T*C*T*A*G
820	C*C*A*T*C*C*A*T*C*A*T*C*C*A*T*C*T*A*G*C
821	C*A*T*C*C*A*T*C*A*T*C*C*A*T*C*T*A*G*C*C
822	A*T*C*C*A*T*C*A*T*C*C*A*T*C*T*A*G*C*C*A
823	T*C*C*A*T*C*A*T*C*C*A*T*C*T*A*G*C*C*A*C
824	G*G*A*G*A*G*A*A*A*G*T*G*T*C*A*T*G*G*A*G
825	G*A*G*A*G*A*A*A*G*T*G*T*C*A*T*G*G*A*G*A
826	A*G*A*G*A*A*A*G*T*G*T*C*A*T*G*G*A*G*A*G
827	G*A*G*A*A*A*G*T*G*T*C*A*T*G*G*A*G*A*G*T
828	A*G*A*A*A*G*T*G*T*C*A*T*G*G*A*G*A*G*T*G
829	G*A*A*A*G*T*G*T*C*A*T*G*G*A*G*A*G*T*G*C
830	G*G*C*A*G*C*T*T*A*C*A*T*C*A*T*C*C*A*T*C
831	G*C*A*G*C*T*T*A*C*A*T*C*A*T*C*C*A*T*C*T
832	C*A*G*C*T*T*A*C*A*T*C*A*T*C*C*A*T*C*T*G
833	A*G*C*T*T*A*C*A*T*C*A*T*C*C*A*T*C*T*G*C
834	G*C*T*T*A*C*A*T*C*A*T*C*C*A*T*C*T*G*C*C
835	C*T*T*A*C*A*T*C*A*T*C*C*A*T*C*T*G*C*C*T
836	T*T*A*C*A*T*C*A*T*C*C*A*T*C*T*G*C*C*T*G
837	T*A*C*A*T*C*A*T*C*C*A*T*C*T*G*C*C*T*G*T
838	A*C*A*T*C*A*T*C*C*A*T*C*T*G*C*C*T*G*T*T
839	C*A*T*C*A*T*C*C*A*T*C*T*G*C*C*T*G*T*T*T
840	A*T*C*A*T*C*C*A*T*C*T*G*C*C*T*G*T*T*T*A
841	T*C*A*T*C*C*A*T*C*T*G*C*C*T*G*T*T*T*A*T
842	C*A*T*C*C*A*T*C*T*G*C*C*T*G*T*T*T*A*T*T
843	A*T*C*C*A*T*C*T*G*C*C*T*G*T*T*T*A*T*T*C
844	T*C*C*A*T*C*T*G*C*C*T*G*T*T*T*A*T*T*C*A
845	C*C*A*T*C*T*G*C*C*T*G*T*T*T*A*T*T*C*A*T
846	C*T*A*C*T*C*T*T*T*T*A*T*C*C*A*T*C*C*A*C
847	A*C*T*C*T*T*T*T*A*T*C*C*A*T*C*C*A*C*A*C
848	C*T*C*T*T*T*T*A*T*C*C*A*T*C*C*A*C*A*C*A

849	T*C*T*T*T*T*A*T*C*C*A*T*C*C*A*C*A*C*A*C
850	C*T*T*T*T*A*T*C*C*A*T*C*C*A*C*A*C*A*C*C
851	T*T*T*T*A*T*C*C*A*T*C*C*A*C*A*C*A*C*C*C
852	T*T*T*A*T*C*C*A*T*C*C*A*C*A*C*A*C*C*C*A
853	T*T*A*T*C*C*A*T*C*C*A*C*A*C*A*C*C*C*A*C
854	T*A*T*C*C*A*T*C*C*A*C*A*C*A*C*C*C*A*C*C
855	A*T*C*C*A*T*C*C*A*C*A*C*A*C*C*C*A*C*C*C
856	T*C*C*A*T*C*C*A*C*A*C*A*C*C*C*A*C*C*C*A
857	C*C*A*T*C*C*A*C*A*C*A*C*C*C*A*C*C*C*A*T
858	C*A*T*C*C*A*C*A*C*A*C*C*C*A*C*C*C*A*T*C
859	A*T*C*C*A*C*A*C*A*C*C*C*A*C*C*C*A*T*C*T
860	T*C*C*A*C*A*C*A*C*C*C*A*C*C*C*A*T*C*T*A
861	C*C*A*C*A*C*A*C*C*C*A*C*C*C*A*T*C*T*A*A
862	C*A*C*A*C*A*C*C*C*A*C*C*C*A*T*C*T*A*A*C
863	A*C*A*C*A*C*C*C*A*C*C*C*A*T*C*T*A*A*C*T
864	C*A*C*A*C*C*C*A*C*C*C*A*T*C*T*A*A*C*T*A
865	A*C*A*C*C*C*A*C*C*C*A*T*C*T*A*A*C*T*A*C
866	C*A*C*C*C*A*C*C*C*A*T*C*T*A*A*C*T*A*C*C
867	A*C*C*C*A*C*C*C*A*T*C*T*A*A*C*T*A*C*C*C
868	C*C*C*A*C*C*C*A*T*C*T*A*A*C*T*A*C*C*C*C
869	C*C*A*C*C*C*A*T*C*T*A*A*C*T*A*C*C*C*C*A
870	C*A*C*C*C*A*T*C*T*A*A*C*T*A*C*C*C*C*A*A
871	A*C*C*C*A*T*C*T*A*A*C*T*A*C*C*C*C*A*A*A
872	C*C*C*A*T*C*T*A*A*C*T*A*C*C*C*C*A*A*A*T
873	C*C*A*T*C*T*A*A*C*T*A*C*C*C*C*A*A*A*T*T
874	A*A*T*T*T*C*A*C*C*C*A*T*C*C*A*C*T*C*T*T
875	A*T*T*T*C*A*C*C*C*A*T*C*C*A*C*T*C*T*T*C
876	T*T*T*C*A*C*C*C*A*T*C*C*A*C*T*C*T*T*C*C
877	T*T*C*A*C*C*C*A*T*C*C*A*C*T*C*T*T*C*C*A
878	T*C*A*C*C*C*A*T*C*C*A*C*T*C*T*T*C*C*A*A
879	C*A*C*C*C*A*T*C*C*A*C*T*C*T*T*C*C*A*A*C
880	A*C*C*C*A*T*C*C*A*C*T*C*T*T*C*C*A*A*C*C
881	C*C*C*A*T*C*C*A*C*T*C*T*T*C*C*A*A*C*C*T
882	C*C*A*T*C*C*A*C*T*C*T*T*C*C*A*A*C*C*T*T
883	C*A*T*C*C*A*C*T*C*T*T*C*C*A*A*C*C*T*T*T
884	A*T*C*C*A*C*T*C*T*T*C*C*A*A*C*C*T*T*T*C

885	T*C*C*A*C*T*C*T*T*C*C*A*A*C*C*T*T*T*C*A
886	C*C*A*C*T*C*T*T*C*C*A*A*C*C*T*T*T*C*A*G
887	C*A*C*T*C*T*T*C*C*A*A*C*C*T*T*T*C*A*G*T
888	A*C*T*C*T*T*C*C*A*A*C*C*T*T*T*C*A*G*T*A
889	C*T*C*T*T*C*C*A*A*C*C*T*T*T*C*A*G*T*A*A
890	C*C*T*T*T*C*A*G*T*A*A*T*T*C*A*A*C*C*A*C
891	C*A*G*T*A*A*T*T*C*A*A*C*C*A*C*A*C*A*T*C
892	A*G*T*A*A*T*T*C*A*A*C*C*A*C*A*C*A*T*C*C
893	G*T*A*A*T*T*C*A*A*C*C*A*C*A*C*A*T*C*C*A
894	A*A*T*T*C*A*A*C*C*A*C*A*C*A*T*C*C*A*T*C
895	A*T*T*C*A*A*C*C*A*C*A*C*A*T*C*C*A*T*C*C
896	T*T*C*A*A*C*C*A*C*A*C*A*T*C*C*A*T*C*C*A
897	T*C*A*A*C*C*A*C*A*C*A*T*C*C*A*T*C*C*A*T
898	C*A*A*C*C*A*C*A*C*A*T*C*C*A*T*C*C*A*T*C
899	A*A*C*C*A*C*A*C*A*T*C*C*A*T*C*C*A*T*C*C
900	A*C*C*A*C*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A
901	C*C*A*C*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T
902	C*A*C*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C
903	A*C*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C
904	C*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A
905	A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T
906	C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*T
907	A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*T*C
908	T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*T*C*A
909	C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*T*C*A*T
910	C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*T*C*A*T*C
911	A*T*C*C*A*T*C*C*A*T*C*C*A*T*T*C*A*T*C*C
912	T*C*C*A*T*C*C*A*T*C*C*A*T*T*C*A*T*C*C*A
913	C*C*A*T*C*C*A*T*C*C*A*T*T*C*A*T*C*C*A*T
914	C*A*T*C*C*A*T*C*C*A*T*T*C*A*T*C*C*A*T*C
915	A*T*C*C*A*T*C*C*A*T*T*C*A*T*C*C*A*T*C*C
916	T*C*C*A*T*C*C*A*T*T*C*A*T*C*C*A*T*C*C*C
917	C*C*A*T*C*C*A*T*T*C*A*T*C*C*A*T*C*C*C*A
918	C*A*T*C*C*A*T*T*C*A*T*C*C*A*T*C*C*C*A*T
919	A*T*C*C*A*T*T*C*A*T*C*C*A*T*C*C*C*A*T*A
920	T*C*C*A*T*T*C*A*T*C*C*A*T*C*C*C*A*T*A*C

921	C*C*A*T*T*C*A*T*C*C*A*T*C*C*C*A*T*A*C*A
922	C*A*T*T*C*A*T*C*C*A*T*C*C*C*A*T*A*C*A*T
923	T*T*C*A*T*C*C*A*T*C*C*C*A*T*A*C*A*T*T*G
924	T*C*A*T*C*C*A*T*C*C*C*A*T*A*C*A*T*T*G*A
925	C*A*T*C*C*A*T*C*C*C*A*T*A*C*A*T*T*G*A*T
926	A*T*C*C*A*T*C*C*C*A*T*A*C*A*T*T*G*A*T*C
927	T*C*C*A*T*C*C*C*A*T*A*C*A*T*T*G*A*T*C*C
928	G*C*A*A*C*T*T*A*A*T*C*C*A*C*C*T*A*C*C*C
929	C*A*A*C*T*T*A*A*T*C*C*A*C*C*T*A*C*C*C*A
930	A*A*C*T*T*A*A*T*C*C*A*C*C*T*A*C*C*C*A*A
931	A*C*T*T*A*A*T*C*C*A*C*C*T*A*C*C*C*A*A*T
932	C*T*T*A*A*T*C*C*A*C*C*T*A*C*C*C*A*A*T*C
933	T*T*A*A*T*C*C*A*C*C*T*A*C*C*C*A*A*T*C*A
934	T*A*A*T*C*C*A*C*C*T*A*C*C*C*A*A*T*C*A*T
935	A*A*T*C*C*A*C*C*T*A*C*C*C*A*A*T*C*A*T*T
936	A*T*C*C*A*C*C*T*A*C*C*C*A*A*T*C*A*T*T*C
937	T*C*C*A*C*C*T*A*C*C*C*A*A*T*C*A*T*T*C*A
938	C*C*A*C*C*T*A*C*C*C*A*A*T*C*A*T*T*C*A*T
939	C*A*C*C*T*A*C*C*C*A*A*T*C*A*T*T*C*A*T*T
940	A*C*C*T*A*C*C*C*A*A*T*C*A*T*T*C*A*T*T*C
941	C*C*T*A*C*C*C*A*A*T*C*A*T*T*C*A*T*T*C*T
942	C*T*T*T*C*A*T*A*C*A*A*C*C*A*A*C*C*A*T*C
943	T*T*T*C*A*T*A*C*A*A*C*C*A*A*C*C*A*T*C*C
944	T*T*C*A*T*A*C*A*A*C*C*A*A*C*C*A*T*C*C*A
945	T*C*A*T*A*C*A*A*C*C*A*A*C*C*A*T*C*C*A*T
946	C*A*T*A*C*A*A*C*C*A*A*C*C*A*T*C*C*A*T*C
947	A*T*A*C*A*A*C*C*A*A*C*C*A*T*C*C*A*T*C*C
948	T*A*C*A*A*C*C*A*A*C*C*A*T*C*C*A*T*C*C*A
949	A*C*A*A*C*C*A*A*C*C*A*T*C*C*A*T*C*C*A*C
950	C*A*A*C*C*A*A*C*C*A*T*C*C*A*T*C*C*A*C*C
951	A*A*C*C*A*A*C*C*A*T*C*C*A*T*C*C*A*C*C*C
952	A*C*C*A*A*C*C*A*T*C*C*A*T*C*C*A*C*C*C*A
953	C*C*A*A*C*C*A*T*C*C*A*T*C*C*A*C*C*C*A*T
954	C*A*A*C*C*A*T*C*C*A*T*C*C*A*C*C*C*C*A*T*C
955	A*A*C*C*A*T*C*C*A*T*C*C*A*C*C*C*A*T*C*A
956	A*C*C*A*T*C*C*A*T*C*C*A*C*C*C*A*T*C*A*A

