(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 August 2022 (25.08.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/178160 A1

(51) International Patent Classification:
C12N 15/113 (2010.01) *A61K 31/7088* (2006.01)
C07H 21/00 (2006.01)

(21) International Application Number:
PCT/US2022/016842

(22) International Filing Date:
17 February 2022 (17.02.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/150,188 17 February 2021 (17.02.2021) US

(71) Applicant: **Q-STATE BIOSCIENCES, INC.** [US/US]; 179 Sidney Street, Cambridge, MA 02139-4238 (US).

(72) Inventors: **FINK, James**; Q-State Biosciences, Inc., 179 Sidney Street, Cambridge, MA 02139-4238 (US). **WILLIAMS, Luis**; Q-State Biosciences, Inc., 179 Sidney Street, Cambridge, MA 02139-4238 (US). **LEWARCH, Caitlin**; Q-State Biosciences, Inc., 179 Sidney Street, Cambridge, MA 02139-4238 (US). **GERBER, David**; 33 Burnham St., Somerville, MA 02144 (US). **BROWN, Duncan**; Q-State Biosciences, Inc., 179 Sidney Street, Cambridge, MA 02139-4238 (US). **AGRAWAL, Sudhir**; Q-State Biosciences, Inc., 179 Sidney Street, Cambridge, MA 02139-4238 (US). **DEMPSEY, Graham, T.**; 27 Partridge Lane, Sudbury, MA 01776 (US).

(74) Agent: **MEYERS, Thomas, C.** et al.; Brown Rudnick LLP, One Financial Center, Boston, MA 02111 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2022/178160 A1

(54) Title: UBE3A ANTISENSE THERAPEUTICS

(57) Abstract: The invention provides compositions useful to knock down overexpression of UBE3A and treat conditions associated with Dup15q syndrome. The compositions include antisense oligonucleotides, preferably short oligonucleotides that are complementary to, and hybridize to, UBE3A transcripts in vivo. The ASOs prevent or inhibit successful translation of UBE3A mRNA into protein. Specifically, preferred embodiments include anti-UBE3A gapmers-oligos that include a central DNA portion flanked by RNA wings. When the gapmer hybridizes to UBE3A pre-mRNA or mRNA, the duplex hybrid recruits RNaseH, which cleaves, or digests, the UBE3A pre-mRNA or mRNA, preventing expression of the UBE3A protein. Because the ASOs prevent expression of the UBE3A protein, treatment with a composition including ASOs of the disclosure may be effective to knock down overexpression of UBE3A.

UBE3A ANTISENSE THERAPEUTICS

TECHNICAL FIELD

The disclosure relates to treatments for neurological disorders.

SEQUENCE LISTING

This application contains a sequence listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. The ASCII-formatted sequence listing, created on February 17, 2022, is named "QSTA-036-01WO-Sequence-Listing", and is 44048 bytes in size.

BACKGROUND

Ubiquitin ligase proteins, such as the E3 ligase E6-associated protein (E6AP, also known as UBE3A), are implicated in neurological and neurodevelopmental disorders. For example, E6AP is encoded by the *UBE3A* gene and expression of the *UBE3A* gene is regulated via genetic imprinting. Loss of E6AP expression leads to the development of Angelman syndrome, typically characterized by impaired speech and motor development, as well as seizures. Conversely, copy number variations (CNVs) of *UBE3A* may be linked to overexpression of E6AP and consequent development of autism spectrum disorders (ASDs).

In some clinical presentations, a portion of chromosome 15 is duplicated. This Dup15q syndrome most commonly occurs in one of two forms, an extra isodicentric chromosome 15 or an interstitial duplication in chromosome 15. Dup15q syndrome is characterized by hypotonia and gross and fine motor delays, intellectual disability, autism spectrum disorder (ASD), and epilepsy, including infantile spasms. It is thought that increased copy number for methylated maternal 15q duplications leads to increased protein expression and that overexpression of UBE3A is linked to severity in Dup15q, where the increased number of maternal alleles is thought to be the primary driver of Dup15q pathology.

SUMMARY

The invention provides compositions for treating disorders associated with CNVs of the *UBE3A* gene. Specifically, the disclosure provides antisense oligonucleotides useful to knock down overexpression of *UBE3A* for treatment of seizures, hypotonia, motor delays, intellectual

disability, disorders presenting seizures, and autism spectrum disorders (ASD) that arise in subjects affected by Dup15q syndrome. Compositions of the invention include antisense oligonucleotides that are complementary to, and hybridize to, *UBE3A* transcripts *in vivo*. The ASOs prevent translation of UBE3A mRNA into protein. Specifically, preferred embodiments include anti-UBE3A gapmers—oligos that include a central DNA portion flanked by RNA wings. When the gapmer hybridizes to *UBE3A* pre-mRNA or mRNA, the hybrid duplex recruits RNaseH, which cleaves, or digests, the *UBE3A* pre-mRNA or mRNA, preventing expression of the UBE3A protein. Because the ASOs prevent expression of the UBE3A protein, treatment with a composition including ASOs of the disclosure is effective to knock down overexpression of UBE3A. Accordingly, compositions of the disclosure are useful to treat Dup15q syndrome and its symptoms.

Oligonucleotides of the disclosure are designed to bind to certain targets in the RNAs used in synthesis of ubiquitin ligase proteins. Binding of the oligonucleotides prevents protein synthesis and downregulates expression of the ubiquitin ligase. Specifically, oligonucleotides of the invention have a sequence that is substantially or entirely complementary to one of the identified targets on a ubiquitin protein ligase E3A pre-mRNA or mRNA. That is, the oligonucleotides are antisense to the identified target. When the antisense oligonucleotide (ASO) hybridizes to its target RNA, it forms a double-stranded ASO:RNA duplex that recruits an enzyme (RNase H) that degrades a portion of the double-stranded duplex. Degrading the ASO:RNA duplex depletes the cell of E6AP mRNA, which decreases the amount of E6AP synthesized by the cell.

Thus, when a composition that includes oligonucleotides that are antisense to the identified targets in E6AP pre-mRNA or mRNA is administered to a patient, the composition will decrease expression of E6AP that may otherwise result from copy number variations of *UBE3A* or the chromosome 15q11.2-q13.1 duplication syndrome known as Dup15q syndrome.

In certain aspects, the disclosure provides compositions for treating Dup15q. Such compositions include a synthetic antisense oligonucleotide (ASO) that inhibits expression of a ubiquitin ligase protein. Preferably, the protein is ubiquitin protein ligase E3A. The ASO hybridizes to a complementary target in a transcript from a *UBE3A* gene. The sequence of bases in the ASO may have at least 80% identity to one of SEQ ID NOS: 1-219, preferably one of SEQ ID NOS: 1-40, and more preferably one of SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164 169,

174, 175, 178, 179, 213, and 214. In some embodiments, a sequence of bases in the ASO is at least 90%, 95%, or 100% identical to one of SEQ ID NOS: 1-219, 1-40, or 146, 155, 156, 158, 159, 161, 164 169, 174, 175, 178, 179, 213, and 214, and the oligonucleotide can hybridize to, and induce RNase cleavage of, *UBE3A* pre-mRNA or mRNA.

In some embodiments, the oligonucleotide comprises two RNA wings flanking a central region of at least 10 DNA bases, preferably about 12 bases. At least one of the two wings of the ASO comprises modified RNA bases. Each modified RNA base may be selected from the group consisting of 2'-O-methoxyethyl RNA and 2'-O-methyl RNA. The ASO may include at least about 20 bases, preferably between about 15 about 25 bases. In certain embodiments, the ASO has a backbone comprising a plurality of phosphorothioate bonds. The ASOs provided herein include a central region of 10-12 bases and flanking regions of 4-5 bases.

A preferred ASO has a base sequence that has been screened and determined to not meet a threshold match for any non-target transcripts in humans. Optionally the ASO has a base sequence with 0 mismatches to a homologous segment in a non-human primate genome and no more than about 5 mismatches in a homologous segment in a rodent genome.

In certain embodiments, a composition of the invention comprises a plurality of ASOs, each having a base sequence at least about 80% identical to one of SEQ ID NOS: 1-219, wherein each of the ASOs has a gapmer structure that comprises a central DNA segment flanked by RNA wings. In certain preferred embodiments, the composition comprises a plurality of ASOs each having a base sequence at least about 80% identical to one of SEQ ID NOS: 1-40, and more preferably to one of SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214, wherein each of the ASOs has a gapmer structure that comprises a central DNA segment flanked by RNA wings. Each oligonucleotide may have a base sequence with at least about a 90% (or 95%, or 100%) match to one of SEQ ID NO: 1-219 (preferably 1-40 and more preferably 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214), with bases linked only by phosphorothioate linkages, the oligonucleotide further comprising a central 10 DNA bases flanked by a 5' wing and a 3' wing, the 5' wing and the 3' wing each comprising five consecutive 2' modified RNA bases.

In some embodiments, each oligonucleotide has a base sequence matching one of SEQ ID NO: 1-219, with at least a majority of inter-base linkages comprising phosphorothioate linkages, the oligonucleotide further comprising a central 10 DNA bases flanked by a 5' wing

and a 3' wing, the 5' wing and the 3' wing each comprising five consecutive 2'-O-methoxyethyl (2'-MOE) 2'- MOE RNA bases. In preferred embodiments, each oligonucleotide has a base sequence matching one of SEQ ID NO: 1-40, with at least a majority of inter-base linkages comprising phosphorothioate linkages, the oligonucleotide further comprising a central 10 DNA bases flanked by a 5' wing and a 3' wing, the 5' wing and the 3' wing each comprising five consecutive 2' MOE RNA bases. In more preferred embodiments, each oligonucleotide has a base sequence matching one of SEQ ID NO: 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214, with at least a majority of inter-base linkages comprising phosphorothioate linkages, the oligonucleotide further comprising a central 10 DNA bases flanked by a 5' wing and a 3' wing, the 5' wing and the 3' wing each comprising five consecutive 2' MOE RNA bases.

In related aspects, the invention provides methods for treating Dup15q syndrome, which methods include delivering one of the disclosed compositions to a subject in need thereof, e.g., to downregulate overexpression of UBE3A. Therapeutic oligonucleotides of the disclosure may have a gapmer structure that includes a central DNA segment flanked by modified RNA wings. Such a therapeutic oligonucleotide may include two wings flanking a central region of DNA bases (e.g., about 10 to 14 DNA bases, e.g., central region of about 12 DNA bases). Preferably at least one end of the oligonucleotide comprises modified RNA bases, e.g., any number or any combination of 2'-O-methoxyethyl RNA ("2'-MOE") and/or 2'-O-methyl RNA ("2' O-Me"). In addition, compositions of the invention may be designed to target an exon-exon junction to differentially target cytoplasmic mRNA versus nuclear pre-mRNA. Thus, ASOs of the invention can be designed to interact with RNA prior to or after splicing, adding specificity and versatility to the compositions.

In various embodiments, therapeutic oligonucleotide may be provided in a solution or carrier formulated for delivery via any suitable route including, for example, intravenously or intrathecally. The oligonucleotide may be of any suitable length, e.g., at least about 18 bases, and preferably between about 15 and about 25 bases. The oligonucleotide may have phosphorothioate bonds in its backbone. In preferred embodiments, the oligonucleotide has a base sequence that has been screened and determined to not meet a threshold match for any long, non-coding RNA or other off-target sequences or transcripts in humans. The oligonucleotide

may have a base sequence with 0 mismatches to a homologous segment in a non-human primate genome and no more than about 5 mismatches in a homologous segment in a rodent genome.

When the composition is delivered to cells *in vitro*, the cells exhibit a dose-dependent knockdown of UBE3A. The oligonucleotide may be a gapmer having a base sequence with at least about a 90% match to one of SEQ ID NO: 1-219, with at least some phosphorothioate linkages. The linkages may be all phosphorothioate or a mixture of phosphorothioate and phosphodiester bonds. The oligonucleotide may further have a central 12 DNA bases flanked by a 5' wing and a 3' wing, the 5' wing and the 3' wing each comprising four consecutive 2' modified RNA bases. Preferably, the oligonucleotide has a base sequence matching one of SEQ ID NO: 1-219, with bases linked by phosphorothioate linkages, and a structure having central DNA bases flanked by a 5' wing and a 3' wing. The number of RNA bases in the wings and DNA bases in the central segment may be 5-10-5 or 4-12-4, or a similar suitable pattern. The 5' wing and the 3' wing may each include several 2'-MOE RNA bases. For example, the oligonucleotide may have 4 consecutive 2'-MOE RNA bases in each wing with a central 12 DNA bases (a "4-12-4" structure), with phosphorothioate linkages throughout the central DNA segment and a mixture of phosphorothioate and phosphodiester bonds in the wings. Alternatively, the oligonucleotide may have 5 consecutive 2'-MOE RNA bases in each wing with a central 10 DNA bases (a "5-10-5" structure), with phosphorothioate linkages throughout the central DNA segment and a mixture of phosphorothioate and phosphodiester bonds in the wings. The 5' and 3' wings could also be of different length in the same ASO, e.g., a "4-11-5" or a "5-11-4" structure.

In combination embodiments, the invention provides compositions that include a plurality of copies of a plurality of distinct therapeutic gapmers, each according to the descriptions above, in a suitable formulation or carrier.

Aspects of the disclosure relate to use of an antisense oligonucleotide (ASO) for the manufacture of a medicament for treating Dup15q syndrome. In such embodiments, the ASO has at least about 75% identity with one of SEQ ID NOS: 1-219, and more preferably at least about 90% identity, e.g., 95% or 100% identity. Preferred embodiments use an ASO that is between about 15 and 25 bases in length, preferably between about 18 and 22, or between about 19 and 21 (inclusive). In general, reference to "an ASO" includes numerous copies of substantially identical molecules. Accordingly, "an ASO" may be any number, e.g., hundreds of thousands, or

millions, of copies of the indicated ASO. In preferred embodiments, the ASO is 20 bases in length and has the sequence of one of SEQ ID NOS: 1-219 and is used in the manufacture of a medicament for the treatment of Dup15q syndrome. The ASO may be provided in any suitable format such as, for example, lyophilized in a tube or in solution in a tube, such as a microcentrifuge tube or a test tube. Preferred embodiments of the use target transcripts of the *UBE3A* gene. One or more (e.g., two, three, four, or five, or more) ASOs may be used in manufacture of the medicament. The one or more ASOs may hybridize to a target in the *UBE3A* pre-mRNA or mRNA. In certain embodiments, a sequence of bases in the ASO is at least about 90% identical to one of SEQ ID NOS: 1-219. In other embodiments, the ASO may have a gapmer structure with a central DNA segment flanked by RNA wings, e.g., a central region of 12 DNA bases with 4 modified RNA bases on both sides of the central region. Each modified RNA base may be 2'-MOE. Preferably a backbone of the ASO has a plurality of phosphorothioate bonds. Accordingly, the ASO may initially be in a form suitable for mixing into a formulation suitable for introduction by injection or a pump. For example, the ASO (thousands or millions or more of copies of one ASO) may be lyophilized in a tube or in solution at a known quantity, molality, or concentration. The ASO may be dissolved or diluted into a pharmaceutically acceptable composition in which a carrier, such as a solvent and/or excipient, includes the ASO and may be loaded in an IV bag, syringe, or pump. The medicament may be made using more than one ASO, e.g., any combination of 2, 3, 4, or 5, or more. Bases in compositions of the invention may be modified or wobble bases, which may be used in order to increase the breadth and effectiveness of compositions of the invention. In one example, ASOs for use in the invention may contain methylated bases (e.g., 5-methylcytosine, 5-methyluracil (thymine) and others).

Compositions of the invention may be formulated to accommodate serial dosing. For example, formulations may provide dosages to be administered at two or more separate times and, optionally, with two or more different ASOs, in order to take advantage of optimal therapeutic windows and to avoid potential competition between ASOs. In addition, compositions of the invention, whether administered serially or not, may interact with more than one target, depending on the composition of the ASOs involved. For example, ASOs may comprise targeted mismatches that allow interaction with multiple targets (both within and across mRNA and pre-mRNA species), thus allowing the associated treatment to impact transcripts

from more than one gene copy. Compositions of the invention may also be delivered in a time-release format and/or comprising adjuvants to increase serum half-life.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a composition for treating Dup15q Syndrome.

FIG. 2 shows an oligonucleotide (ASO) with a gapmer structure.

FIG. 3 shows results from screening 40 *UBE3A* exonic ASOs.

FIG. 4 gives results showing dose-response of ten ASO candidates.

FIG. 5 shows results from screening human exonic ASOs with mouse homology.

FIG. 6 shows a table summarizing qPCR readouts of UBE3A knockdown, expressed as percent of UBE3A knockdown, for certain screened ASOs of the invention.

FIG. 7 shows a table summarizing qPCR readouts of UBE3A knockdown, expressed as percent of UBE3A knockdown, for certain screened ASOs of the invention.

FIG. 8 shows a table summarizing qPCR readouts of UBE3A knockdown, expressed as percent of UBE3A knockdown, for certain screened ASOs of the invention.

FIG. 9 shows a table summarizing qPCR readouts of UBE3A knockdown, expressed as percent of UBE3A knockdown, for certain screened ASOs of the invention.

FIG. 10 shows UBE3A ASO dose-response modulation of target expression for 2 lead candidate example ASOs and their PO-modified daughter molecules in Dup15q patient fibroblasts (top) or mouse embryonic fibroblasts (bottom).

FIG. 11 shows plots of the dose-response and indicates EC50 for the same 2 example lead candidate ASOs from FIG. 10.

FIG. 12 shows dose-response data for lead all-PS backbone ASO candidates of the invention that target UBE3A exons.

FIG. 13 shows dose-response data for lead all-PS backbone ASO candidates of the invention that target UBE3A introns.

FIG. 14 shows dose-response data for lead all-PS backbone ASO candidates of the invention that have 100% mouse homology for rodent *in vivo* efficacy studies.

FIG. 15 shows dose-response data for PO-modified daughter lead ASO candidates that have 100% mouse homology for rodent *in vivo* efficacy studies.

FIG. 16 shows dose-response data for PO-modified daughter lead ASO candidates of the invention for human clinical candidate studies.

FIG. 17 shows a western blot for a certain candidate lead UBE3A ASO and 3 PO-modified daughter molecules with identical ASO sequences.

FIG. 18 show a quantification of the UBE3A protein knockdown for the ASOs of FIG. 17.

FIG. 19 provides a table summarizing UBE3A protein knockdown results for lead all-PS backbone ASO candidates targeting UBE3A.

FIG. 20 provides a table summarizing UBE3A protein knockdown results for lead all-PS backbone ASO candidates with 100% mouse homology for rodent in vivo efficacy studies.

FIG. 21 provides a table summarizing UBE3A protein knockdown results for PO-modified daughter lead ASO candidates with 100% mouse homology for rodent in vivo efficacy studies.

FIG. 22 provides a table summarizing UBE3A protein knockdown results for PO-modified daughter lead ASO candidates for human clinical candidates.

FIG. 23 provides data showing the knockdown of UBE3A transcript in human NGN2 stem cell-derived neurons using UBE3A lead candidate ASOs of the invention.

FIG. 24 provides data showing the knockdown of UBE3A transcript in human primary neurons using UBE3A lead candidate ASOs of the invention.

FIG. 25 provides data showing the knockdown of UBE3A transcript in non-human primate primary fibroblast cultures using UBE3A lead ASO candidates of the invention.

FIG. 26 provides data showing the knockdown of UBE3A transcript in mouse primary cortical neurons using UBE3A lead candidate ASOs of the invention.

FIG. 27 provides data showing the knockdown of UBE3A transcript in rat primary cortical neurons using UBE3A lead ASO candidates of the invention where the cells were harvested for qPCR after four days.

FIG. 28 provides data showing the knockdown of UBE3A transcript in rat primary cortical neurons using UBE3A lead ASO candidates of the invention where the cells were harvested for qPCR after eight days.

DETAILED DESCRIPTION

FIG. 1 shows a composition 101 for treating Dup15q Syndrome. The composition 101 includes an antisense oligonucleotide 107 that hybridizes to a target segment 115 in an mRNA 117 or a pre-mRNA. The RNA 117 encodes a ubiquitin ligase protein such as ubiquitin protein ligase E3A. The segment 115 of the RNA 117 that includes the target is at least about 75% complementary to one of SEQ ID NOS: 1-219. Hybridization of the ASO 107 to the segment 115 of the RNA 117 prevents translation of the mRNA into the UBE3A protein. Preferably, a sequence of bases in the oligonucleotide has at least 80% identity to one of SEQ ID NOS: 1-219, and more preferably at least about 90 % identity. In certain embodiments, a sequence of bases in the oligonucleotide is at least about 90% identical to one of SEQ ID NOS: 1-219, wherein the oligonucleotide can hybridize to, and induce RNase H cleavage of *UBE3A* pre-mRNA or mRNA.