957	C*C*A*T*C*C*A*T*C*C*A*C*C*C*A*T*C*A*A*T
958	C*A*T*C*C*A*T*C*C*A*C*C*C*A*T*C*A*A*T*T
959	A*T*C*C*A*T*C*C*A*C*C*C*A*T*C*A*A*T*T*T
960	T*C*C*A*T*C*C*A*C*C*C*A*T*C*A*A*T*T*T*A
961	C*C*A*T*C*C*A*C*C*C*A*T*C*A*A*T*T*T*A*T
962	C*A*T*C*C*A*C*C*C*A*T*C*A*A*T*T*T*A*T*C
963	A*T*C*C*A*C*C*C*A*T*C*A*A*T*T*T*A*T*C*C
964	T*C*C*A*C*C*C*A*T*C*A*A*T*T*T*A*T*C*C*A
965	C*C*A*C*C*C*A*T*C*A*A*T*T*T*A*T*C*C*A*A
966	C*A*C*C*C*A*T*C*A*A*T*T*T*A*T*C*C*A*A*C
967	A*C*C*C*A*T*C*A*A*T*T*T*A*T*C*C*A*A*C*C
968	C*C*C*A*T*C*A*A*T*T*T*A*T*C*C*A*A*C*C*A
969	A*T*C*C*A*A*C*C*A*T*C*C*A*T*T*T*T*T*C*G
970	T*C*C*A*A*C*C*A*T*C*C*A*T*T*T*T*T*C*G*T
971	C*C*A*A*C*C*A*T*C*C*A*T*T*T*T*T*C*G*T*C
972	C*A*A*C*C*A*T*C*C*A*T*T*T*T*T*C*G*T*C*T
973	A*A*C*C*A*T*C*C*A*T*T*T*T*T*C*G*T*C*T*G
974	A*C*C*A*T*C*C*A*T*T*T*T*T*C*G*T*C*T*G*T
975	C*C*A*T*C*C*A*T*T*T*T*T*C*G*T*C*T*G*T*C
976	C*A*T*C*C*A*T*T*T*T*T*C*G*T*C*T*G*T*C*C
977	A*T*C*C*A*T*T*T*T*T*C*G*T*C*T*G*T*C*C*A
978	T*C*C*A*T*T*T*T*T*C*G*T*C*T*G*T*C*C*A*C
979	C*C*A*T*T*T*T*T*C*G*T*C*T*G*T*C*C*A*C*C
980	C*A*T*T*T*T*T*C*G*T*C*T*G*T*C*C*A*C*C*A
981	A*T*T*T*T*T*C*G*T*C*T*G*T*C*C*A*C*C*A*G
982	T*T*T*T*T*C*G*T*C*T*G*T*C*C*A*C*C*A*G*C
983	T*T*T*T*C*G*T*C*T*G*T*C*C*A*C*C*A*G*C*C
984	T*T*T*C*G*T*C*T*G*T*C*C*A*C*C*A*G*C*C*A
985	T*T*C*G*T*C*T*G*T*C*C*A*C*C*A*G*C*C*A*C
986	T*C*G*T*C*T*G*T*C*C*A*C*C*A*G*C*C*A*C*T
987	G*T*C*T*G*T*C*C*A*C*C*A*G*C*C*A*C*T*C*A
988	T*C*T*G*T*C*C*A*C*C*A*G*C*C*A*C*T*C*A*C
989	C*T*G*T*C*C*A*C*C*A*G*C*C*A*C*T*C*A*C*A
990	T*G*T*C*C*A*C*C*A*G*C*C*A*C*T*C*A*C*A*A
991	G*T*C*C*A*C*C*A*G*C*C*A*C*T*C*A*C*A*A*C
992	T*C*C*A*C*C*A*G*C*C*A*C*T*C*A*C*A*A*C*C

993	C*C*A*C*C*A*G*C*C*A*C*T*C*A*C*A*A*C*C*A
994	C*A*C*C*A*G*C*C*A*C*T*C*A*C*A*A*C*C*A*T
995	A*C*C*A*G*C*C*A*C*T*C*A*C*A*A*C*C*A*T*C
996	C*C*A*G*C*C*A*C*T*C*A*C*A*A*C*C*A*T*C*C
997	C*A*G*C*C*A*C*T*C*A*C*A*A*C*C*A*T*C*C*A
998	A*G*C*C*A*C*T*C*A*C*A*A*C*C*A*T*C*C*A*T
999	G*C*C*A*C*T*C*A*C*A*A*C*C*A*T*C*C*A*T*C
1000	C*C*A*C*T*C*A*C*A*A*C*C*A*T*C*C*A*T*C*T
1001	C*A*C*T*C*A*C*A*A*C*C*A*T*C*C*A*T*C*T*A
1002	A*C*T*C*A*C*A*A*C*C*A*T*C*C*A*T*C*T*A*A
1003	C*T*C*A*C*A*A*C*C*A*T*C*C*A*T*C*T*A*A*A
1004	G*C*A*A*T*A*G*T*T*C*A*A*C*C*A*C*A*C*A*T
1005	C*A*A*T*A*G*T*T*C*A*A*C*C*A*C*A*C*A*T*C
1006	A*A*T*A*G*T*T*C*A*A*C*C*A*C*A*C*A*T*C*C
1007	A*T*A*G*T*T*C*A*A*C*C*A*C*A*C*A*T*C*C*T
1008	T*A*G*T*T*C*A*A*C*C*A*C*A*C*A*T*C*C*T*T
1009	A*G*T*T*C*A*A*C*C*A*C*A*C*A*T*C*C*T*T*C
1010	G*T*T*C*A*A*C*C*A*C*A*C*A*T*C*C*T*T*C*C
1011	T*T*C*A*A*C*C*A*C*A*C*A*T*C*C*T*T*C*C*A
1012	T*C*A*A*C*C*A*C*A*C*A*T*C*C*T*T*C*C*A*T
1013	C*A*A*C*C*A*C*A*C*A*T*C*C*T*T*C*C*A*T*T
1014	A*A*C*C*A*C*A*C*A*T*C*C*T*T*C*C*A*T*T*C
1015	A*C*C*A*C*A*C*A*T*C*C*T*T*C*C*A*T*T*C*A
1016	C*C*A*C*A*C*A*T*C*C*T*T*C*C*A*T*T*C*A*T
1017	C*A*C*A*C*A*T*C*C*T*T*C*C*A*T*T*C*A*T*C
1018	A*C*A*C*A*T*C*C*T*T*C*C*A*T*T*C*A*T*C*C
1019	C*A*C*A*T*C*C*T*T*C*C*A*T*T*C*A*T*C*C*A
1020	A*C*A*T*C*C*T*T*C*C*A*T*T*C*A*T*C*C*A*C
1021	C*A*T*C*C*T*T*C*C*A*T*T*C*A*T*C*C*A*C*C
1022	A*T*C*C*T*T*C*C*A*T*T*C*A*T*C*C*A*C*C*C
1023	T*C*C*T*T*C*C*A*T*T*C*A*T*C*C*A*C*C*C*A
1024	C*C*T*T*C*C*A*T*T*C*A*T*C*C*A*C*C*C*A*C
1025	C*T*T*C*C*A*T*T*C*A*T*C*C*A*C*C*C*A*C*C
1026	T*T*C*C*A*T*T*C*A*T*C*C*A*C*C*C*A*C*C*C
1027	T*C*C*A*T*T*C*A*T*C*C*A*C*C*C*A*C*C*C*A
1028	C*A*T*T*C*A*T*C*C*A*C*C*C*A*C*C*C*A*T*T

1029	A*T*T*C*A*T*C*C*A*C*C*C*A*C*C*C*A*T*T*C
1030	T*T*C*A*T*C*C*A*C*C*C*A*C*C*C*A*T*T*C*A
1031	T*C*A*T*C*C*A*C*C*C*A*C*C*C*A*T*T*C*A*T
1032	C*A*T*C*C*A*C*C*C*A*C*C*C*A*T*T*C*A*T*C
1033	A*T*C*C*A*C*C*C*A*C*C*C*A*T*T*C*A*T*C*C
1034	T*C*C*A*C*C*C*A*C*C*C*A*T*T*C*A*T*C*C*A
1035	C*C*A*C*C*C*A*C*C*C*A*T*T*C*A*T*C*C*A*T
1036	C*A*C*C*C*A*C*C*C*A*T*T*C*A*T*C*C*A*T*T
1037	A*C*C*C*A*C*C*C*A*T*T*C*A*T*C*C*A*T*T*T
1038	C*C*C*A*C*C*C*A*T*T*C*A*T*C*C*A*T*T*T*G
1039	C*C*A*C*C*C*A*T*T*C*A*T*C*C*A*T*T*T*G*T
1040	C*A*C*C*C*A*T*T*C*A*T*C*C*A*T*T*T*G*T*C
1041	A*C*C*C*A*T*T*C*A*T*C*C*A*T*T*T*G*T*C*C
1042	C*C*A*T*T*C*A*T*C*C*A*T*T*T*G*T*C*C*A*T
1043	C*A*T*T*C*A*T*C*C*A*T*T*T*G*T*C*C*A*T*C
1044	T*T*C*A*T*C*C*A*T*T*T*G*T*C*C*A*T*C*T*G
1045	T*C*A*T*C*C*A*T*T*T*G*T*C*C*A*T*C*T*G*C
1046	C*A*T*C*C*A*T*T*T*G*T*C*C*A*T*C*T*G*C*C
1047	A*T*C*C*A*T*T*T*G*T*C*C*A*T*C*T*G*C*C*T
1048	T*C*C*A*T*T*T*G*T*C*C*A*T*C*T*G*C*C*T*A
1049	C*C*A*T*T*T*G*T*C*C*A*T*C*T*G*C*C*T*A*T
1050	C*A*T*T*T*G*T*C*C*A*T*C*T*G*C*C*T*A*T*A
1051	A*T*T*T*G*T*C*C*A*T*C*T*G*C*C*T*A*T*A*C
1052	T*T*T*G*T*C*C*A*T*C*T*G*C*C*T*A*T*A*C*A
1053	T*T*G*T*C*C*A*T*C*T*G*C*C*T*A*T*A*C*A*T
1054	T*G*T*C*C*A*T*C*T*G*C*C*T*A*T*A*C*A*T*C
1055	G*T*C*C*A*T*C*T*G*C*C*T*A*T*A*C*A*T*C*C
1056	T*C*C*A*T*C*T*G*C*C*T*A*T*A*C*A*T*C*C*A
1057	C*C*A*T*C*T*G*C*C*T*A*T*A*C*A*T*C*C*A*T
1058	C*A*T*C*T*G*C*C*T*A*T*A*C*A*T*C*C*A*T*C
1059	A*T*C*T*G*C*C*T*A*T*A*C*A*T*C*C*A*T*C*C
1060	T*C*T*G*C*C*T*A*T*A*C*A*T*C*C*A*T*C*C*A
1061	C*T*G*C*C*T*A*T*A*C*A*T*C*C*A*T*C*C*A*T
1062	T*G*C*C*T*A*T*A*C*A*T*C*C*A*T*C*C*A*T*C
1063	G*C*C*T*A*T*A*C*A*T*C*C*A*T*C*C*A*T*C*C
1064	C*C*T*A*T*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A

1065	C*T*A*T*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T
1066	T*A*T*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C
1067	A*T*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C
1068	T*A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A
1069	A*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T
1070	C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C
1071	A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C
1072	T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A
1073	C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T
1074	C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C
1075	A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*T
1076	T*C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*T*A
1077	C*C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*T*A*C
1078	C*A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*T*A*C*C
1079	A*T*C*C*A*T*C*C*A*T*C*C*A*T*C*T*A*C*C*T
1080	T*C*C*A*T*C*C*A*T*C*C*A*T*C*T*A*C*C*T*A
1081	C*C*A*T*C*C*A*T*C*C*A*T*C*T*A*C*C*T*A*T
1082	C*A*T*C*C*A*T*C*C*A*T*C*T*A*C*C*T*A*T*C
1083	A*T*C*C*A*T*C*C*A*T*C*T*A*C*C*T*A*T*C*T
1084	T*C*C*A*T*C*C*A*T*C*T*A*C*C*T*A*T*C*T*A
1085	C*C*A*T*C*C*A*T*C*T*A*C*C*T*A*T*C*T*A*C
1086	C*A*T*C*C*A*T*C*T*A*C*C*T*A*T*C*T*A*C*C
1087	A*T*C*C*A*T*C*T*A*C*C*T*A*T*C*T*A*C*C*C
1088	T*C*C*A*T*C*T*A*C*C*T*A*T*C*T*A*C*C*C*A
1089	C*C*A*T*C*T*A*C*C*T*A*T*C*T*A*C*C*C*A*T
1090	C*A*T*C*T*A*C*C*T*A*T*C*T*A*C*C*C*A*T*C
1091	A*T*C*T*A*C*C*T*A*T*C*T*A*C*C*C*A*T*C*T
1092	T*C*T*A*C*C*T*A*T*C*T*A*C*C*C*A*T*C*T*G
1093	C*T*A*C*C*T*A*T*C*T*A*C*C*C*A*T*C*T*G*A
1094	T*A*C*C*T*A*T*C*T*A*C*C*C*A*T*C*T*G*A*C
1095	A*C*C*T*A*T*C*T*A*C*C*C*A*T*C*T*G*A*C*T
1096	C*C*T*A*T*C*T*A*C*C*C*A*T*C*T*G*A*C*T*A
1097	C*T*A*T*C*T*A*C*C*C*A*T*C*T*G*A*C*T*A*T
1098	T*A*T*C*T*A*C*C*C*A*T*C*T*G*A*C*T*A*T*C
1099	A*T*C*T*A*C*C*C*A*T*C*T*G*A*C*T*A*T*C*A
1100	T*C*T*A*C*C*C*A*T*C*T*G*A*C*T*A*T*C*A*A