The oligonucleotide 107 hybridizes to the segment 115 in the mRNA 117 because the oligonucleotide 107 is substantially or entirely antisense to the target segment 115 of the mRNA 117. In that aspect, the composition includes an antisense oligonucleotide (ASO). Compositions 101 include ASOs that bind to target RNA with base pair complementarity and exert various effects, based on the ASO chemical structure and design. Various mechanisms, commonly employed in preclinical models of neurological disease and human clinical trial development, may be employed. Those mechanisms include RNA target degradation via recruitment of the RNase H enzyme; alternative splicing modification to include or exclude exons, and miRNA inhibition to inhibit miRNA binding to its target.

Preferred embodiments of the disclosure include ASOs that hybridize to the *UBE3A* pre-mRNA or mRNA and recruit the RNase H enzyme. The RNase H enzyme cleaves the RNA, which downregulates expression of the UBE3A protein. Thus, oligonucleotide 107 of the disclosure addresses *UBE3A* CNVs as targets for Dup15q syndrome. The disclosure builds on the insights that data suggest that one of the most common genetic variants associated with autism spectrum disorder (ASD) are duplications of chromosome 15q11.2-q13.1 (Dup15q syndrome). The chromosome 15q11.2-q13.1 region includes the imprinted Prader-Willi/Angelman syndrome critical region (PWACR) as well as several genes critical for brain development and synaptic function, such as ubiquitin protein ligase E3A (UBE3A), small nuclear ribonucleoprotein polypeptide N (SNRPN), and three GABAA receptor genes

(GABRB3, GABRA5, and GABRG3). Dup15q syndrome includes two primary types of duplications of 15q11.2-13.1: (1) an isodicentric chromosome 15 (idic(15)) that results in two additional maternally derived copies on a supernumerary chromosome that includes 15p and the proximal region of 15q11, most commonly leading to four copies of the region, or (2) an interstitial 15q duplication in which one extra copy of the 15q11.2-q13.1 region occurs on the same chromosome arm, typically resulting in three copies of the region, and has an overall milder phenotype. See Hogart, 2010, The comorbidity of autism with the genomic disorders of chromosome 15q11.2-13, *Neurobiol Dis* 38:181-91, incorporated by reference. Increased copy number for methylated maternal 15q duplications leads to changes in gene and protein expression and overexpression of UBE3A is linked to severity in Dup15q, where the increased number of maternal alleles is thought to be the primary driver of Dup15q pathology. See Scoles, 2011, Increased copy number for methylated maternal 15q duplications leads to changes in gene and protein expression in human cortical samples, *Mol Autism* 2:19 and Baker, 2020, Relationships between UBE3A and SNORD116 expression and features of autism in chromosome 15 imprinting disorders, *Translational Psychiatry* 10:362, both incorporated by reference. Here, compositions that include *UBE3A* ASOs are administered to a subject to treat Dup15q syndrome.

Thus, the disclosure provides a use of an antisense oligonucleotide (ASO) for the manufacture of a medicament for treating Dup15q syndrome in a patient. In the use, the ASO has at least about 75% identity with one of SEQ ID NOS: 1-219, and more preferably at least 90% identity, e.g., 95% or greater identity. Preferred embodiments use an ASO that is between about 15 and 25 bases in length, preferably between about 18 and 22 (inclusive). In general, reference to "an ASO" includes numerous copies of substantially identical molecules. Accordingly, "an ASO" may be more than hundreds of thousands or millions of copies of the defined ASO. In preferred embodiments, the ASO is 20 bases in length and has the sequence of one of SEQ ID NOS: 1-219 and is used in the manufacture of a medicament for the treatment of Dup15q syndrome. The ASO may be provided in any suitable format such as, for example, lyophilized in a tube or in solution in a tube, such as a microcentrifuge tube or a test tube. Preferred embodiments of the use target *UBE3A*. One or more (e.g., two, three, four, or five, or more) ASOs may be used in manufacture of the medicament. The one or more ASOs may hybridize to a target in a *UBE3A* mRNA. In certain embodiments of the use, a sequence of bases in the ASO

is at least 90% identical to one of SEQ ID NOS: 1-219. In embodiments of the use, an ASO may have a gapmer structure with a central DNA segment flanked by RNA wings, e.g., a central region of 10-12 DNA bases with 4-5 modified RNA bases on both sides of the central region. Each modified RNA base may be 2'-MOE RNA, 2'-O-methyl RNA, or other suitable sugar. Preferably a backbone of the ASO has a plurality of phosphorothioate bonds, either exclusively or also including phosphodiester linkages, e.g., most or all of the sugar linkages may be phosphorothioate in the use embodiments. The ASO may initially be in a form suitable for mixing into a formulation suitable for introduction by injection. For example, the ASO (thousands or millions or more of copies of one ASO) may be lyophilized in a tube or in solution at a known quantity, molality, or concentration. The ASO may be dissolved or diluted into a pharmaceutically acceptable composition in which a carrier, such as a solvent or excipient, includes the ASO and may be loaded in an IV bag, syringe, or vial. The medicament may be made using more than one ASO, e.g., any combination of 2, 3, 4, or 5, or more.

Any ASO(s) described in the use embodiment may be included in a composition of the disclosure. Preferred embodiments of compositions of the disclosure include one or a plurality of therapeutic oligonucleotides each having a base sequence at least 80% identical to one of SEQ ID NOS: 1-219 wherein each of the therapeutic oligonucleotides has a gapmer structure that comprises a central DNA segment flanked by modified RNA wings, wherein the plurality of therapeutic oligonucleotides are provided in a solution or carrier formulated for injection.

FIG. 2 shows an oligonucleotide 207 with a gapmer structure. The oligonucleotide 207 includes two wings (first wing 215 and second wing 216) flanking a central region 221 of about 10-12 DNA bases. In preferred embodiments, the wings 215, 216 are all or predominantly RNA bases whereas the central region 221 is all or predominantly DNA bases. Preferably, the wings are all RNA bases (modified or unmodified) and the central region is all DNA bases. In some embodiments, each wing consists of 5 RNA bases, all or most of which are modified RNA bases, e.g., in which each modified RNA base is selected from the group consisting of 2'-O-methoxyethyl RNA and 2'-O-methyl RNA. A modified RNA base may include a substitution on a 2' hydroxyl group of a ribose sugar. A 2'-O-Methoxyethyl ("2'-MOE") modified sugar may be included in an RNA base. The oligonucleotide 207 preferably includes at least about 15 bases and may include between about 15 about 25 bases. In some embodiments, the oligonucleotide 207 has a backbone comprising a plurality of phosphorothioate bonds. One or any number of

phosphorothioate bonds may be included in the backbone of a segment of DNA, such as the central region 221 of the oligonucleotide 207. The oligonucleotide 207 may include one or any number of the phosphorothioate bonds. For example, every backbone linkage within the oligonucleotide 207 may be phosphorothioate, or most, or about half may be phosporothioate. In addition, there may be other modifications to the phosphodiester backbone.

The composition 101 may be formulated for delivery. Accordingly, the oligonucleotide 107 may initially be in a form suitable for mixing into a formulation suitable for introduction into a syringe, bag, or injection pump. For example, the oligonucleotide 107 (thousands or millions or more of copies of one oligonucleotide 107) may be lyophilized in a tube or in solution at a known molality of concentration. The oligonucleotide 107 may be dissolved or diluted into a pharmaceutically acceptable composition in which a carrier, such as a solvent or excipient, includes the oligonucleotide 107 and may be loaded in an IV bag, syringe, or vial. As described, the composition 101 includes at least one oligonucleotide 107 with a sequence that is defined by comparison to one of SEQ ID NO: 1-219. Thus, compositions of the disclosure are defined and illustrated by the identified targets.

Specifically, the oligonucleotide 107 hybridizes to an mRNA encoding a UBE3A protein along a segment of the mRNA that is at least about 75% complementary to one of SEQ ID NOS: 1-219 to thereby prevent translation of the mRNA into the UBE3A protein. This is accomplished where the oligonucleotide has at least about 75% identity to one of SEQ ID NOS: 1-219, preferably at least about 90% or 95% or 100% identity. In certain embodiments, the oligonucleotide has the sequence of one of SEQ ID NOS: 1-219, although one of skill in the art will understand that oligonucleotides with 90 or preferably 95% identity to a complementary target will still tend to hybridize in a sequence-specific manner to the target. Forming a double stranded structure is energetically favorable enough through Watson-Crick base pairing and base stacking that the double stranded structure can tolerate approximately about 1 mismatched base pair every ten or so. Accordingly, under moderately stringent physiological conditions in a cell, 95% identity should be effective, especially where an oligonucleotide has a gapmer structure with at least a few modified RNA bases or phosphorothioate backbone linkages to protect the oligonucleotide from enzymatic degradation.

In fact, a feature and benefit of compositions of the disclosure is that the targets (of SEQ ID NOS: 1-219) have been substantially screened to rule out sequences for which the

complement is present in molecules other than *UBE3A* transcripts. For example, the sequences have been screened against databases of RNA transcripts including long, non-coding RNA (lncRNA), and initial sequences that matched non-target sequences were excluded. Thus, ASOs with sequences of SEQ ID Nos. 1-219 when administered to a patient should have a minimized chance of hybridizing to non-target sequences. Accordingly, in preferred embodiments, the oligonucleotide 107 has a base sequence that has been screened and determined to not meet a threshold match for any off-target coding or long, non-coding RNA in humans. A composition or use that meets the criteria stated above should not bind to off-target material such as lncRNA or other off-target RNA transcripts in vivo, as the included sequences have been screened against a database of lncRNA and other RNA transcripts. Sequences of the disclosure have been screened for target specificity. Preferably, the oligonucleotide 107 has a base sequence with 0 mismatches to a homologous segment in a human or non-human primate genome and no more than about 5 mismatches in a homologous segment in a rodent genome.

When the composition is delivered to cells, the cells exhibit a dose-dependent knockdown of UBE3A.

FIG. 3 shows results from screening 40 *UBE3A* exonic ASOs (with 1 control fibroblast line; results taken 48 hours post treatment). The indicated results correspond to SEQ ID Nos. 1-40. In the figure, bars for ASOs that were tested in concentration response (CR)are marked by circles.

FIG. 4 gives results showing dose-response of ten ASO candidates of SEQ ID NOS: 14, 17, 4, 7, 8, 18, 21, 26, 34, and 35 (at 6 concentrations each) designed according to embodiments of the disclosure (about 20 bases, about 12 base DNA central region flanked by RNA wings with 2'-O modified RNA and phosphorothioate linkages through ASO). All ten ASOs decreased *UBE3A* expression, relative to controls in a dose-dependent manner (vehicle-only treated cells and untreated "cells only" conditions).