1101	C*T*A*C*C*C*A*T*C*T*G*A*C*T*A*T*C*A*A*C
1102	T*A*C*C*C*A*T*C*T*G*A*C*T*A*T*C*A*A*C*A
1103	A*C*C*C*A*T*C*T*G*A*C*T*A*T*C*A*A*C*A*A
1104	C*C*C*A*T*C*T*G*A*C*T*A*T*C*A*A*C*A*A*A
1105	C*A*C*C*T*A*T*C*T*A*C*T*C*A*A*T*C*T*T*C
1106	A*C*C*T*A*T*C*T*A*C*T*C*A*A*T*C*T*T*C*C
1107	C*C*T*A*T*C*T*A*C*T*C*A*A*T*C*T*T*C*C*T
1108	C*C*T*T*C*T*A*A*T*A*A*C*T*C*A*A*C*C*A*C
1109	A*A*T*A*A*C*T*C*A*A*C*C*A*C*A*C*T*T*C*C
1110	A*T*A*A*C*T*C*A*A*C*C*A*C*A*C*T*T*C*C*A
1111	T*A*A*C*T*C*A*A*C*C*A*C*A*C*T*T*C*C*A*T
1112	A*A*C*T*C*A*A*C*C*A*C*A*C*T*T*C*C*A*T*C
1113	A*C*T*C*A*A*C*C*A*C*A*C*T*T*C*C*A*T*C*C
1114	C*T*C*A*A*C*C*A*C*A*C*T*T*C*C*A*T*C*C*A
1115	T*C*A*A*C*C*A*C*A*C*T*T*C*C*A*T*C*C*A*T
1116	C*A*A*C*C*A*C*A*C*T*T*C*C*A*T*C*C*A*T*C
1117	A*A*C*C*A*C*A*C*T*T*C*C*A*T*C*C*A*T*C*C
1118	A*C*C*A*C*A*C*T*T*C*C*A*T*C*C*A*T*C*C*C
1119	C*C*A*C*A*C*T*T*C*C*A*T*C*C*A*T*C*C*C*A
1120	C*A*C*A*C*T*T*C*C*A*T*C*C*A*T*C*C*C*A*T
1121	A*C*A*C*T*T*C*C*A*T*C*C*A*T*C*C*C*A*T*C
1122	C*A*C*T*T*C*C*A*T*C*C*A*T*C*C*C*A*T*C*C
1123	A*C*T*T*C*C*A*T*C*C*A*T*C*C*C*A*T*C*C*A
1124	C*T*T*C*C*A*T*C*C*A*T*C*C*C*A*T*C*C*A*A
1125	T*T*C*C*A*T*C*C*A*T*C*C*C*A*T*C*C*A*A*T
1126	T*C*C*A*T*C*C*A*T*C*C*C*A*T*C*C*A*A*T*A
1127	C*C*A*T*C*C*A*T*C*C*C*A*T*C*C*A*A*T*A*C
1128	C*A*T*C*C*A*T*C*C*C*A*T*C*C*A*A*T*A*C*A
1129	A*T*C*C*A*T*C*C*C*A*T*C*C*A*A*T*A*C*A*A
1130	T*C*C*A*T*C*C*C*A*T*C*C*A*A*T*A*C*A*A*C
1131	C*C*A*T*C*C*C*A*T*C*C*A*A*T*A*C*A*A*C*T
1132	C*A*T*C*C*C*A*T*C*C*A*A*T*A*C*A*A*C*T*T
1133	A*C*A*A*C*T*T*A*A*T*C*T*G*C*T*C*A*T*C*C
1134	C*A*A*C*T*T*A*A*T*C*T*G*C*T*C*A*T*C*C*A
1135	A*C*T*T*A*A*T*C*T*G*C*T*C*A*T*C*C*A*A*C
1136	C*T*T*A*A*T*C*T*G*C*T*C*A*T*C*C*A*A*C*A

1137	A*T*C*T*G*C*T*C*A*T*C*C*A*A*C*A*T*T*T*C
1138	T*C*T*G*C*T*C*A*T*C*C*A*A*C*A*T*T*T*C*A
1139	C*T*G*C*T*C*A*T*C*C*A*A*C*A*T*T*T*C*A*T
1140	T*G*C*T*C*A*T*C*C*A*A*C*A*T*T*T*C*A*T*C
1141	G*C*T*C*A*T*C*C*A*A*C*A*T*T*T*C*A*T*C*T
1142	C*C*A*A*C*A*T*T*T*C*A*T*C*T*A*T*C*C*A*C
1143	C*A*A*C*A*T*T*T*C*A*T*C*T*A*T*C*C*A*C*C
1144	A*A*C*A*T*T*T*C*A*T*C*T*A*T*C*C*A*C*C*C
1145	A*C*A*T*T*T*C*A*T*C*T*A*T*C*C*A*C*C*C*A
1146	C*A*T*T*T*C*A*T*C*T*A*T*C*C*A*C*C*C*A*G
1147	A*T*T*T*C*A*T*C*T*A*T*C*C*A*C*C*C*A*G*T
1148	T*T*T*C*A*T*C*T*A*T*C*C*A*C*C*C*A*G*T*C
1149	T*T*C*A*T*C*T*A*T*C*C*A*C*C*C*A*G*T*C*A
1150	T*C*A*T*C*T*A*T*C*C*A*C*C*C*A*G*T*C*A*A
1151	C*A*T*C*T*A*T*C*C*A*C*C*C*A*G*T*C*A*A*T
1152	A*T*C*T*A*T*C*C*A*C*C*C*A*G*T*C*A*A*T*C
1153	T*C*T*A*T*C*C*A*C*C*C*A*G*T*C*A*A*T*C*A
1154	C*T*A*T*C*C*A*C*C*C*A*G*T*C*A*A*T*C*A*T
1155	T*A*T*C*C*A*C*C*C*A*G*T*C*A*A*T*C*A*T*C
1156	A*T*C*C*A*C*C*C*A*G*T*C*A*A*T*C*A*T*C*T
1157	T*C*C*A*C*C*C*A*G*T*C*A*A*T*C*A*T*C*T*A
1158	C*C*A*C*C*C*A*G*T*C*A*A*T*C*A*T*C*T*A*T
1159	C*A*C*C*C*A*G*T*C*A*A*T*C*A*T*C*T*A*T*C
1160	A*C*C*C*A*G*T*C*A*A*T*C*A*T*C*T*A*T*C*C
1161	C*C*C*A*G*T*C*A*A*T*C*A*T*C*T*A*T*C*C*A
1162	C*C*A*G*T*C*A*A*T*C*A*T*C*T*A*T*C*C*A*G
1163	C*A*G*T*C*A*A*T*C*A*T*C*T*A*T*C*C*A*G*C
1164	A*G*T*C*A*A*T*C*A*T*C*T*A*T*C*C*A*G*C*A
1165	G*T*C*A*A*T*C*A*T*C*T*A*T*C*C*A*G*C*A*A
1166	C*A*A*T*C*A*T*C*T*A*T*C*C*A*G*C*A*A*T*C
1167	C*A*T*C*T*A*T*C*C*A*G*C*A*A*T*C*T*A*T*C
1168	A*T*C*C*A*G*C*A*A*T*C*T*A*T*C*T*A*T*C*C
1169	T*C*C*A*G*C*A*A*T*C*T*A*T*C*T*A*T*C*C*A
1170	C*C*A*G*C*A*A*T*C*T*A*T*C*T*A*T*C*C*A*C
1171	C*A*G*C*A*A*T*C*T*A*T*C*T*A*T*C*C*A*C*T
1172	A*G*C*A*A*T*C*T*A*T*C*T*A*T*C*C*A*C*T*C

1173	G*C*A*A*T*C*T*A*T*C*T*A*T*C*C*A*C*T*C*A
1174	C*T*A*T*C*T*A*T*C*C*A*C*T*C*A*T*C*A*A*G
1175	A*T*C*C*A*C*T*C*A*T*C*A*A*G*T*T*A*T*C*C
1176	T*C*C*A*C*T*C*A*T*C*A*A*G*T*T*A*T*C*C*A
1177	C*C*A*C*T*C*A*T*C*A*A*G*T*T*A*T*C*C*A*T
1178	C*A*C*T*C*A*T*C*A*A*G*T*T*A*T*C*C*A*T*C
1179	A*C*T*C*A*T*C*A*A*G*T*T*A*T*C*C*A*T*C*C
1180	C*T*C*A*T*C*A*A*G*T*T*A*T*C*C*A*T*C*C*A
1181	C*A*T*C*A*A*G*T*T*A*T*C*C*A*T*C*C*A*T*C
1182	C*C*A*T*C*A*T*C*T*A*A*C*A*A*T*T*A*C*C*C
1183	C*A*T*C*A*T*C*T*A*A*C*A*A*T*T*A*C*C*C*C
1184	A*T*C*A*T*C*T*A*A*C*A*A*T*T*A*C*C*C*C*C
1185	T*C*A*T*C*T*A*A*C*A*A*T*T*A*C*C*C*C*C*A
1186	C*A*T*C*T*A*A*C*A*A*T*T*A*C*C*C*C*C*A*A
1187	A*C*A*A*T*T*A*C*C*C*C*C*A*A*A*T*T*C*A*C
1188	C*A*A*T*T*A*C*C*C*C*C*A*A*A*T*T*C*A*C*C
1189	C*C*A*T*C*C*C*A*T*A*C*A*T*T*G*A*T*C*C*G
1190	C*A*T*C*C*C*A*T*A*C*A*T*T*G*A*T*C*C*G*C
1191	A*T*C*C*C*A*T*A*C*A*T*T*G*A*T*C*C*G*C*A
1192	T*C*C*C*A*T*A*C*A*T*T*G*A*T*C*C*G*C*A*A
1193	C*C*C*A*T*A*C*A*T*T*G*A*T*C*C*G*C*A*A*C
1194	C*C*A*T*A*C*A*T*T*G*A*T*C*C*G*C*A*A*C*T
1195	C*A*T*A*C*A*T*T*G*A*T*C*C*G*C*A*A*C*T*T
1196	C*A*T*T*G*A*T*C*C*G*C*A*A*C*T*T*A*A*T*C
1197	A*T*T*G*A*T*C*C*G*C*A*A*C*T*T*A*A*T*C*C
1198	T*T*G*A*T*C*C*G*C*A*A*C*T*T*A*A*T*C*C*A
1199	T*G*A*T*C*C*G*C*A*A*C*T*T*A*A*T*C*C*A*C
1200	G*A*T*C*C*G*C*A*A*C*T*T*A*A*T*C*C*A*C*C
1201	A*T*C*C*G*C*A*A*C*T*T*A*A*T*C*C*A*C*C*T
1202	T*C*C*G*C*A*A*C*T*T*A*A*T*C*C*A*C*C*T*A
1203	C*C*G*C*A*A*C*T*T*A*A*T*C*C*A*C*C*T*A*C
1204	C*G*C*A*A*C*T*T*A*A*T*C*C*A*C*C*T*A*C*C
1205	C*C*A*T*T*C*A*T*C*C*A*C*C*C*A*C*C*C*A*T
1206	C*C*C*A*T*T*C*A*T*C*C*A*T*T*T*G*T*C*C*A
1207	C*C*A*T*C*A*T*C*C*A*T*C*T*A*G*C*C*A*C*G
1208	C*A*T*C*A*T*C*C*A*T*C*T*A*G*C*C*A*C*G*A

1209	A*T*C*A*T*C*C*A*T*C*T*A*G*C*C*A*C*G*A*A
1210	T*C*A*T*C*C*A*T*C*T*A*G*C*C*A*C*G*A*A*T
1211	C*A*T*C*C*A*T*C*T*A*G*C*C*A*C*G*A*A*T*C
1212	A*T*C*C*A*T*C*T*A*G*C*C*A*C*G*A*A*T*C*T
1213	T*C*C*A*T*C*T*A*G*C*C*A*C*G*A*A*T*C*T*A
1214	C*C*A*T*C*T*A*G*C*C*A*C*G*A*A*T*C*T*A*C
1215	C*A*T*C*T*A*G*C*C*A*C*G*A*A*T*C*T*A*C*C
1216	A*T*C*T*A*G*C*C*A*C*G*A*A*T*C*T*A*C*C*C
1217	T*C*T*A*G*C*C*A*C*G*A*A*T*C*T*A*C*C*C*A
1218	C*T*A*G*C*C*A*C*G*A*A*T*C*T*A*C*C*C*A*C
1219	T*A*G*C*C*A*C*G*A*A*T*C*T*A*C*C*C*A*C*C
1220	A*G*C*C*A*C*G*A*A*T*C*T*A*C*C*C*A*C*C*A
1221	G*C*C*A*C*G*A*A*T*C*T*A*C*C*C*A*C*C*A*A
1222	C*C*A*C*G*A*A*T*C*T*A*C*C*C*A*C*C*A*A*C
1223	C*A*C*G*A*A*T*C*T*A*C*C*C*A*C*C*A*A*C*T
1224	A*C*G*A*A*T*C*T*A*C*C*C*A*C*C*A*A*C*T*C
1225	C*G*A*A*T*C*T*A*C*C*C*A*C*C*A*A*C*T*C*A
1226	G*A*C*A*T*A*C*C*C*A*G*A*C*A*C*A*A*A*C*G
1227	A*C*A*T*A*C*C*C*A*G*A*C*A*C*A*A*A*C*G*G
1228	C*A*T*A*C*C*C*A*G*A*C*A*C*A*A*A*C*G*G*C
1229	G*C*C*A*G*A*A*A*G*A*G*G*A*A*G*A*G*C*T*G
1230	C*C*A*G*A*A*A*G*A*G*G*A*A*G*A*G*C*T*G*G
1231	G*G*C*A*G*G*C*A*G*G*A*A*T*G*G*T*G*A*G*T
1232	G*C*A*G*G*C*A*G*G*A*A*T*G*G*T*G*A*G*T*G
1233	C*A*G*G*C*A*G*G*A*A*T*G*G*T*G*A*G*T*G*G
1234	A*G*G*C*A*G*G*A*A*T*G*G*T*G*A*G*T*G*G*A
1235	G*G*C*A*G*G*A*A*T*G*G*T*G*A*G*T*G*G*A*A
1236	G*C*A*G*G*A*A*T*G*G*T*G*A*G*T*G*G*A*A*G
1237	C*A*G*G*A*A*T*G*G*T*G*A*G*T*G*G*A*A*G*T
1238	A*G*G*A*A*T*G*G*T*G*A*G*T*G*G*A*A*G*T*G
1239	G*G*A*A*T*G*G*T*G*A*G*T*G*G*A*A*G*T*G*G
1240	G*A*A*T*G*G*T*G*A*G*T*G*G*A*A*G*T*G*G*C
1241	A*A*T*G*G*T*G*A*G*T*G*G*A*A*G*T*G*G*C*A
1242	A*T*G*G*T*G*A*G*T*G*G*A*A*G*T*G*G*C*A*T
1243	T*G*G*T*G*A*G*T*G*G*A*A*G*T*G*G*C*A*T*G
1244	G*G*T*G*A*G*T*G*G*A*A*G*T*G*G*C*A*T*G*G

1245	T*C*A*T*T*C*A*T*C*T*G*T
1246	C*T*C*A*T*T*C*A*T*C*T*G
1247	A*C*T*C*A*T*T*C*A*T*C*T
1248	C*A*C*T*C*A*T*T*C*A*T*C
1249	T*C*A*C*T*C*A*T*T*C*A*T
1250	A*T*C*A*C*T*C*A*T*T*C*A
1251	C*A*T*C*A*C*T*C*A*T*T*C
1252	T*C*A*T*C*A*C*T*C*A*T*T
1253	C*T*C*A*T*C*A*C*T*C*A*T
1254	C*A*C*T*C*A*T*T*C*A*T*C*T*G*T
1255	T*C*A*C*T*C*A*T*T*C*A*T*C*T*G
1256	A*T*C*A*C*T*C*A*T*T*C*A*T*C*T
1257	C*A*T*C*A*C*T*C*A*T*T*C*A*T*C
1258	T*C*A*TC*A*C*T*C*A*T*T*C*A*T
1259	C*T*C*A*T*C*A*C*T*C*A*T*T*C*A
1260	T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T
1261	A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G
1262	C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T
1263	T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C
1264	C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T
1265	A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T
1266	C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G
1267	T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T
1268	C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C
1269	C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T
1270	T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G
1271	C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T
1272	T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T
1273	C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G
1274	C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T*T
1275	A*C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T
1276	C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T*T*C
1277	A*C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T*T
1278	T*A*C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T
1279	C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T*T*C*A
1280	A*C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T*T*C

1281	T*A*C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T*T
1282	C*T*A*C*T*C*A*T*C*A*C*T*C*A*T*T*C*A*T*C*T*G*T

[0099] ASO 55 (SEQ ID. NO. 55) variations with a motif where each nucleobase has a 2'-OCH_2CH_2-OCH_3 group (i.e., 2'-MOE) and each internucleoside linkage is a phosphorothioate linkage are alternately labeled as below, to be more clearly identifiable as variations of ASO 55 with the aforementioned motif and internucleoside linkage:

SEQ ID NO.	Alternate Identifier
1245	hUNC13A-ASO55_12-1
1246	hUNC13A-ASO55_12-2
1247	hUNC13A-ASO55_12-3
1248	hUNC13A-ASO55_12-4
1249	hUNC13A-ASO55_12-5
1250	hUNC13A-ASO55_12-6
1251	hUNC13A-ASO55_12-7
1252	hUNC13A-ASO55_12-8
1253	hUNC13A-ASO55_12-9
1254	hUNC13A-ASO55_15-1
1255	hUNC13A-ASO55_15-2
1256	hUNC13A-ASO55_15-3
1257	hUNC13A-ASO55_15-4
1258	hUNC13A-ASO55_15-5
1259	hUNC13A-ASO55_15-6
1260	hUNC13A-ASO55_16-1
1261	hUNC13A-ASO55_16-2
1262	hUNC13A-ASO55_16-3
1263	hUNC13A-ASO55_16-4
1264	hUNC13A-ASO55_16-5
1265	hUNC13A-ASO55_17-1
1266	hUNC13A-ASO55_17-2
1267	hUNC13A-ASO55_17-3
1268	hUNC13A-ASO55_17-4
1269	hUNC13A-ASO55_18-1

1270	hUNC13A-ASO55_18-2
1271	hUNC13A-ASO55_18-3
1272	hUNC13A-ASO55_19-1
1273	hUNC13A-ASO55_19-2
1274	hUNC13A-ASO55_21-1
1275	hUNC13A-ASO55_21-2
1276	hUNC13A-ASO55_22-1
1277	hUNC13A-ASO55_22-2
1278	hUNC13A-ASO55_22-3
1279	hUNC13A-ASO55_23-1
1280	hUNC13A-ASO55_23-2
1281	hUNC13A-ASO55_23-3
1282	hUNC13A-ASO55_23-4

[0100] The UNC13A antisense or inhibitory nucleic acids of the disclosure can inhibit the expression of the cryptic exon between canonical exons 20 and 21 of UNC13A and increase UNC13A protein expression. The UNC13A antisense or inhibitory nucleic acids can include any combination of the oligonucleotides set forth in Table 2 and sequences that are 98%-99% identical thereto.

[0101] In one embodiment, the ASO or oligonucleotide is 100% complementary to SEQ ID NO: 1283 (chr19:17641557 – 17642844).

SEQ ID NO: 1283	GUGAGGGUCA UUGCUCGGCC CCUCCCAUGC CACUUCCACU CACCAUUCCU GCCUGCCCAG CUCUUCCUCU UUCUGGCCAC ACCAUCCACA CUCUCCUGGC CCUCUGAGAC UGCCCGCCAU GCCAUUCCCU UUACCUGGAA AACUCCUCCC UAUCCAUCAA AGUCCAGAUU CAGGGUCACC UCCUCUGGGA AGCCCACCUU GGCCUCCAGG UUGACUCUCA CUACUCAUCA UCAGGUUCUU CCUUCUAUUC CAGCCCUAAC CACUCAGGAU UGGGCCGUUU GUGUCUGGGU AUGUCUCUUC CAGCUGCCUG GGUUUCCUGG AAAGAACUCU UAUCCCCAGG AACUAGUUUG

	UUGAAUAAAU GCUGGUGAAU GAAUGAAUGA UUGAACAGAU GAAUGAGUGA UGAGUAGAUA AAAGGAUGGA UGGAGAGAUG GGUGAGUACA UGGAUGGAUA GAUGGAUGAG UUGGUGGGUA GAUUCGUGGC UAGAUGGAUG AUGGAUGGAU GGACAGAUGG AUGGAUAUAU GAUUGAACUA UUGAAAGUAU AGAUGUAUGG AUGGGUGAAU UUGGGGGUAA UUGUUAGAUG AUGGAUGAGU AUAGAUGAAU GAUGGAUGGA UAACUUGAUG AGUGGAUAGA UAGAUUGCUG GAUAGAUGAU UGACUGGGUG GAUAGAUGAA AUGUUGGAUG AGCAGAUUAA GUUGUAUUGG AUGGGAUGGA UGGAAGUGUG GUUGAGUUAU UAGAAGGAAG AUUGAGUAGA UAGGUGAAUU UGUUGAUAGU CAGAUGGGUA GAUAGGUAGA UGGAUGGAUG GAUGGAUGGA UGUAUAGGCA GAUGGACAAA UGGAUGAAUG GGUGGGUGGA UGAAUGGAAG GAUGUGUGGU UGAACUAUUG CAAGUAUUGA UAAUUGGGUU CAUAAUUUCU GAAUAUUUAG AUGGAUGGUU GUGAGUGGCU GGUGGACAGA CGAAAAAUGG AUGGUUGGAU AAAUUGAUGG GUGGAUGGAU GGUUGGUUGU AUGAAAGAAU GAAUGAUUGG GUAGGUGGAU UAAGUUGCGG AUCAAUGUAU GGGAUGGAUG AAUGGAUGGA UGGAUGGAUG UGUGGUUGAA UUACUGAAAG GUUGGAAGAG UGGAUGGGUG AAAUUUGGGG UAGUUAGAUG GGUGGGUGUG UGGAUGGAUA AAAGAGUAGA UGAAUGAAUU AAUGAAUAAA CAGGCAGAUG GAUGAUGUAA GCUGCCCCAG ACCCUGGGAC CUCUGACCCC CGGCGACCCC UUGCACUCUC CAUGACACUU UCUCUCCCAU GGUGGCAG

[0102] Methods of treatment may include any number of modes of administering a disclosed composition. Modes of administration may include aqueous, lipid, oily or other solutions, solutions in simulated cerebrospinal fluid, emulsions such as oil-in-water emulsions, liposomes,

aqueous or oily suspensions and the like. Typically, an ASO of the disclosure will be administered directly to the CNS of the subject. Accordingly, the formulation or composition will be sterile and more preferably be suitable for injection. The following formulations and methods are merely exemplary and are in no way limiting.

[0103] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which may contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that may include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations may be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and may be stored as liquids or in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets. The formulation may be provided in a pre-filled syringe.

[0104] Additional therapeutic agent(s) may be administered simultaneously or sequentially with the disclosed one or more antisense or inhibitory nucleic acids and compositions. Sequential administration includes administration before or after the disclosed one or more antisense or inhibitory nucleic acids or compositions. In some embodiments, the additional therapeutic agent or agents may be administered in the same composition as the disclosed one or more antisense or inhibitory nucleic acids. In other embodiments, there may be an interval of time between administration of the additional therapeutic agent and the disclosed one or more antisense or inhibitory nucleic acids. In some embodiments, administration of an additional therapeutic agent with a disclosed one or more antisense or inhibitory nucleic acids may allow lower doses of the

other therapeutic agents and/or administration at less frequent intervals. When used in combination with one or more other active ingredients, the one or more antisense or inhibitory nucleic acids of the disclosure and the other active ingredients may be used in lower doses than when each is used singly. Accordingly, the pharmaceutical compositions of the disclosure include those that contain one or more other active ingredients, in addition to one or more antisense or inhibitory nucleic acids of the disclosure. The above combinations include combinations of one or more antisense or inhibitory nucleic acids of the disclosure not only with one other active compound, but also with two or more other active compounds. For example, the compound of the disclosure may be combined with a variety of drugs to treat neurological diseases. The antisense oligonucleotide may be covalently linked to another oligonucleotide, such as one with a target other than PIKFYVE. The antisense oligonucleotide may be covalently linked to an antibody.

[0105] The disclosed one or more antisense or inhibitory nucleic acids can be combined with the following, but are not limited, anticholinergic drugs, anticonvulsants, antidepressants, benzodiazepines, decongestants, muscle relaxants, pain medications, and/or stimulants. Additional types of therapy and treatment include, but are not limited to digital communication devices, feeding tubes, mechanical ventilation, nutritional support, deep brain stimulation, occupational therapy, physical therapy, and/or speech therapy.

[0106] The disclosed composition(s) may be incorporated into a pharmaceutical composition suitable for administration to a subject (such as a patient, which may be a human or non-human). The pharmaceutical compositions may comprise a carrier (e.g., a pharmaceutically acceptable carrier). Any suitable carrier can be used within the context of the disclosure, and such carriers are well known in the art. The choice of carrier will be determined, in part, by the particular use

of the composition (e.g., administration to an animal) and the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the composition of the present invention.

[0107] The pharmaceutical compositions may include a therapeutically effective amount or a prophylactically effective amount of the antisense oligonucleotide. A therapeutically effective amount of the composition may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the composition to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of one or more antisense or inhibitory nucleic acids of the disclosure are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0108] The pharmaceutical compositions may include one or more pharmaceutically acceptable carriers. The term "pharmaceutically acceptable carrier," as used herein, means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as, but not limited to, lactose, glucose and sucrose; starches such as, but not limited to, corn starch and potato starch; cellulose and its derivatives such as, but not limited to, sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as, but not limited to, cocoa butter and suppository waxes; oils such as, but not limited to, peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and

soybean oil; glycols; such as propylene glycol; esters such as, but not limited to, ethyl oleate and ethyl laurate; agar; buffering agents such as, but not limited to, magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogenfree water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as, but not limited to, sodium lauryl sulfate and magnesium stearate, as well as releasing agents, coating agents, preservatives and antioxidants may also be present in the composition, according to the judgment of the formulator.