Because nucleic acid hybridization has some tolerance for mis-matches, it may be found that an oligonucleotide 107 with a base sequence that is at least a 90% match to one of SEQ ID NOS: 1-219, with bases linked only by phosphorothioate linkages, and in which the oligonucleotide 107 has a central segment of DNA bases flanked by a 5' wing and a 3' wing (e.g., a 4-12-4 structure in which the 5' wing and the 3' wing each comprise four consecutive 2' modified RNA bases flanking 12 DNA bases, or a 5-10-5 structure, or similar) exhibits dose-

dependent knockdown according to the pattern shown in the chart. In some embodiments, the oligonucleotide 107 specifically has a base sequence matching one of SEQ ID NOS: 1-219 (more preferably one of SEQ ID NOS: 1-40 or more preferably SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, or 214), with bases linked by phosphorothioate linkages (optionally with some phosphodiester linkages), in which the oligonucleotide 107 has a central 12 DNA bases flanked by a 5' wing and a 3' wing, and in which the 5' wing and the 3' wing each include four consecutive 2'-MOE RNA bases.

FIG. 5 shows results from screening mouse exonic *Ube3a* ASOs and human exonic ASOs with mouse homology in mouse fibroblasts. The screened human ASOs included those of SEQ ID NOS: 1, 4, 5, 9, 15, 16, 21, 25, 28, and 29. The results tend to show that it is possible to design ASOs against human targets for which there exist homologous targets in rodent models.

Because these compositions are effective at knocking down expression of *UBE3A*, the compositions of the disclosure may be used to treat Dup15q syndrome in patients. Methods of the disclosure include administering to a patient in need thereof any composition of the disclosure to thereby treat or alleviate Dup15q syndrome.

Compositions of the disclosure may be tested on in vitro samples of living neurons. For example, neurons in vitro may include optogenetic constructs that provide neural activation under optical stimulus (e.g., a modified algal channelrhodopsin that causes the neuron to fire in response to light) and optical reporters of neural activity (modified archaerhodopsins that emit light in proportion to neuronal membrane voltage and yield signals of neuronal activity). The in vitro neurons may be assayed in a fluorescence microscopy instrument and optionally treated with neural stimulant composition that causes neurons to fire in a predictable manner. Any suitable optogenetic constructs, optogenetic microscope, or neural stimulant compositions may be used. For example, suitable optogenetic constructs include those described in U.S. Pat. 9,594,075, incorporated by reference. Suitable optogenetic microscopes include those described in U.S. Pat. 10,288,863, incorporated by reference.

Methods and compositions of the disclosure may beneficially be used for delivery of therapeutic oligonucleotides 107 described herein to neurons in vivo in subjects with Dup15q syndrome. Any suitable delivery approach may be used including, for example, systemic delivery (e.g., by injection) or local delivery (e.g., by subcutaneous, intrathecal, orimplantation of a slow-release device). Methods of the disclosure may involve delivering a composition of the

disclosure once, several times over days or weeks, every few months, e.g., about 3 or 4 times per year.

An oligonucleotide of the disclosure, such as a gapmer, ASO, or therapeutic oligonucleotide 107 in a composition 101 may have a sequence defined with reference to one of the sequences set forth in Table 1. For example, an oligonucleotide of the disclosure may have a sequence that is at least about 75%, 80%, 90%, 95%, or perfectly identical to one of SEQ ID NOS: 1-219 as set forth in Table 1. Certain preferred embodiments against *UBE3A* include those in Table 1 labeled as SEQ ID NOS: 1-40.

Further, as described in the Examples presented below, the inventors screened ASOs of the invention. Based on the resulting data, ASOs corresponding to SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214 were identified as lead candidate ASOs based on single dose and dose-response efficacy, sequence motif liabilities, and off-target alignment analyses. Those ASOs showed the greatest *in vitro* efficacy, lowest off-target alignments, and limited sequence motif concerns. Accordingly, in certain aspects, preferred ASOs against *UBE3A* according to the invention include ASOs having a sequence that is at least about 75%, 80%, 90%, 95%, or perfectly identical to a sequence corresponding to SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164 169, 174, 175, 178, 179, 213, and 214.

Table 1: Sequences for ASOs

Sequence Identifier	Sequence	Start position in negative strand of chromosome 15
SEQ ID NO: 1	TCATTTCCACAGCCCTCAGT	25375694
SEQ ID NO: 2	TCAGAGCAGGAGTTGTTGGG	25375505
SEQ ID NO: 3	GATTTCAGTTCTTCCTTGGT	25371643
SEQ ID NO: 4	TCCATAGCAGCAGCAGAACA	25371571
SEQ ID NO: 5	GCTTCTGAGTCTTCTTCCAT	25371556
SEQ ID NO: 6	GTGAGCTATCACCTATCCTT	25371527
SEQ ID NO: 7	TTGTTGTCTCCCTGTGAGCT	25371514
SEQ ID NO: 8	GCAATCTGGTGTAGACCCTT	25371443
SEQ ID NO: 9	TCCCCTCCCACTACATTTGC	25371022

SEQ ID NO: 10	TTTGTGTCCACTTCCCCTCC	25371010
SEQ ID NO: 11	GGGATGGGCTCTTCATCATC	25370977
SEQ ID NO: 12	AGGACCTTTCTTGTTTCTTC	25370913
SEQ ID NO: 13	ACCAAGTTCAGTTTCCAGGG	25370883
SEQ ID NO: 14	ACCTCATTCAGTGGTTCATT	25370812
SEQ ID NO: 15	GGATTCAACTGCTGTCCTTG	25370620
SEQ ID NO: 16	TCATCAACTCCTTGTTCTCC	25360444
SEQ ID NO: 17	ATTTCCTCCACAACCAGCTG	25360399
SEQ ID NO: 18	GCCAGACCCAGTACTATGCC	25356793
SEQ ID NO: 19	CCACATTCCCTTCATACTCC	25356007
SEQ ID NO: 20	GAGTCCCTGGTATAGCCACC	25354364
SEQ ID NO: 21	AGTCTTTTCTGTTCATCTGT	25340180
SEQ ID NO: 22	CAGGTGCTCTGTCTGTGCCC	25340142
SEQ ID NO: 23	CCCACAGGTGCTCTGTCTGT	25340138
SEQ ID NO: 24	CCTAGTCCTCCCACAGGTGC	25340129
SEQ ID NO: 25	AACCTTTCTGTGTCTGGGCC	25339254
SEQ ID NO: 26	CAGCCTTTTTGTACTGGGAC	25339012
SEQ ID NO: 27	TTCCAGCCCACATGTCCCCA	25338942
SEQ ID NO: 28	GAAATCTGCTGTTCCAGCCC	25338931
SEQ ID NO: 29	AGGCTCAACCTCAAGCAGTA	25338769
SEQ ID NO: 30	GGGAGAGTAGTTCTGTTGGT	25338727
SEQ ID NO: 31	CATTCCAATTTCTCCCTTCC	25338489
SEQ ID NO: 32	CCCTGTCCTTTCATATACTA	25338344
SEQ ID NO: 33	GGCCAAATGCACTTTCCCCA	25338284
SEQ ID NO: 34	GCACAGTAGCCATCTTTTTC	25338041
SEQ ID NO: 35	TCATTCATTTCCAGGTCAGC	25337996
SEQ ID NO: 36	AGGCACAAGCTCAGCACATT	25337708
SEQ ID NO: 37	GCATTGTCTTCTTTTTCCAC	25337455
SEQ ID NO: 38	CCCCATGTTACCTTATCACA	25337426
SEQ ID NO: 39	GTCCCTTTCATCAAGGTAGC	25337365

SEQ ID NO: 40	GCACAGTGGATGAGAAGCCT	25337320
SEQ ID NO: 41	GCTGCTCGCTTCCTGTACCA	25375752
SEQ ID NO: 42	CTTACTGGGTGAGAGTCTCC	25356686
SEQ ID NO: 43	TTCTTACCCGGCTTCCACAT	25354521
SEQ ID NO: 44	TTTCTTACCCGGCTTCCACA	25354520
SEQ ID NO: 45	CTTTCTTACCCGGCTTCCAC	25354519
SEQ ID NO: 46	TACCTTTCTGTGTCTGGGCC	25340082
SEQ ID NO: 47	ACCTTCCTGTTTTCATTTGT	25355890
SEQ ID NO: 48	ACTTACTGGGTGAGAGTCTC	25356685
SEQ ID NO: 49	TACCTTCCTGTTTTCATTTG	25355889
SEQ ID NO: 50	AACTTACTGGGTGAGAGTCT	25356684
SEQ ID NO: 51	GCCCTCCCTTCCCATCAATC	25438011
SEQ ID NO: 52	TCCCCACACCTCTGACTAGT	25436704
SEQ ID NO: 53	GGGTGGTGGGCTGGGACCAA	25435050
SEQ ID NO: 54	ACTGACCCCTAGTTCTGCCT	25430565
SEQ ID NO: 55	CCTTGGCTCTCCCCTCCCTT	25425998
SEQ ID NO: 56	GGACCCATGGCCTTTGAGCT	25415877
SEQ ID NO: 57	TGACACCATACCTCCCCTCT	25415825
SEQ ID NO: 58	CCCAGCACTACTGCCCACTA	25415373
SEQ ID NO: 59	ACCCCAGCCATCCCAGCACT	25415362
SEQ ID NO: 60	GAGTCTCTCTCTTTCCCAGT	25414672
SEQ ID NO: 61	CCTCTGACCCTTGAGTCTCC	25412413
SEQ ID NO: 62	CACCCTACCTGGGTCCCTCA	25411743
SEQ ID NO: 63	CCTCTCTTCCAGTCCCCTCT	25411061
SEQ ID NO: 64	GGTCAACTCTCAGGCCCACT	25408962
SEQ ID NO: 65	GGTGCAGCTTCTCCATCCTG	25408633
SEQ ID NO: 66	CCCTCCAGCATCAGATGTCA	25407191
SEQ ID NO: 67	GACACACCTGGTCTCCACCA	25407060
SEQ ID NO: 68	CTTCACCCATTCCCCTCAGT	25403266
SEQ ID NO: 69	TGGGCTCCTGTGTCTGTCAG	25393846

SEQ ID NO: 70	GCCCTCCAGTGACCCTGCCA	25380443
SEQ ID NO: 71	GTCCAGGAGTCTTTCAGCTT	25378642
SEQ ID NO: 72	CTGCATTCCACTGTGCCAGC	25374354
SEQ ID NO: 73	GGGTCTTCCTAGTTTGTTCC	25372328
SEQ ID NO: 74	GTTTCCTTATGCCAGTTCCC	25362783
SEQ ID NO: 75	ATGAGCAGGGTCCAGCAGGA	25342721
SEQ ID NO: 76	TTGCCACTTCCCTTCCCTGC	25341989
SEQ ID NO: 77	GACTCTACACTGTCCAGCCA	25432729
SEQ ID NO: 78	CTCCATTAGCTCCTCAGAGT	25413636
SEQ ID NO: 79	TCCTCCTAACCTCTTCCAGA	25397434
SEQ ID NO: 80	CCACATCTCAGCCATTCCTT	25366556
SEQ ID NO: 81	GCTATCACCTATCCTTGA	25371531
SEQ ID NO: 82	GTCTCCCTGTGAGCTATC	25371519
SEQ ID NO: 83	TCTGGTGTAGACCCTTCT	25371447
SEQ ID NO: 84	CCTCCCACTACATTTGCA	25371025
SEQ ID NO: 85	ATTCAACTGCTGTCCTTG	25370622
SEQ ID NO: 86	TGCAGGATTTTCCATAGC	25360497
SEQ ID NO: 87	TAGCCAGACCCAGTACTA	25356791
SEQ ID NO: 88	GTGAGAGTCTCCCAAGTC	25356693
SEQ ID NO: 89	CACATTCCCTTCATACTC	25356008
SEQ ID NO: 90	GGCTTCCACATATAAGCA	25354529
SEQ ID NO: 91	ATCTGCTGTTCCAGCCCA	25338934
SEQ ID NO: 92	GAGAGTAGTTCTGTTGGT	25338729
SEQ ID NO: 93	ACATACTGTGGCATGAGT	25338414
SEQ ID NO: 94	GCACTTTCCCCAGTAAAC	25338292
SEQ ID NO: 95	GCAATAGGCTTGACTACC	25338257
SEQ ID NO: 96	GGGAGACTTTGGATTGTC	25338130
SEQ ID NO: 97	CCAGGTCAGCTTACTGTA	25338006
SEQ ID NO: 98	GCTCAGCACATTAGCTAT	25337716
SEQ ID NO: 99	CCCCATGTTACCTTATCA	25337426

SEQ ID NO: 100	GGTCCCTTTCATCAAGGT	25337364
SEQ ID NO: 101	GGAGGGATGAGGATCACAGA	
SEQ ID NO: 102	GCTTGCTCCTTTCTTGGAGG	
SEQ ID NO: 103	TATCTCAGAGCAGGAGTTGT	
SEQ ID NO: 104	GCTCTGTACCAATGCCTCAG	
SEQ ID NO: 105	CAGAACATGCAGCTTTTTCC	
SEQ ID NO: 106	GCCATTTCCAGATATTCAGG	
SEQ ID NO: 107	TCAGTTTTCCTTGGGCTGCA	
SEQ ID NO: 108	GTTGCTGAAATGTCTCCATC	
SEQ ID NO: 109	CCCTCCCACTACATTTGCAT	
SEQ ID NO: 110	CTAGAACCTCATTCAGTGGT	
SEQ ID NO: 111	GATTCAACTGCTGTCCTTGA	
SEQ ID NO: 112	CCACATACAACTGCTTCTTC	
SEQ ID NO: 113	CCAGACCCAGTACTATGCCA	
SEQ ID NO: 114	TTCCCAGAACTCCCTAATCA	
SEQ ID NO: 115	GGTAACCTTTCTGTGTCTGG	
SEQ ID NO: 116	GGCCTTCAACAATCTCTCTT	
SEQ ID NO: 117	GCCTTTTTGTACTGGGACAC	
SEQ ID NO: 118	TCTGCTGTTCCAGCCCACAT	
SEQ ID NO: 119	ATCTGCTGTTCCAGCCCACA	
SEQ ID NO: 120	CTAAAGTTCTGAGGGCTGCA	
SEQ ID NO: 121	CATACTGTGGCATGAGTTGT	
SEQ ID NO: 122	GACTACCATTTCATTTGGCC	
SEQ ID NO: 123	CATTTCCAGGTCAGCTTACT	
SEQ ID NO: 124	CACCAAGGCACAAGCTCAGC	
SEQ ID NO: 125	AAAGCTGCATTTTTCCTGCC	
SEQ ID NO: 126	ACAGTGTTCTAAAGGCTGGC	
SEQ ID NO: 127	CAGACACATCATCAGGGCCT	
SEQ ID NO: 128	ACAGACACATCATCAGGGCC	
SEQ ID NO: 129	CACAGACACATCATCAGGGC	

SEQ ID NO: 130	GACTCAGGGATGGGCTCTTC	
SEQ ID NO: 131	GGACTCAGGGATGGGCTCTT	
SEQ ID NO: 132	TGGACTCAGGGATGGGCTCT	
SEQ ID NO: 133	TCCCTTCCTTCCATCTTTCT	
SEQ ID NO: 134	CTCCCTTCCTTCCATCTTTC	
SEQ ID NO: 135	ACATACTGTGGCATGAGTTG	
SEQ ID NO: 136	CAATCAGAGTAAACTGACCC	
SEQ ID NO: 137	GACAGGAAGCACAAAACTCA	
SEQ ID NO: 138	GGACAAGTGCATCATCTATG	
SEQ ID NO: 139	TAAATAGCCAGACCCAGTAC	
SEQ ID NO: 140	GGATTCAACTGCTGTCCTTG	
SEQ ID NO: 141	GGATTCAACTGCTGTCCTTG	
SEQ ID NO: 142	GGATTCAACTGCTGTCCTTG	
SEQ ID NO: 143	AACCTTTCTGTGTCTGGGCC	
SEQ ID NO: 144	AACCTTTCTGTGTCTGGGCC	
SEQ ID NO: 145	AACCTTTCTGTGTCTGGGCC	
SEQ ID NO: 146	GCTTGCTCCTTTCTTGGAGG	
SEQ ID NO: 147	GCTTGCTCCTTTCTTGGAGG	
SEQ ID NO: 148	GCTTGCTCCTTTCTTGGAGG	
SEQ ID NO: 149	GGTAACCTTTCTGTGTCTGG	
SEQ ID NO: 150	GGTAACCTTTCTGTGTCTGG	
SEQ ID NO: 151	GGTAACCTTTCTGTGTCTGG	
SEQ ID NO: 152	GGCCTTCAACAATCTCTCTT	
SEQ ID NO: 153	GGCCTTCAACAATCTCTCTT	
SEQ ID NO: 154	GGCCTTCAACAATCTCTCTT	
SEQ ID NO: 155	GCAATCTGGTGTAGACCCTT	
SEQ ID NO: 156	GCAATCTGGTGTAGACCCTT	
SEQ ID NO: 157	GCAATCTGGTGTAGACCCTT	
SEQ ID NO: 158	GGGATGGGCTCTTCATCATC	
SEQ ID NO: 159	GGGATGGGCTCTTCATCATC	

SEQ ID NO: 160	GGGATGGGCTCTTCATCATC	
SEQ ID NO: 161	ACCAAGTTCAGTTTCCAGGG	
SEQ ID NO: 162	ACCAAGTTCAGTTTCCAGGG	
SEQ ID NO: 163	ACCAAGTTCAGTTTCCAGGG	
SEQ ID NO: 164	GGATTCAACTGCTGTCCTTG	
SEQ ID NO: 165	GGATTCAACTGCTGTCCTTG	
SEQ ID NO: 166	ATTTCCTCCACAACCAGCTG	
SEQ ID NO: 167	ATTTCCTCCACAACCAGCTG	
SEQ ID NO: 168	ATTTCCTCCACAACCAGCTG	
SEQ ID NO: 169	CAGCCTTTTTGTACTGGGAC	
SEQ ID NO: 170	CAGCCTTTTTGTACTGGGAC	
SEQ ID NO: 171	CAGCCTTTTTGTACTGGGAC	
SEQ ID NO: 172	GCTTGCTCCTTTCTTGGAGG	
SEQ ID NO: 173	GCTTGCTCCTTTCTTGGAGG	
SEQ ID NO: 174	GCCATTTCCAGATATTCAGG	
SEQ ID NO: 175	GCCATTTCCAGATATTCAGG	
SEQ ID NO: 176	GCCATTTCCAGATATTCAGG	
SEQ ID NO: 177	GGCCTTCAACAATCTCTCTT	
SEQ ID NO: 178	GCCTTTTTGTACTGGGACAC	
SEQ ID NO: 179	GCCTTTTTGTACTGGGACAC	
SEQ ID NO: 180	GCCTTTTTGTACTGGGACAC	
SEQ ID NO: 181	GACTACCATTTCATTTGGCC	
SEQ ID NO: 182	GACTACCATTTCATTTGGCC	
SEQ ID NO: 183	GACTACCATTTCATTTGGCC	
SEQ ID NO: 184	TCATTTCCACAGCCCTCAGT	
SEQ ID NO: 185	CCTTTCTTGGAGGGATGAGG	
SEQ ID NO: 186	CTGAGCTTGCTCCTTTCTTG	
SEQ ID NO: 187	GCAGCTTTTTCCTTTTCATC	
SEQ ID NO: 188	CAGCAGCAGAACATGCAGCT	
SEQ ID NO: 189	TCTTCTTCCATAGCAGCAGC	

SEQ ID NO: 190	GATGCTTCTGAGTCTTCTTC	
SEQ ID NO: 191	TCCCCTCCCACTACATTTGC	
SEQ ID NO: 192	TCTGCAGGATTTTCCATAGC	
SEQ ID NO: 193	ACTGCTTCTTCAAGTCTGCA	
SEQ ID NO: 194	AGTCTTTTCTGTTCATCTGT	
SEQ ID NO: 195	ACAGGTGCTCTGTCTGTGCC	
SEQ ID NO: 196	CTGTGTCTGGGCCATTTTTG	
SEQ ID NO: 197	ACCTTTCTGTGTCTGGGCCA	
SEQ ID NO: 198	GTAGGTAACCTTTCTGTGTC	
SEQ ID NO: 199	ACAGCCTTTTTGTACTGGGA	
SEQ ID NO: 200	TGAAATCTGCTGTTCCAGCC	
SEQ ID NO: 201	AGGCTCAACCTCAAGCAGTA	
SEQ ID NO: 202	TCCCTGTCCTTTCATATACT	
SEQ ID NO: 203	GCACTTTCCCCAGTAAACTT	
SEQ ID NO: 204	CCTTTCTTGGAGGGATGAGG	
SEQ ID NO: 205	CCTTTCTTGGAGGGATGAGG	
SEQ ID NO: 206	CCTTTCTTGGAGGGATGAGG	
SEQ ID NO: 207	ACAGGTGCTCTGTCTGTGCC	
SEQ ID NO: 208	ACAGGTGCTCTGTCTGTGCC	
SEQ ID NO: 209	ACAGGTGCTCTGTCTGTGCC	
SEQ ID NO: 210	ACCTTTCTGTGTCTGGGCCA	
SEQ ID NO: 211	ACCTTTCTGTGTCTGGGCCA	
SEQ ID NO: 212	ACCTTTCTGTGTCTGGGCCA	
SEQ ID NO: 213	ACAGCCTTTTTGTACTGGGA	
SEQ ID NO: 214	ACAGCCTTTTTGTACTGGGA	
SEQ ID NO: 215	ACAGCCTTTTTGTACTGGGA	
SEQ ID NO: 216	GCACTTTCCCCAGTAAACTT	
SEQ ID NO: 217	GCACTTTCCCCAGTAAACTT	
SEQ ID NO: 218	GCACTTTCCCCAGTAAACTT	
SEQ ID NO: 219	ACAGCCTTTTTGTACTGGGA	

Preferred combination embodiments of the disclosure include a composition for treating Dup15q syndrome. The composition includes: a first oligonucleotide that hybridizes to an mRNA encoding the UBE3A protein along a segment of the mRNA that is at least about 90% complementary to one of SEQ ID NO: 1-40; and optionally a second oligonucleotide that hybridizes to an mRNA encoding a UBE3A protein along a segment of the mRNA that is at least about 90% complementary to a different one of SEQ ID NO: 1-40. In the preferred combination embodiments, each of the therapeutic oligonucleotides may have a gapmer structure that includes a central DNA segment flanked by modified RNA wings.