[0109] The route by which the disclosed one or more antisense or inhibitory nucleic acids are administered, and the form of the composition will dictate the type of carrier to be used.

[0110] The pharmaceutical compositions of the disclosure can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be parenteral including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal, intracerebroventricular, or intraventricular, administration. In one embodiment the antisense or inhibitory nucleic acid is administered by intravenous, intraperitoneal, or as a bolus injection or administered directly into the target organ. In another embodiment, the antisense or inhibitory nucleic acid is administered intrathecally or intra-cerebroventricular as a bolus injection.

[0111] Carriers for systemic administration typically include at least one of solvents, diluents, lubricants, binders, disintegrants, colorants, flavors, sweeteners, antioxidants, preservatives, glidants, solvents, suspending agents, wetting agents, surfactants, combinations thereof, and others. All carriers are optional in the compositions.

[0112] Suitable diluents include sugars such as glucose, lactose, dextrose, and sucrose; diols such as propylene glycol; calcium carbonate; sodium carbonate; sugar alcohols, such as glycerin; mannitol; and sorbitol.

[0113] Suitable lubricants include silica, talc, stearic acid and its magnesium salts and calcium salts, calcium sulfate; and liquid lubricants such as polyethylene glycol and vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma. The amount of lubricant(s) in a systemic or topical composition is typically about 5 to about 10%.

[0114] Suitable binders include polyvinyl pyrrolidone; magnesium aluminum silicate; starches such as corn starch and potato starch; gelatin; tragacanth; and cellulose and its derivatives, such as sodium carboxymethylcellulose, ethyl cellulose, methylcellulose, microcrystalline cellulose, and sodium carboxymethylcellulose. The amount of binder(s) in a systemic composition is typically about 5 to about 50%.

[0115] Suitable disintegrants include agar, alginic acid and the sodium salt thereof, effervescent mixtures, croscarmelose, crospovidone, sodium carboxymethyl starch, sodium starch glycolate, clays, and ion exchange resins. The amount of disintegrant(s) in a systemic composition is typically about 0.1 to about 10%.

[0116] Suitable colorants include a colorant such as an FD&C dye. When used, the amount of colorant in a systemic or topical composition is typically about 0.005 to about 0.1%.

[0117] Suitable flavors include menthol, peppermint, and fruit flavors. The amount of flavor(s), when used, in a systemic or topical composition is typically about 0.1 to about 1.0%.

[0118] Suitable antioxidants include butylated hydroxyanisole ("BHA"), butylated hydroxytoluene ("BHT"), and vitamin E. The amount of antioxidant(s) in a systemic or topical composition is typically about 0.1 to about 5%.

[0119] Suitable preservatives include benzalkonium chloride, methyl paraben and sodium benzoate. The amount of preservative(s) in a systemic or topical composition is typically about 0.01 to about 5%.

[0120] Suitable glidants include silicon dioxide. The amount of glidant(s) in a systemic or topical composition is typically about 1 to about 5%.

[0121] Suitable solvents include water, isotonic saline, ethyl oleate, glycerine, hydroxylated castor oils, alcohols such as ethanol, and phosphate buffer solutions. The amount of solvent(s) in a systemic or topical composition is typically from about 0 to about 100%.

[0122] Suitable suspending agents include AVICEL RC-591 (from FMC Corporation of Philadelphia, PA) and sodium alginate. The amount of suspending agent(s) in a systemic or topical composition is typically about 1 to about 8%.

[0123] Suitable surfactants include lecithin, Polysorbate 80, and sodium lauryl sulfate, and the TWEENS from Atlas Powder Company of Wilmington, Delaware. Suitable surfactants include those disclosed in the C.T.F.A. Cosmetic Ingredient Handbook, 1992, pp.587-592; Remington's Pharmaceutical Sciences, 15th Ed. 1975, pp. 335-337; and McCutcheon's Volume 1, Emulsifiers & Detergents, 1994, North American Edition, pp. 236-239. The amount of surfactant(s) in the systemic or topical composition is typically about 0.1% to about 5%.

[0124] Compositions and formulations for parenteral, intrathecal, intra-cerebroventricular, or intraventricular administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients. For example, an intrathecal cerebrospinal fluid (CSF) catheter can be used to deliver antisense formulations of the disclosure. The catheter can be inserted at the L3 or L4 vertebrae. The distal tip of the catheter

extends within the intrathecal space to approximately the L1 vertebrae. Antisense oligonucleotides are dissolved in saline, are sterilized by filtration, and are administered at 0.33 ml/min in a 1.0 ml volume followed by a 0.5 ml sterile water flush. Total infusion time is 4.5 min.

[0125] Although the amounts of components in the systemic compositions may vary depending on the type of systemic composition prepared, in general, systemic compositions include 0.01% to 50% of active compound and 50% to 99.99% of one or more carriers. Compositions for parenteral administration typically include 0.1% to 10% of actives and 90% to 99.9% of a carrier including a diluent and a solvent.

[0126] The amount of the carrier employed in conjunction with a disclosed compound is sufficient to provide a practical quantity of composition for administration per unit dose of the medicament. Techniques and compositions for making dosage forms useful in the methods of this invention are described in the following references: Modern Pharmaceutics, Chapters 9 and 10, Banker & Rhodes, eds. (1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms, 2nd Ed., (1976).

[0127] In vivo testing of candidate antisense or inhibitory nucleic acids may be conducted by means known to one of ordinary skill in the art. For example, the candidate one or more antisense or inhibitory nucleic acids may be administered to a mammal, such as a mouse or a rabbit. The mammal may be administered, by any route deemed appropriate, a dose of a candidate antisense or inhibitory nucleic acids. Conventional methods and criteria can then be used to monitor animals for signs of reduction or improvement of motor neuron activity and/or expression or activity of *UNC13A* gene or protein, respectively. If needed, the results obtained in the presence of the candidate antisense or inhibitory nucleic acids can be compared with

results in control animals that are not treated with the candidate antisense or inhibitory nucleic acids. Dosing studies may be performed in, or in conjunction with, the herein described methods for identifying one or more antisense or inhibitory nucleic acids capable of treating a neurological disease and/or any follow-on testing of candidate antisense or inhibitory nucleic acids in vivo. One of skill in the art of medicine may determine the appropriate dosage of one or more antisense or inhibitory nucleic acids. The dosage may be determined by monitoring the subject for signs of disease inhibition or amelioration. The dosage may be increased or decreased to obtain the desired frequency of treatment. The toxicity and efficacy of one or more antisense or inhibitory nucleic acids may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g. determining the lethal dose to 50% of the population (LD50) and the dose therapeutically effective in 50% of the population (ED50). The dose ratio of LD50/ED50 is the therapeutic index and, indicating the ratio between the toxic and therapeutic effects. A delivery system may be designed to help prevent toxic side effects, by delivering the one or more antisense or inhibitory nucleic acids to specific targets, e.g., delivered specifically to motor or central nervous system neurons. The optimal dose of the one or more antisense or inhibitory nucleic acids may be determined based on results of clinical electrophysiology or electromyography to analyze excitability in peripheral nerves, for example.

[0128] The dosage for use in humans may be determined by evaluating data obtained from animal studies and cell culture assays. The preferred dosage will have little or no toxicity and include the ED50. The dosage may vary depending on the dosage form and route of administration. For any antisense or inhibitory nucleic acid used in the methods described herein, the dosage may be estimated initially in cell culture. A dose may be formulated in animal models that includes the concentration of the test compound which achieves a half maximal

inhibition of symptoms (LD50) as determined in cell culture. Such information obtained from cell cultures and animal models may be used to more accurately determine useful doses in humans.

[0129] The disclosure provides ASOs that suppress expression of a cryptic exon between canonical exons 20 and 21 of UNC13A in human cells. The accompanying data suggest that these ASOs may be capable of preventing neurodegeneration.

Examples

[0130] Example 1

[0131] To assess if patient neurons could recapitulate the cryptic exon inclusion phenotype, the genetic sequencing data of the approximately 80 ALS / FTD patient lines were reviewed, and an ALS patient heterozygous for the UNC13A risk allele was identified. Induced excitatory cortical neurons (iNs) were generated from the patient's iPSCs using the doxycycline-inducible Ngn2 method. Ngn2-iNs express at high levels the telencephalic markers Brn-2, Cux1 and FoxG1, which are characteristic of layer 2/3 excitatory cortical neurons. They form mature pre- and postsynaptic specializations and integrate into existing synaptic networks when transplanted into mouse brains. After generating iNs from this UNC13A risk SNP carrier, quantitative RT-PCR was performed and confirmed the presence of the cryptic exon cryptic exon. Since cryptic exon inclusion is known to only occur in nuclei depleted of TDP-43 postmortem, quantitative RT-PCR was performed on iNS derived from three patient lines in which TDP-43 expression was reduced using siRNA. It was found that the depletion of TDP-43 caused a >1,000 fold increase in cryptic exon levels and significantly reduced levels of the UNC13A regular transcript and protein (FIGs.

3E-3G). Thus, reduced levels of TDP-43 in the context of ALS and FTD disease cause the inclusion of a cryptic exon in UNC13A mRNA that results in lowered UNC13A protein levels.

[0132] Example 2: ASO-Mediated Suppression of Cryptic Exon Inclusion Can Rescue UNC13A Levels in Patient-Derived Neurons

[0133] ASO sequences were identified based on the gene between exon 20 and 21, to tile around the cryptic exon and TDP-43 binding sites. and screened for reduction in cryptic exon inclusion and increase normal transcript expression against a control (NCASO) (data in FIG. 1). Several ASO sequences were identified that potently blocked cryptic exon inclusion (FIGs. 3A-3G), while also identifying a number of ASO sequences that did not.

[0134] FIGs. 1 and 4A-4F are charts of ASOs in UNC13A exon 20-21 to block cryptic exon expression against a control (NCASO).

[0135] The foregoing description and drawings should be considered as illustrative only of the principles of the invention. The invention is not intended to be limited by the preferred embodiment and may be implemented in a variety of ways that will be clear to one of ordinary skill in the art. Numerous applications of the invention will readily occur to those skilled in the art. Therefore, it is not desired to limit the invention to the specific examples disclosed or the exact construction and operation shown and described. Rather, all suitable modifications and equivalents may be resorted to, falling within the scope of the invention. All references cited herein are incorporated by reference.

CLAIMS

1. A single stranded antisense oligonucleotide that suppresses the expression of a cryptic exon in UNC13A, wherein the antisense oligonucleotide has a nucleobase sequence that comprises at least 12 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 1-641.

2. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide has a nucleobase sequence that comprises at least 15 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 1-1282.

3. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide has a nucleobase sequence that comprises at least 15 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 4-6, 9-11, 22-25, 53, 55, 359, or 360.

4. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide has a nucleobase sequence of any one of SEQ ID NOs: 1-1282.

5. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide has a nucleobase sequence of any one of SEQ ID NOs: 4-6, 9-11, 22-25, 53, 55, 359, or 360.

6. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide has a nucleobase sequence of any one of SEQ ID NOs: 645-647, 650-652, 663-666, 694, 696, 1000, and 1001.

7. The antisense oligonucleotide of any one of the preceding claims, wherein the antisense oligonucleotide has 18 to 20 linked nucleosides.

8. The antisense oligonucleotide of any one of the preceding claims, wherein at least one internucleoside linkage is a modified internucleoside linkage.

9. The antisense oligonucleotide of claim 8, wherein at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.

10. The antisense oligonucleotide of claim 8, wherein each modified internucleoside linkage is a phosphorothioate internucleoside linkage.

11. The antisense oligonucleotide of any one of the preceding claims, wherein at least one nucleoside comprises a modified nucleobase.

12. The antisense oligonucleotide of any one of the preceding claims, wherein at least one nucleoside of the antisense oligonucleotide comprises a modified sugar moiety.

13. The antisense oligonucleotide of claim 12, wherein the modified sugar moiety comprises a 2'-O-methoxyethyl group.

14. The antisense oligonucleotide of claim 1, wherein each nucleoside of the antisense oligonucleotide comprises a modified sugar moiety having a 2'-O-methoxyethyl group and each internucleoside linkage is a phosphorothioate internucleoside linkage.

15. The antisense oligonucleotide of any of the preceding claims, wherein the antisense oligonucleotide comprises 15 to 50 nucleosides.

16. A pharmaceutical composition comprising the antisense oligonucleotide of any one of the preceding claims, and a pharmaceutically acceptable carrier, diluent and/or excipient.

17. The pharmaceutical composition of claim 16, wherein the pharmaceutical composition is suitable for parenteral delivery.

18. The pharmaceutical composition of claim 16, wherein the pharmaceutical composition is suitable for intracerebroventricular or intrathecal administration.

19. A method of treating a subject having a neurological or neurodegenerative disease in need thereof comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of any one of claims 16-18.

20. The method of claim 19, wherein the neurological disease is selected from the group consisting of familial and sporadic amyotrophic lateral sclerosis (ALS) and familial and sporadic frontotemporal dementia (FTD).