More preferred combination embodiments of the disclosure include a composition for treating Dup15q syndrome that includes an mRNA encoding a UBE3A protein along a segment of the mRNA that is at least about 90% complementary to one of SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214; and optionally a second oligonucleotide that hybridizes to an mRNA encoding a UBE3A protein along a segment of the mRNA that is at least about 90% complementary to one of SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214.

Either or both wings may include modified RNA bases, e.g., both wings may include 4 consecutive RNA bases with 2'-O-methoxyethyl ribose modifications. The entirety of each oligonucleotide may be connected via phosphodiester or phosphorothioate linkages or others as will be apparent to the skilled artisan. Most preferably, at least the terminal linkages will be non-standard (i.e., not phosphodiester, e.g., phosphorothioate) and more preferably most or all of the linkages within the RNA wings will be non-standard, e.g., phosphorothioate. Preferably the plurality of therapeutic oligonucleotides is provided lyophilized or in solution, for dilution or reconstitution in a clinic for delivery. That is, packaged in one or more tubes, lyophilized or in solution, are at least thousand to millions of copies of the first oligonucleotide and, optionally, at least thousand to millions of copies of the second oligonucleotide. This preferred combination embodiment of the composition may prove to have unexpected benefits as an antisense therapeutic for the treatment of Dup15q syndrome. Embodiments of the disclosure include oligonucleotides, including locked nucleic acid (LNA) antisense oligonucleotides targeting UBE3A which are capable of downregulating overexpression of UBE3A. The invention provides for an oligonucleotide of 10 to 30 nucleotides in length, which comprises a contiguous

nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementarity, such as 100% complementarity, to a UBE3A target nucleic acid, and which is capable of inhibiting the overexpression of UBE3A in vivo. An oligonucleotide 107 may be 100% identical to one of SEQ ID NOS: 1-219, or preferably one of SEQ ID NOS: 1-40 or one of SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214. In certain aspects oligonucleotide 107 may be at least 90%, 95%, 98%, or 99% identical to one of SEQ ID NOS: 1-219, or preferably one of SEQ ID NOS: 1-40 or one of SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214.

Embodiments include a pharmaceutically acceptable salt of the antisense oligonucleotide according to the invention, or the conjugate according to the invention.

The invention provides a pharmaceutical composition comprising the antisense oligonucleotide of the invention or the conjugate of the invention and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

The invention provides for the antisense oligonucleotide of the invention or the conjugate of the invention or the pharmaceutical salt or composition of the invention for use in medicine.

The invention provides for the antisense oligonucleotide of the invention or the conjugate of the invention or the pharmaceutical salt or composition of the invention for use in the treatment or prevention or alleviation of Dup15q syndrome. The invention provides for the use of the antisense oligonucleotide of the invention or the conjugate of the invention or the pharmaceutical salt or composition of the invention, for the preparation of a medicament for the treatment, prevention or alleviation of Dub15q syndrome.

Oligonucleotides are commonly made in the laboratory by solid-phase chemical synthesis followed by purification and isolation. When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucleobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides. The oligonucleotide of the invention may be man-made, i.e., chemically synthesized, and is typically purified or isolated. The oligonucleotide of the invention may comprise one or more modified nucleosides or nucleotides, such as 2' sugar modified nucleosides.

The modified nucleotides may be independently selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide,

an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'- O -allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a glycol modified nucleotide, and a 2'-0-(N-methylacetamide) modified nucleotide, and combinations thereof.

The nitrogenous bases of the ASO may be naturally occurring nucleobases such as adenine, guanine, cytosine, thymidine, uracil, xanthine and hypoxanthine, as well as non-naturally occurring variants, such as substituted purine or substituted pyrimidine, such as nucleobases selected from isocytosine, pseudoisocytosine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, 5-propynyl-uracil, 5-bromouracil 5-thiazolo-uracil, 2-thio-uracil, 2'-thio-thymine, inosine, diaminopurine, 6-aminopurine, 2-aminopurine, 2,6-diaminopurine and 2- chloro-6-aminopurine.

The nucleobase moieties may be indicated by the letter code for each corresponding nucleobase, e.g. A, T, G, C or U, wherein each letter may optionally include modified nucleobases of equivalent function. For example, in the exemplified oligonucleotides, the nucleobase moieties are selected from A, T, G, C, and 5-methyl cytosine. Optionally, for LNA gapmers, 5-methyl cytosine LNA nucleosides may be used.

An oligonucleotide 107 of the disclosure is capable of down-regulating (inhibiting) the expression of UBE3A. In some embodiments the antisense oligonucleotide of the invention is capable of modulating the expression of the target by inhibiting or down-regulating it. Preferably, such modulation produces an inhibition of expression of at least 20% compared to the normal expression level of the target, more preferably at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% inhibition compared to the normal expression level of the target.

An antisense oligonucleotide (ASO) of the disclosure may decrease the level of the target nucleic acid (e.g., via RNase H cleavage) or may decrease the functionality (or alter the functionality) of the target nucleic acid, e.g., via modulation of splicing of a pre-mRNA.

An oligonucleotide 107 of the disclosure may comprise one or more nucleosides which have a modified sugar moiety, i.e., a modification of the sugar moiety when compared to the ribose sugar moiety found in DNA and RNA. Numerous nucleosides with modification of the ribose sugar moiety have been made, primarily with the aim of improving certain properties of oligonucleotides, such as affinity and/or nuclease resistance. Such modifications include those where the ribose ring structure is modified, e.g., by replacement with a hexose ring (HNA), or a bicyclic ring, which typically have a bridge between the C2 and C4 carbons on the ribose ring (LNA), or an unlinked ribose ring which typically lacks a bond between the C2 and C3 carbons (e.g., UNA). Modified nucleosides also include nucleosides where the sugar moiety is replaced with a non-sugar moiety, for example in the case of peptide nucleic acids (PNA), or morpholino nucleic acids.

Sugar modifications also include modifications made via altering the substituent groups on the ribose ring to groups other than hydrogen, or the 2'-OH group naturally found in DNA and RNA nucleosides. Substituents may, for example be introduced at the 2', 3', 4' or 5' positions.

The oligonucleotide may include one or more Locked Nucleic Acid (LNA) bases. An LNA may include a 2'- modified nucleoside which comprises a biradical linking the C2' and C4' of the ribose sugar ring of said nucleoside (also referred to as a "2'-4' bridge"), which restricts or locks the conformation of the ribose ring. These nucleosides are also termed bridged nucleic acid or bicyclic nucleic acid (BNA) in the literature. The locking of the conformation of the ribose is associated with an enhanced affinity of hybridization (duplex stabilization) when the LNA is incorporated into an oligonucleotide for a complementary RNA or DNA molecule. This can be routinely determined by measuring the melting temperature of the oligonucleotide/complement duplex. Non limiting, exemplary LNA nucleosides are disclosed in WO 99/014226, WO 00/66604, WO 98/039352, WO 2004/046160, WO 00/047599, WO 2007/134181, WO 2010/077578, WO 2010/036698, WO 2007/090071, WO 2009/006478, WO 2011/156202, WO 2008/154401, WO 2009/067647, and WO 2008/150729, all incorporated by reference.

Pharmaceutically acceptable salts of oligonucleotides of the disclosure include those salts that retain the biological effectiveness and properties of the free bases or free acids, which are not biologically or otherwise undesirable. The salts are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, particularly hydrochloric acid, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic

acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, a sulfonic acid, or salicylic acid. In addition, those salts may be prepared from addition of an inorganic base or an organic base to the free acid. Salts derived from an inorganic base include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium salts. Salts derived from organic bases include, but are not limited to salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, lysine, arginine, N-ethylpiperidine, piperidine, polyamine resins.

An oligonucleotide 107 may mediate or promote nuclease mediated degradation of UBE3A pre-mRNA or mRNA transcripts. Nuclease mediated degradation refers to an oligonucleotide capable of mediating degradation of a complementary nucleotide sequence when forming a duplex with such a sequence. In some embodiments, the oligonucleotide may function via nuclease mediated degradation of the target nucleic acid, where the oligonucleotides of the invention are capable of recruiting a nuclease, particularly an endonuclease, preferably endoribonuclease (RNase), such as RNase H. Examples of oligonucleotide designs which operate via nuclease mediated mechanisms are oligonucleotides which typically comprise a region of at least 5 or 6 consecutive DNA nucleosides and are flanked on one side or both sides by affinity enhancing nucleosides, for example gapmers. The RNase H activity of an antisense oligonucleotide 107 refers to its ability to recruit RNase H when in a duplex with a complementary RNA molecule.