21. A method of increasing UNC13A protein expression in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of any one of claims 16-18.

22. The method of any one of claims 19-21, wherein the pharmaceutical composition is administered by intracerebroventricular or intrathecal administration.

23. The method of any one of claims 19-21, wherein the subject possesses a SNP variant associated with rs12973192 (C>G), rs12608932 (A>C), or both.

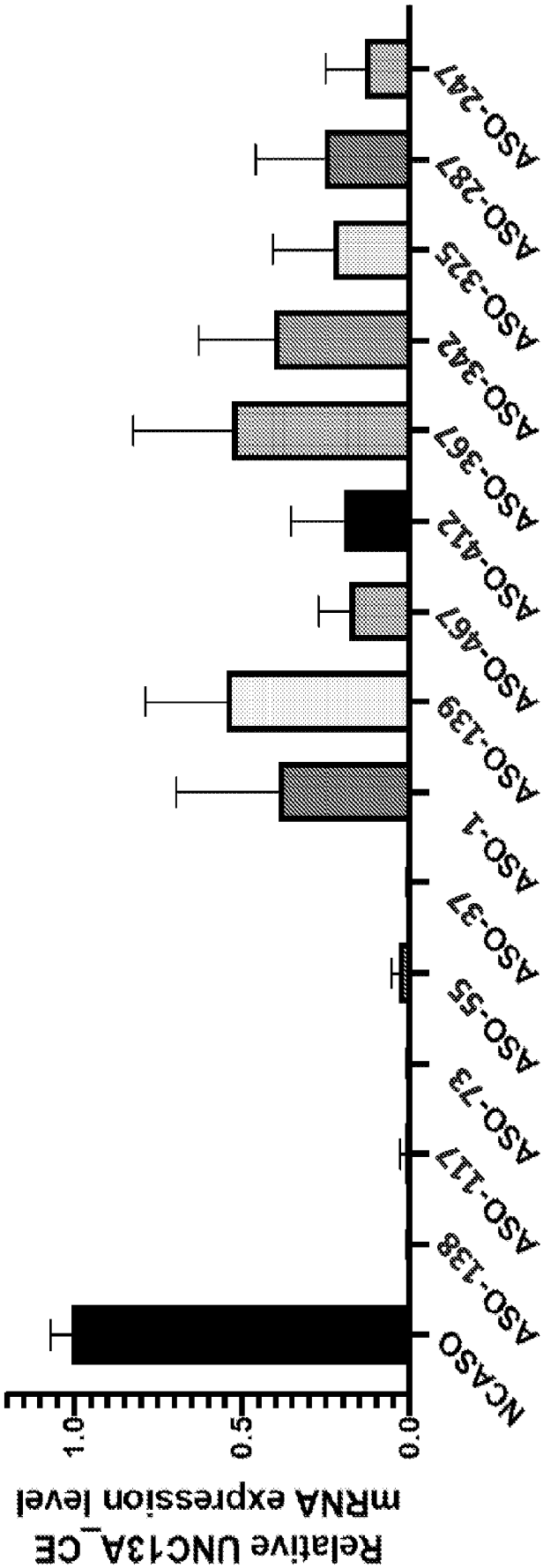
Relative UNC13A_CE
mRNA expression level
1.0
0.5
0.0
NCASO
ASO-138
ASO-117
ASO-73
ASO-55
ASO-37
ASO-1
ASO-139
ASO-467
ASO-412
ASO-367
ASO-342
ASO-325
ASO-287
ASO-247

FIG. 1

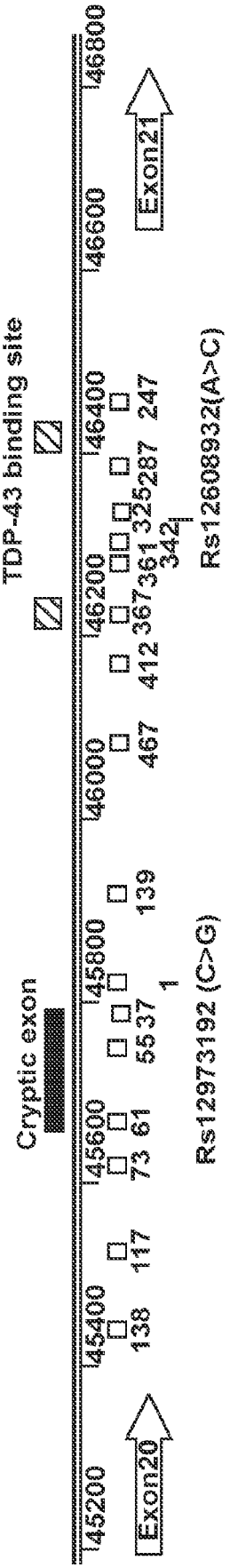
45200
45400
45600
45800
46000
46200
46400
46600
46800
Exon20
Exon21
TDP-43 binding site
Cryptic exon
138
117
73
61
55
37
1
139
467
412
367
361
342
325
287
247
Rs12973192 (C>G)
Rs12608932(A>C)

FIG. 2

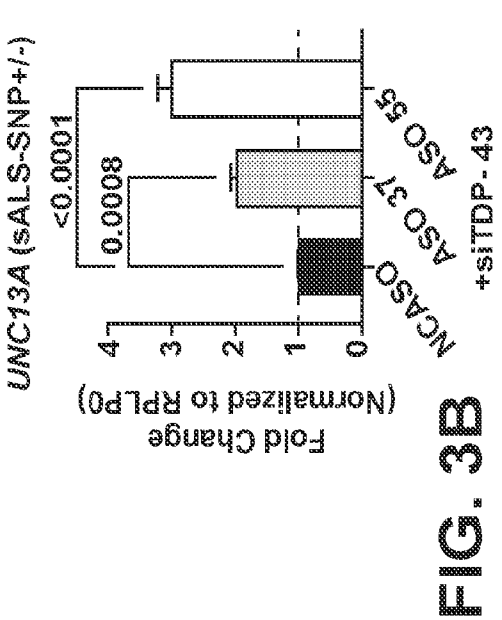

FIG. 3B

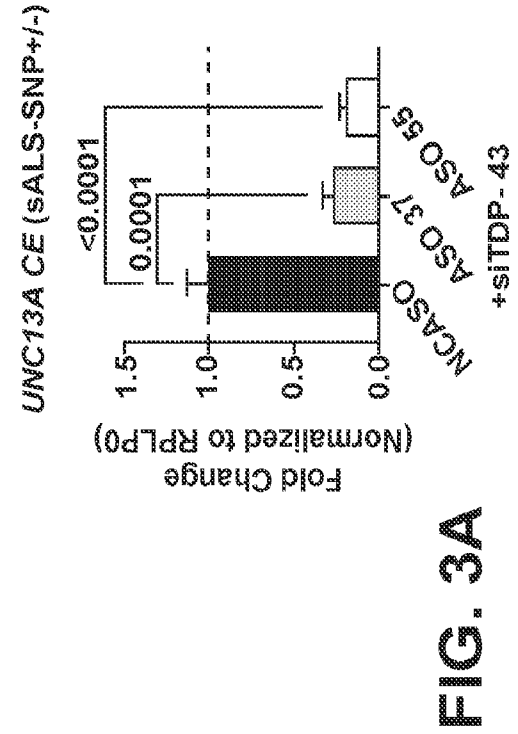

FIG. 3A

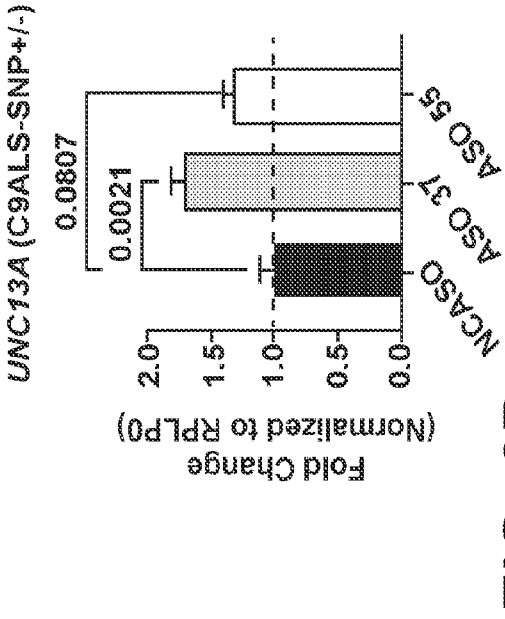

FIG. 3D

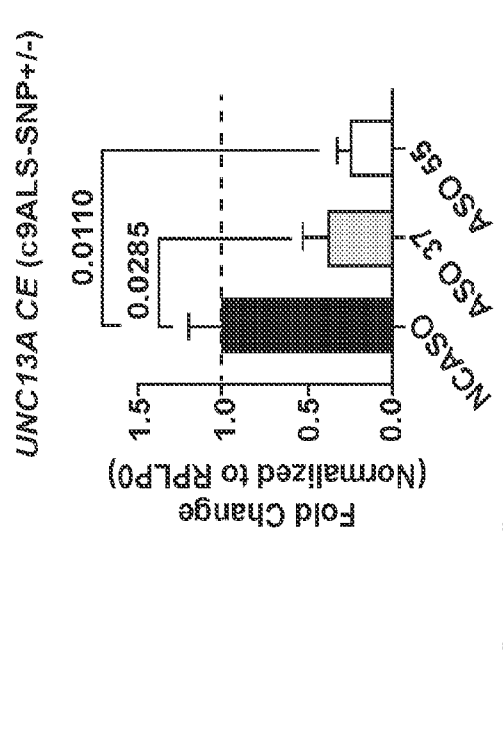

FIG. 3C

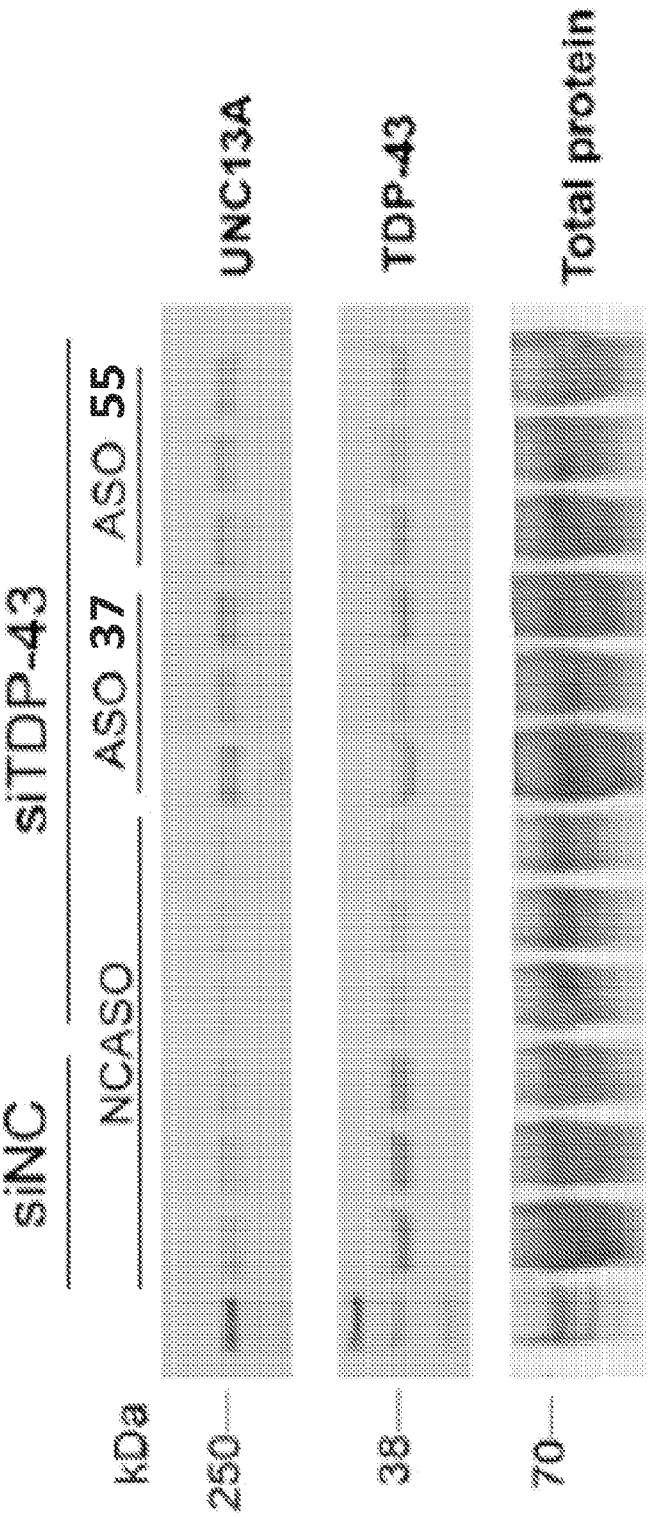
siNC
siTDP-43
NCASO
ASO 37
ASO 55
UNC13A
TDP-43
Total protein
kDa
250
38
70

FIG. 3E

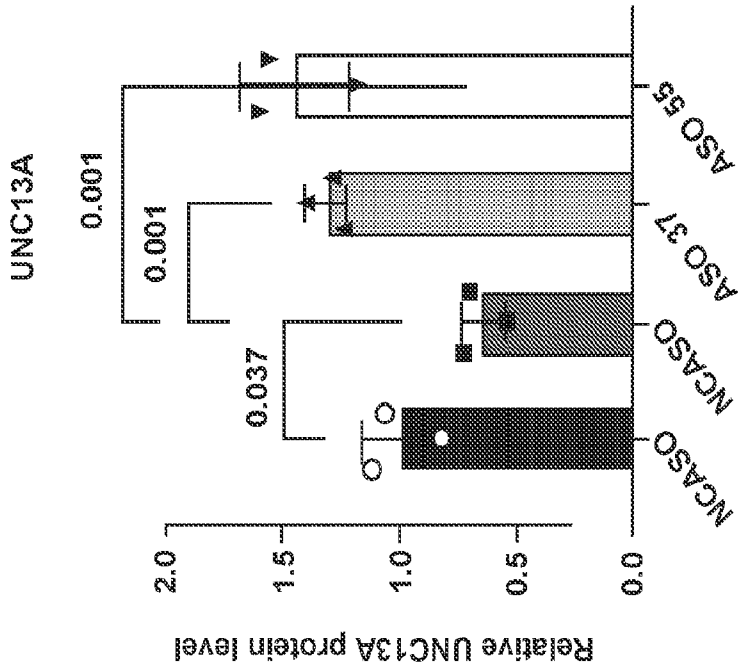
UNC13A
0.001
0.001
0.037
Relative UNC13A protein level
2.0
1.5
1.0
0.5
0.0
NCASO
NCASO
ASO 37
ASO 55
+siNC
+siTDP-43

FIG. 3G

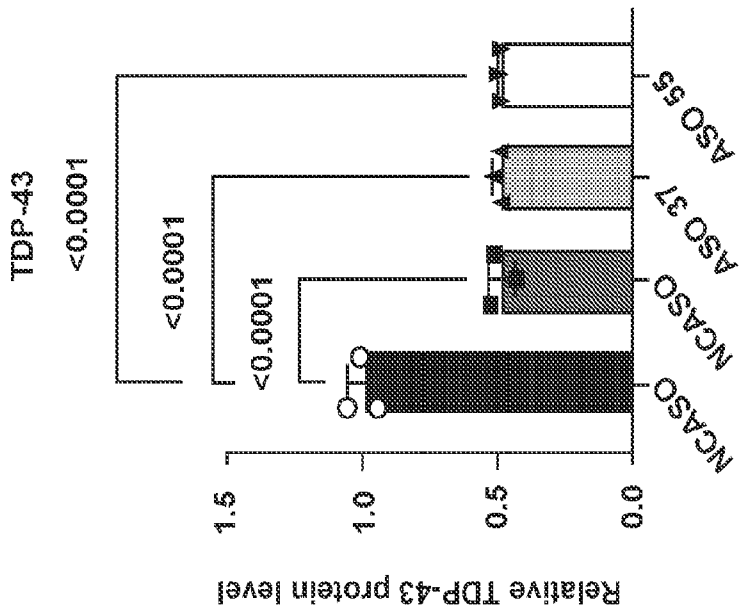
TDP-43
<0.0001
<0.0001
<0.0001
Relative TDP-43 protein level
1.5
1.0
0.5
0.0
NCASO
NCASO
ASO 37
ASO 55
+siNC
+siTDP-43

FIG. 3F

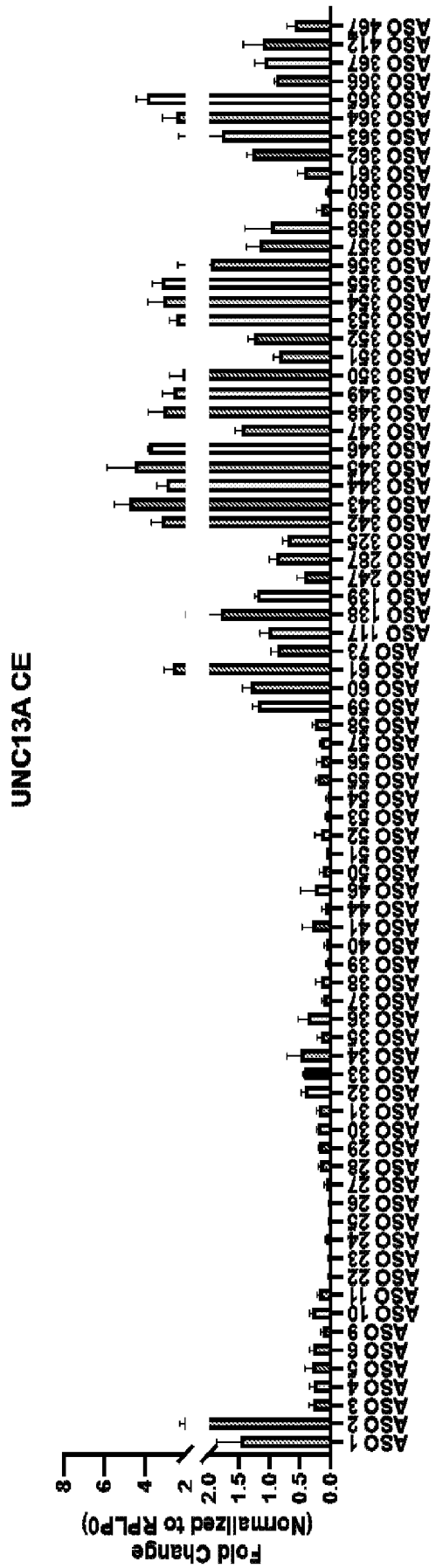
UNC13A CE
Fold Change
(Normalized to RPLP0)
8
6
4
2
2.0
1.5
1.0
0.5
0.0
ASO 1
ASO 2
ASO 3
ASO 4
ASO 5
ASO 6
ASO 9
ASO 10
ASO 11
ASO 22
ASO 23
ASO 24
ASO 25
ASO 26
ASO 27
ASO 28
ASO 29
ASO 30
ASO 31
ASO 32
ASO 33
ASO 34
ASO 35
ASO 36
ASO 37
ASO 38
ASO 39
ASO 40
ASO 41
ASO 44
ASO 45
ASO 46
ASO 50
ASO 51
ASO 52
ASO 53
ASO 54
ASO 55
ASO 56
ASO 57
ASO 58
ASO 59
ASO 60
ASO 61
ASO 73
ASO 117
ASO 138
ASO 139
ASO 247
ASO 287
ASO 325
ASO 342
ASO 343
ASO 344
ASO 345
ASO 346
ASO 347
ASO 348
ASO 349
ASO 350
ASO 351
ASO 352
ASO 353
ASO 354
ASO 355
ASO 356
ASO 357
ASO 358
ASO 359
ASO 360
ASO 361
ASO 362
ASO 363
ASO 364
ASO 365
ASO 366
ASO 367
ASO 412
ASO 467

FIG. 4A

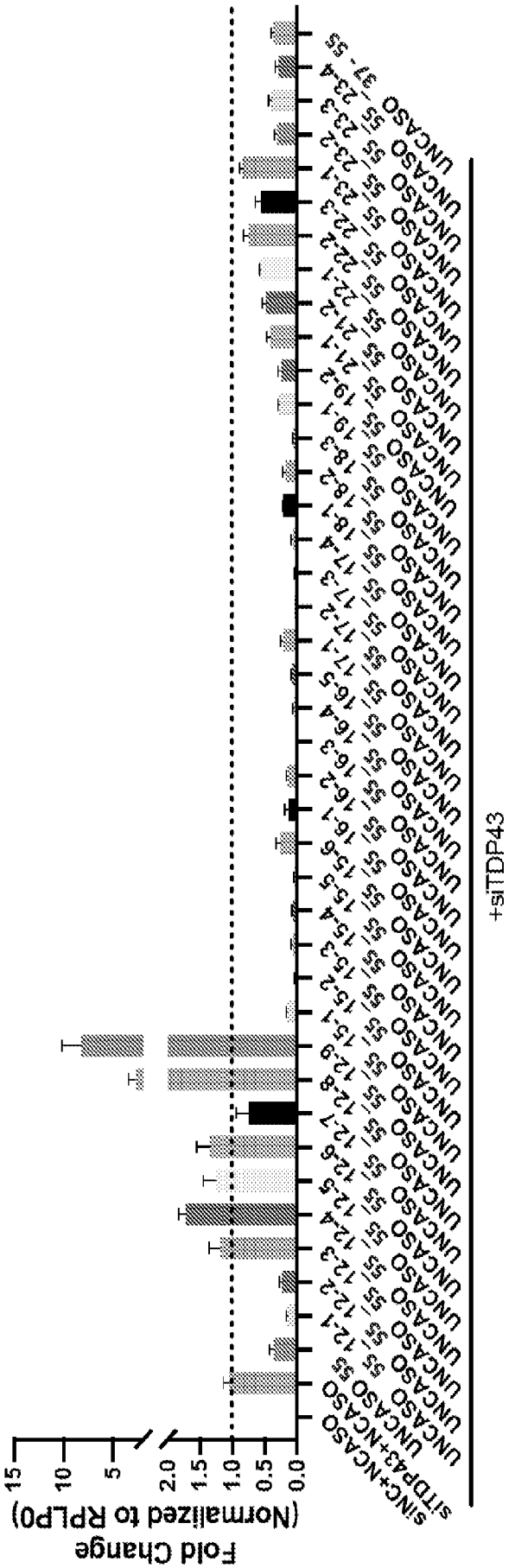
UNC13A CE
Fold Change
(Normalized to RPLP0)
15
10
5
2.0
1.5
1.0
0.5
0.0
siNC+NCASO
siTDP43+NCASO
UNCASO 55
UNCASO 55_12-1
UNCASO 55_12-2
UNCASO 55_12-3
UNCASO 55_12-4
UNCASO 55_12-5
UNCASO 55_12-6
UNCASO 55_12-7
UNCASO 55_12-8
UNCASO 55_12-9
UNCASO 55_15-1
UNCASO 55_15-2
UNCASO 55_15-3
UNCASO 55_15-4
UNCASO 55_15-5
UNCASO 55_15-6
UNCASO 55_16-1
UNCASO 55_16-2
UNCASO 55_16-3
UNCASO 55_16-4
UNCASO 55_16-5
UNCASO 55_17-1
UNCASO 55_17-2
UNCASO 55_17-3
UNCASO 55_17-4
UNCASO 55_18-1
UNCASO 55_18-2
UNCASO 55_18-3
UNCASO 55_19-1
UNCASO 55_19-2
UNCASO 55_21-1
UNCASO 55_21-2
UNCASO 55_22-1
UNCASO 55_22-2
UNCASO 55_22-3
UNCASO 55_23-1
UNCASO 55_23-2
UNCASO 55_23-3
UNCASO 55_23-4
UNCASO 37-55
+siTDP43

FIG. 4B

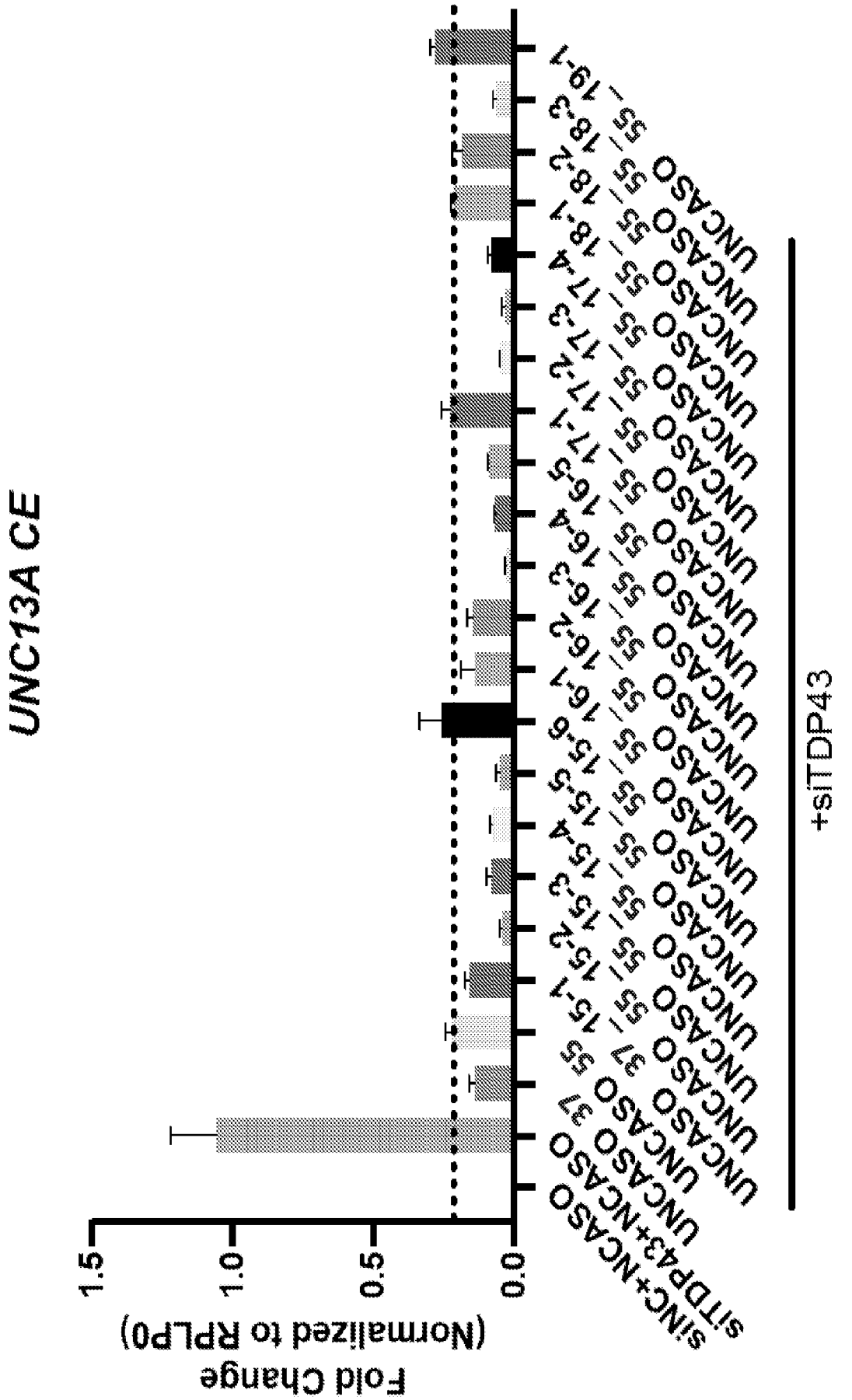
UNC13A CE
Fold Change
(Normalized to RPLP0)
1.5
1.0
0.5
0.0
siNC+NCASO
siTDP43+NCASO
UNCASO 37
UNCASO 55
UNCASO 37 55 / 15-1
UNCASO 55 / 15-2
UNCASO 55 / 15-3
UNCASO 55 / 15-4
UNCASO 55 / 15-5
UNCASO 55 / 15-6
UNCASO 55 / 16-1
UNCASO 55 / 16-2
UNCASO 55 / 16-3
UNCASO 55 / 16-4
UNCASO 55 / 16-5
UNCASO 55 / 17-1
UNCASO 55 / 17-2
UNCASO 55 / 17-3
UNCASO 55 / 17-4
UNCASO 55 / 18-1
UNCASO 55 / 18-2
UNCASO 55 / 18-3
UNCASO 55 / 19-1
+siTDP43