The antisense oligonucleotide 107 of the invention, or contiguous nucleotide sequence thereof, may be a gapmer, also termed gapmer oligonucleotide or gapmer designs. The antisense gapmers are commonly used to inhibit a target nucleic acid via RNase H mediated degradation. A gapmer oligonucleotide comprises at least three distinct structural regions a 5'-flank, a gap and a 3'-flank, F-G-F' in the '5 -> 3' orientation. The "gap" region (G) comprises a stretch of contiguous DNA nucleotides which enable the oligonucleotide to recruit RNase H. The gap region is flanked by a 5' flanking region (F) comprising one or more sugar modified nucleosides, advantageously high affinity sugar modified nucleosides, and by a 3' flanking region (F') comprising one or more sugar modified nucleosides, advantageously high affinity sugar modified nucleosides. The one or more sugar modified nucleosides in region F and F' enhance the affinity

of the oligonucleotide for the target nucleic acid (i.e., are affinity enhancing sugar modified nucleosides). In some embodiments, the one or more sugar modified nucleosides in region F and F' are 2' sugar modified nucleosides, such as high affinity 2' sugar modifications, such as independently selected from LNA and 2'-MOE.

A mixed wing gapmer is an LNA gapmer wherein one or both of region F and F' comprise a 2' substituted nucleoside, such as a 2' substituted nucleoside independently selected from the group consisting of 2'-O-alkyl-RNA units, 2'-O-methyl-RNA, 2'-amino-DNA units, 2'-fluoro-DNA units, 2'-alkoxy-RNA, 2'-MOE units, arabino nucleic acid (ANA) units, 2'-fluoro-ANA units, or combinations thereof. In some embodiments wherein at least one of region F and F', or both region F and F' comprise at least one LNA nucleoside, the remaining nucleosides of region F and F' are independently selected from the group consisting of 2'-MOE and LNA. In some embodiments wherein at least one of region F and F', or both region F and F' comprise at least two LNA nucleosides, the remaining nucleosides of region F and F' are independently selected from the group consisting of 2'-MOE and LNA. In some mixed wing embodiments, one or both of region F and F' may further comprise one or more DNA nucleosides. Gapmer designs are discussed in WO 2008/049085 and WO 2012/109395, both incorporated by reference.

Table 2 shows examples of antisense oligonucleotides of the invention that incorporate modified bases and other modifications as described herein. As explained, numerous non-standard nucleic acid monomers are commercially available from custom oligonucleotide vendors and are easily incorporated into the antisense oligonucleotides of the invention. These monomer units are described using well-known oligonucleotide synthesis nomenclature to indicate the non-standard monomer units, for example as set forth by Integrated DNA Technologies (Iowa, US). For example, in the sequences provided in Table 2, the non-standard monomer units are enclosed in forward slashes "/" and an asterisk "*" between units indicates a PS linkage, while a lack of an asterisk indicates a PO linkage. Table 2 also provides the SEQ ID NO. of the ASO.

Table 2: Exemplary ASOs of the invention with modified nucleotides and linkages.

SEQ ID	Sequence Showing Modifications
SEQ ID NO: 1	/52MOErT/*/i2MOErC/*/i2MOErA/*/i2MOErT/*T*T*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*A*G*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*/i2MOErC/*/i2MOErA/*/i2MOErG/*/32MOErT/

SEQ ID NO: 2	/52MOErT/*/i2MOErC/*/i2MOErA/*/i2MOErG/*A*G*/iMe-dC/*A*G*G*A*G*T*T*G*T*/i2MOErT/*/i2MOErG/*/i2MOErG/*/32MOErG/
SEQ ID NO: 3	/52MOErG/*/i2MOErA/*/i2MOErT/*/i2MOErT/*T*/iMe-dC/*A*G*T*T*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*T*/i2MOErT/*/i2MOErG/*/i2MOErG/*/32MOErT/
SEQ ID NO: 4	/52MOErT/*/i2MOErC/*/i2MOErC/*/i2MOErA/*T*A*G*/iMe-dC/*A*G*/iMe-dC/*A*G*/iMe-dC/*A*G*/i2MOErA/*/i2MOErA/*/i2MOErC/*/32MOErA/
SEQ ID NO: 5	/52MOErG/*/i2MOErC/*/i2MOErT/*/i2MOErT/*/iMe-dC/*T*G*A*G*T*/iMe-dC/*T*T*/iMe-dC/*T*T*/i2MOErC/*/i2MOErC/*/i2MOErA/*/32MOErT/
SEQ ID NO: 6	/52MOErG/*/i2MOErT/*/i2MOErG/*/i2MOErA/*G*/iMe-dC/*T*A*T*/iMe-dC/*A*/iMe-dC/*/iMe-dC/*T*A*T*/i2MOErC/*/i2MOErC/*/i2MOErT/*/32MOErT/
SEQ ID NO: 7	/52MOErT/*/i2MOErT/*/i2MOErG/*/i2MOErT/*T*G*T*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*G*T*G*/i2MOErA/*/i2MOErG/*/i2MOErC/*/32MOErT/
SEQ ID NO: 8	/52MOErG/*/i2MOErC/*/i2MOErA/*/i2MOErA/*T*/iMe-dC/*T*G*G*T*G*T*A*G*A*/iMe-dC/*/i2MOErC/*/i2MOErC/*/i2MOErT/*/32MOErT/
SEQ ID NO: 9	/52MOErT/*/i2MOErC/*/i2MOErC/*/i2MOErC/*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*T*A*/iMe-dC/*A*T*/i2MOErT/*/i2MOErT/*/i2MOErG/*/32MOErC/
SEQ ID NO: 10	/52MOErT/*/i2MOErT/*/i2MOErT/*/i2MOErG/*T*G*T*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErC/
SEQ ID NO: 11	/52MOErG/*/i2MOErG/*/i2MOErG/*/i2MOErA/*T*G*G*G*/iMe-dC/*T*/iMe-dC/*T*T*/iMe-dC/*A*T*/i2MOErC/*/i2MOErA/*/i2MOErT/*/32MOErC/
SEQ ID NO: 12	/52MOErA/*/i2MOErG/*/i2MOErG/*/i2MOErA/*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*T*G*T*T*T*/i2MOErC/*/i2MOErT/*/i2MOErT/*/32MOErC/
SEQ ID NO: 13	/52MOErA/*/i2MOErC/*/i2MOErC/*/i2MOErA/*A*G*T*T*/iMe-dC/*A*G*T*T*T*/iMe-dC/*/iMe-dC/*/i2MOErA/*/i2MOErG/*/i2MOErG/*/32MOErG/
SEQ ID NO: 14	/52MOErA/*/i2MOErC/*/i2MOErC/*/i2MOErT/*/iMe-dC/*A*T*T*/iMe-dC/*A*G*T*G*G*T*T*/i2MOErC/*/i2MOErA/*/i2MOErT/*/32MOErT/
SEQ ID NO: 15	/52MOErG/*/i2MOErG/*/i2MOErA/*/i2MOErT/*T*/iMe-dC/*A*A*/iMe-dC/*T*G*/iMe-dC/*T*G*T*/iMe-dC/*/i2MOErC/*/i2MOErT/*/i2MOErT/*/32MOErG/
SEQ ID NO: 16	/52MOErT/*/i2MOErC/*/i2MOErA/*/i2MOErT/*/iMe-dC/*A*A*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*T*T*G*T*T*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErC/

SEQ ID NO: 17	/52MOErA/*/i2MOErT/*/i2MOErT/*/i2MOErT/*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*A*A*/iMe-dC/*/iMe-dC/*A*/i2MOErG/*/i2MOErC/*/i2MOErT/*/32MOErG/
SEQ ID NO: 18	/52MOErG/*/i2MOErC/*/i2MOErC/*/i2MOErA/*G*A*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*G*T*A*/iMe-dC/*T*A*/i2MOErT/*/i2MOErG/*/i2MOErC/*/32MOErC/
SEQ ID NO: 19	/52MOErC/*/i2MOErC/*/i2MOErA/*/i2MOErC/*A*T*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*T*/iMe-dC/*A*T*A*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErC/
SEQ ID NO: 20	/52MOErG/*/i2MOErA/*/i2MOErG/*/i2MOErT/*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*G*G*T*A*T*A*G*/iMe-dC/*/i2MOErC/*/i2MOErA/*/i2MOErC/*/32MOErC/
SEQ ID NO: 21	/52MOErA/*/i2MOErG/*/i2MOErT/*/i2MOErC/*T*T*T*T*/iMe-dC/*T*G*T*T*/iMe-dC/*A*T*/i2MOErC/*/i2MOErT/*/i2MOErG/*/32MOErT/
SEQ ID NO: 22	/52MOErC/*/i2MOErA/*/i2MOErG/*/i2MOErG/*T*G*/iMe-dC/*T*/iMe-dC/*T*G*T*/iMe-dC/*T*G*T*/i2MOErG/*/i2MOErC/*/i2MOErC/*/32MOErC/
SEQ ID NO: 23	/52MOErC/*/i2MOErC/*/i2MOErC/*/i2MOErA/*/iMe-dC/*A*G*G*T*G*/iMe-dC/*T*/iMe-dC/*T*G*T*/i2MOErC/*/i2MOErT/*/i2MOErG/*/32MOErT/
SEQ ID NO: 24	/52MOErC/*/i2MOErC/*/i2MOErT/*/i2MOErA/*G*T*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*A*G*/i2MOErG/*/i2MOErT/*/i2MOErG/*/32MOErC/
SEQ ID NO: 25	/52MOErA/*/i2MOErA/*/i2MOErC/*/i2MOErC/*T*T*T*/iMe-dC/*T*G*T*G*T*/iMe-dC/*T*G*/i2MOErG/*/i2MOErG/*/i2MOErC/*/32MOErC/
SEQ ID NO: 26	/52MOErC/*/i2MOErA/*/i2MOErG/*/i2MOErC/*/iMe-dC/*T*T*T*T*T*G*T*A*/iMe-dC/*T*G*/i2MOErG/*/i2MOErG/*/i2MOErA/*/32MOErC/
SEQ ID NO: 27	/52MOErT/*/i2MOErT/*/i2MOErC/*/i2MOErC/*A*G*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*A*T*G*T*/iMe-dC/*/i2MOErC/*/i2MOErC/*/i2MOErC/*/32MOErA/
SEQ ID NO: 28	/52MOErG/*/i2MOErA/*/i2MOErA/*/i2MOErA/*T*/iMe-dC/*T*G*/iMe-dC/*T*G*T*T*/iMe-dC/*/iMe-dC/*A*/i2MOErG/*/i2MOErC/*/i2MOErC/*/32MOErC/
SEQ ID NO: 29	/52MOErA/*/i2MOErG/*/i2MOErG/*/i2MOErC/*T*/iMe-dC/*A*A*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*A*A*G*/iMe-dC/*/i2MOErA/*/i2MOErG/*/i2MOErT/*/32MOErA/
SEQ ID NO: 30	/52MOErG/*/i2MOErG/*/i2MOErG/*/i2MOErA/*G*A*G*T*A*G*T*T*/iMe-dC/*T*G*T*/i2MOErT/*/i2MOErG/*/i2MOErG/*/32MOErT/
SEQ ID NO: 31	/52MOErC/*/i2MOErA/*/i2MOErT/*/i2MOErT/*/iMe-dC/*/iMe-dC/*A*A*T*T*T*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*/i2MOErT/*/i2MOErT/*/i2MOErC/*/32MOErC/