FIG. 4C

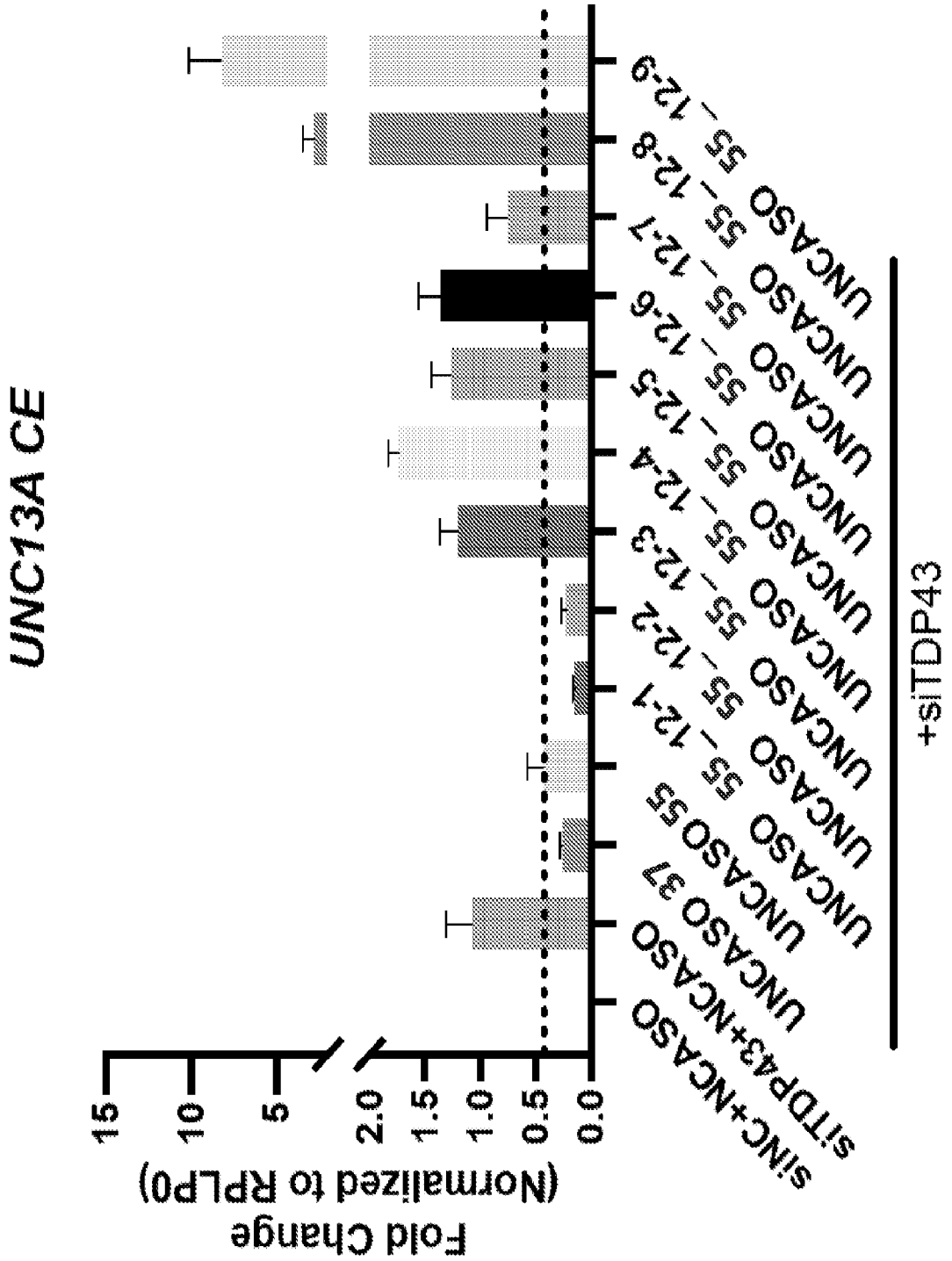
UNC13A CE
Fold Change
(Normalized to RPLP0)
15
10
5
2.0
1.5
1.0
0.5
0.0
siNC+NCASO
siTDP43+NCASO
UNCASO 37
UNCASO 55
UNCASO 55 - 12-1
UNCASO 55 - 12-2
UNCASO 55 - 12-3
UNCASO 55 - 12-4
UNCASO 55 - 12-5
UNCASO 55 - 12-6
UNCASO 55 - 12-7
UNCASO 55 - 12-8
UNCASO 55 - 12-9
+siTDP43

FIG. 4D

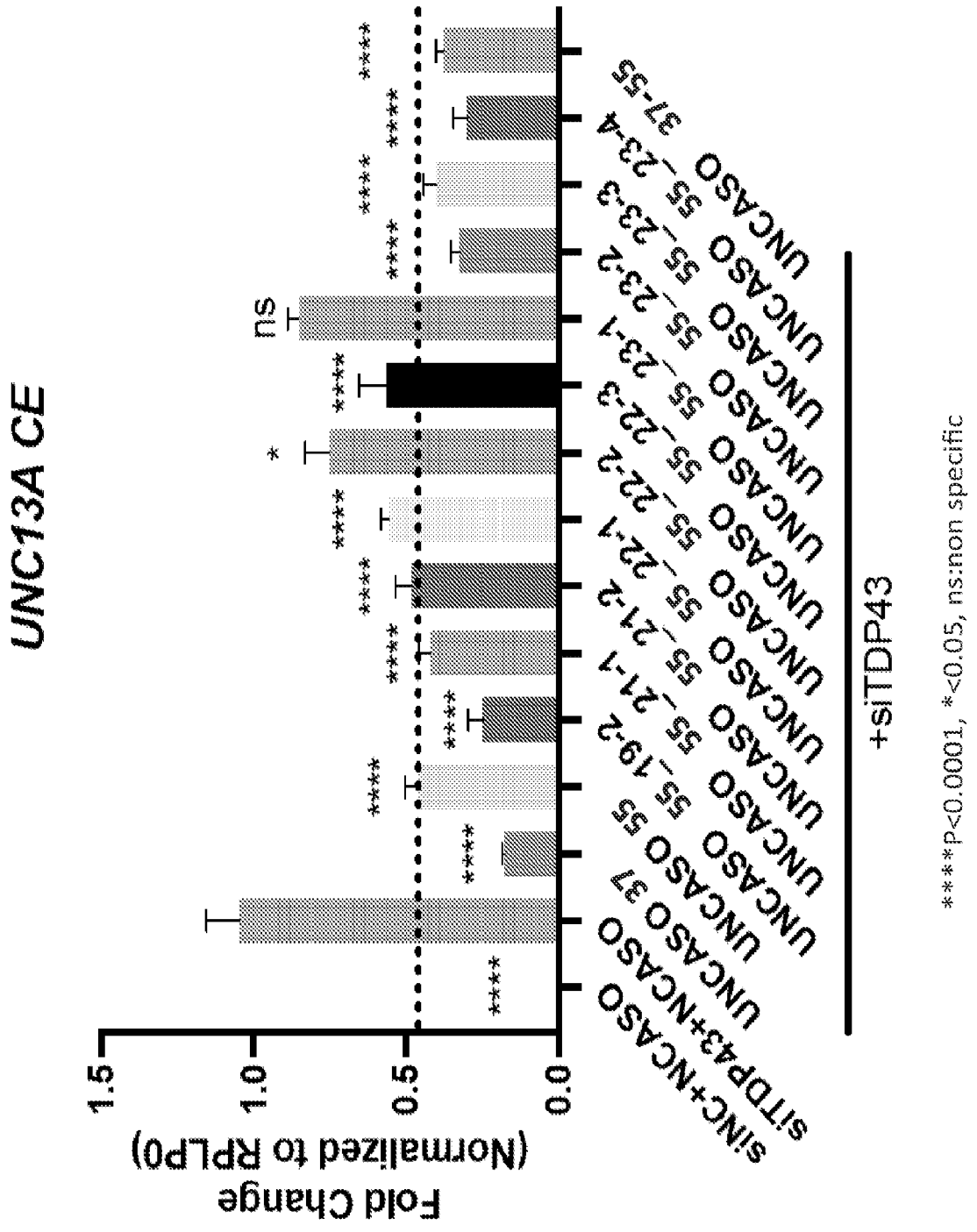
UNC13A CE
Fold Change (Normalized to RPLP0)
1.5
1.0
0.5
0.0
siNC+NCASO
siTDP43+NCASO
UNCASO 37
UNCASO 55
UNCASO 55_19-2
UNCASO 55_21-1
UNCASO 55_21-2
UNCASO 55_22-1
UNCASO 55_22-2
UNCASO 55_22-3
UNCASO 55_23-1
UNCASO 55_23-2
UNCASO 55_23-3
UNCASO 55_23-4
UNCASO 37-55
ns
+siTDP43
****P<0.0001, *<0.05, ns:non specific

FIG. 4E

ID	Sequence
hUNC13A-ASO55_12-1	TCATTCATCTGT
hUNC13A-ASO55_12-2	CTCATTCATCTG
hUNC13A-ASO55_12-3	ACTCATTCATCT
hUNC13A-ASO55_12-4	CACTCATTCATC
hUNC13A-ASO55	CTCATCACTCATTCATCTGT
hUNC13A-ASO55_22-3	TACTCATCACTCATTCATCTGT
hUNC13A-ASO55_23-1	ACTCATCACTCATTCATCTGTTC

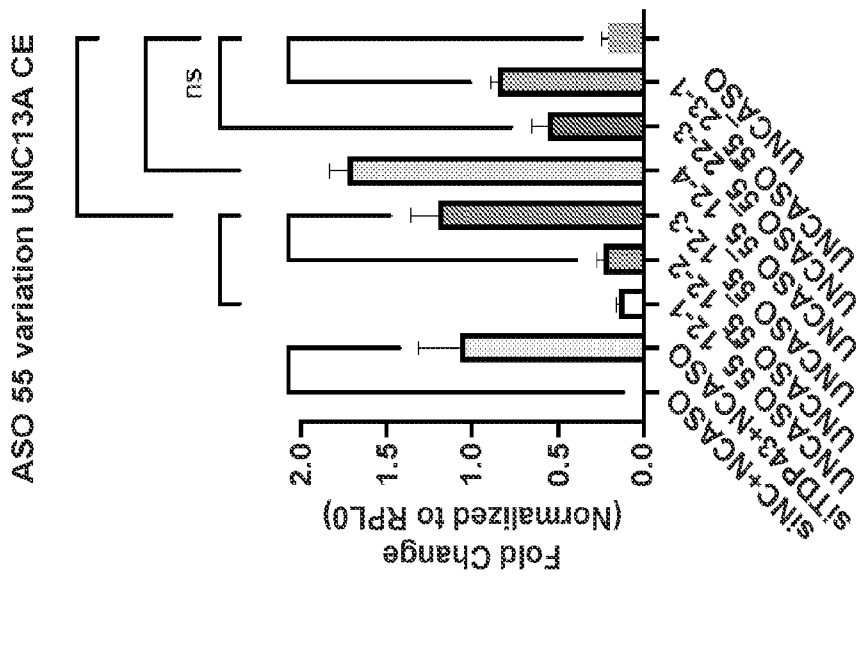

FIG. 4F