SEQ ID NO: 32	/52MOErC/*/i2MOErC/*/i2MOErC/*/i2MOErT/*G*T*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*A*T*A*T*/i2MOErA/*/i2MOErC/*/i2MOErT/*/32MOErA/
SEQ ID NO: 33	/52MOErG/*/i2MOErG/*/i2MOErC/*/i2MOErC/*A*A*A*T*G*/iMe-dC/*A*/iMe-dC/*T*T*T*/iMe-dC/*/i2MOErC/*/i2MOErC/*/i2MOErC/*/32MOErA/
SEQ ID NO: 34	/52MOErG/*/i2MOErC/*/i2MOErA/*/i2MOErC/*A*G*T*A*G*/iMe-dC/*/iMe-dC/*A*T*/iMe-dC/*T*T*/i2MOErT/*/i2MOErT/*/i2MOErT/*/32MOErC/
SEQ ID NO: 35	/52MOErT/*/i2MOErC/*/i2MOErA/*/i2MOErT/*T*/iMe-dC/*A*T*T*T*/iMe-dC/*/iMe-dC/*A*G*G*T*/i2MOErC/*/i2MOErA/*/i2MOErG/*/32MOErC/
SEQ ID NO: 36	/52MOErA/*/i2MOErG/*/i2MOErG/*/i2MOErC/*A*/iMe-dC/*A*A*G*/iMe-dC/*T*/iMe-dC/*A*G*/iMe-dC/*A*/i2MOErC/*/i2MOErA/*/i2MOErT/*/32MOErT/
SEQ ID NO: 37	/52MOErG/*/i2MOErC/*/i2MOErA/*/i2MOErT/*T*G*T*/iMe-dC/*T*T*/iMe-dC/*T*T*T*T*T*/i2MOErC/*/i2MOErC/*/i2MOErA/*/32MOErC/
SEQ ID NO: 38	/52MOErC/*/i2MOErC/*/i2MOErC/*/i2MOErC/*A*T*G*T*T*A*/iMe-dC/*/iMe-dC/*T*T*A*T*/i2MOErC/*/i2MOErA/*/i2MOErC/*/32MOErA/
SEQ ID NO: 39	/52MOErG/*/i2MOErT/*/i2MOErC/*/i2MOErC/*/iMe-dC/*T*T*T*/iMe-dC/*A*T*/iMe-dC/*A*A*G*G*/i2MOErT/*/i2MOErA/*/i2MOErG/*/32MOErC/
SEQ ID NO: 40	/52MOErG/*/i2MOErC/*/i2MOErA/*/i2MOErC/*A*G*T*G*G*A*T*G*A*G*A*A*/i2MOErG/*/i2MOErC/*/i2MOErC/*/32MOErT/
SEQ ID NO: 41	/52MOErG/*/i2MOErC/*/i2MOErT/*/i2MOErG/*/iMe-dC/*T*/iMe-dC/*G*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*T*G*T*/i2MOErA/*/i2MOErC/*/i2MOErC/*/32MOErA/
SEQ ID NO: 42	/52MOErC/*/i2MOErT/*/i2MOErT/*/i2MOErA/*/iMe-dC/*T*G*G*G*T*G*A*G*A*G*T*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErC/
SEQ ID NO: 43	/52MOErT/*/i2MOErT/*/i2MOErC/*/i2MOErT/*T*A*/iMe-dC/*/iMe-dC/*/iMe-dC/*G*G*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*/i2MOErA/*/i2MOErC/*/i2MOErA/*/32MOErT/
SEQ ID NO: 44	/52MOErT/*/i2MOErT/*/i2MOErT/*/i2MOErC/*T*T*A*/iMe-dC/*/iMe-dC/*/iMe-dC/*G*G*/iMe-dC/*T*T*/iMe-dC/*/i2MOErC/*/i2MOErA/*/i2MOErC/*/32MOErA/
SEQ ID NO: 45	/52MOErC/*/i2MOErT/*/i2MOErT/*/i2MOErT/*/iMe-dC/*T*T*A*/iMe-dC/*/iMe-dC/*G*G*/iMe-dC/*T*T/i2MOErC/*/i2MOErC/*/i2MOErA/*/32MOErC/
SEQ ID NO: 46	/52MOErT/*/i2MOErA/*/i2MOErC/*/i2MOErC/*T*T*T*/iMe-dC/*T*G*T*G*T*/iMe-dC/*T*G*/i2MOErG/*/i2MOErG/*/i2MOErC/*/32MOErC/

SEQ ID NO: 47	/52MOErA/*/i2MOErC/*/i2MOErC/*/i2MOErT/*T*/iMe-dC/*/iMe-dC/*T*G*T*T*T*T*/iMe-dC/*A*T*/i2MOErT/*/i2MOErT/*/i2MOErG/*/32MOErT/
SEQ ID NO: 48	/52MOErA/*/i2MOErC/*/i2MOErT/*/i2MOErT/*A*/iMe-dC/*T*G*G*G*T*G*A*G*A*G*/i2MOErT/*/i2MOErC/*/i2MOErT/*/32MOErC/
SEQ ID NO: 49	/52MOErT/*/i2MOErA/*/i2MOErC/*/i2MOErC/*T*T*/iMe-dC/*/iMe-dC/*T*G*T*T*T*T*/iMe-dC/*A*/i2MOErT/*/i2MOErT/*/i2MOErT/*/32MOErG/
SEQ ID NO: 50	/52MOErA/*/i2MOErA/*/i2MOErC/*/i2MOErT/*T*A*/iMe-dC/*T*G*G*G*T*G*A*G*A*/i2MOErG/*/i2MOErT/*/i2MOErC/*/32MOErT/
SEQ ID NO: 51	/52MOErG/*/i2MOErC/*/i2MOErC/*/i2MOErC/*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*T*/iMe-dC/*/i2MOErA/*/i2MOErA/*/i2MOErT/*/32MOErC/
SEQ ID NO: 52	/52MOErT/*/i2MOErC/*/i2MOErC/*/i2MOErC/*/iMe-dC/*A*/iMe-dC/*A*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*T*G*A*/iMe-dC/*/i2MOErT/*/i2MOErA/*/i2MOErG/*/32MOErT/
SEQ ID NO: 53	/52MOErG/*/i2MOErG/*/i2MOErG/*/i2MOErT/*G*G*T*G*G*G*/iMe-dC/*T*G*G*G*A*/i2MOErC/*/i2MOErC/*/i2MOErC/*/32MOErA/
SEQ ID NO: 54	/52MOErA/*/i2MOErC/*/i2MOErT/*/i2MOErG/*A*/iMe-dC/*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*A*G*T*T*/iMe-dC/*T*/i2MOErG/*/i2MOErC/*/i2MOErC/*/32MOErT/
SEQ ID NO: 55	/52MOErC/*/i2MOErC/*/i2MOErT/*/i2MOErT/*G*G*/iMe-dC/*T*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*/i2MOErC/*/i2MOErC/*/i2MOErT/*/32MOErT/
SEQ ID NO: 56	/52MOErG/*/i2MOErG/*/i2MOErA/*/i2MOErC/*/iMe-dC/*/iMe-dC/*A*T*G*G*/iMe-dC/*/iMe-dC/*T*T*T*T*G*/i2MOErA/*/i2MOErG/*/i2MOErC/*/32MOErT/
SEQ ID NO: 57	/52MOErT/*/i2MOErG/*/i2MOErA/*/i2MOErC/*A*/iMe-dC/*/iMe-dC/*A*T*A*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErT/
SEQ ID NO: 58	/52MOErC/*/i2MOErC/*/i2MOErC/*/i2MOErA/*G*/iMe-dC/*A*/iMe-dC/*T*A*/iMe-dC/*T*G*/iMe-dC/*/iMe-dC/*/iMe-dC/*/i2MOErA/*/i2MOErC/*/i2MOErT/*/32MOErA/
SEQ ID NO: 59	/52MOErA/*/i2MOErC/*/i2MOErC/*/i2MOErC/*/iMe-dC/*A*G*/iMe-dC/*/iMe-dC/*A*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*G*/i2MOErC/*/i2MOErA/*/i2MOErC/*/32MOErT/
SEQ ID NO: 60	/52MOErG/*/i2MOErA/*/i2MOErG/*/i2MOErT/*/iMe-dC/*T*/iMe-dC/*T*/iMe-dC/*T*/iMe-dC/*T*T*T*/iMe-dC/*/iMe-dC/*/i2MOErC/*/i2MOErA/*/i2MOErG/*/32MOErT/
SEQ ID NO: 61	/52MOErC/*/i2MOErC/*/i2MOErT/*/i2MOErC/*T*G*A*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*T*G*A*G*T*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErC/

SEQ ID NO: 62	/52MOErC/*/i2MOErA/*/i2MOErC/*/i2MOErC/*/iMe-dC/*T*A*/iMe-dC/*/iMe-dC/*T*G*G*G*T*/iMe-dC/*/iMe-dC/*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErA/
SEQ ID NO: 63	/52MOErC/*/i2MOErC/*/i2MOErT/*/i2MOErC/*T*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*A*G*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErT/
SEQ ID NO: 64	/52MOErG/*/i2MOErG/*/i2MOErT/*/i2MOErC/*A*A*/iMe-dC/*T*/iMe-dC/*T*/iMe-dC/*A*G*G*/iMe-dC/*/iMe-dC/*/i2MOErC/*/i2MOErA/*/i2MOErC/*/32MOErT/
SEQ ID NO: 65	/52MOErG/*/i2MOErG/*/i2MOErT/*/i2MOErG/*/iMe-dC/*A*G*/iMe-dC/*T*T*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*A*T*/i2MOErC/*/i2MOErC/*/i2MOErT/*/32MOErG/
SEQ ID NO: 66	/52MOErC/*/i2MOErC/*/i2MOErC/*/i2MOErT/*/iMe-dC/*/iMe-dC/*A*G*/iMe-dC/*A*T*/iMe-dC/*A*G*A*T*/i2MOErG/*/i2MOErT/*/i2MOErC/*/32MOErA/
SEQ ID NO: 67	/52MOErG/*/i2MOErA/*/i2MOErC/*/i2MOErA/*/iMe-dC/*A*/iMe-dC/*/iMe-dC/*T*G*G*T*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*/i2MOErA/*/i2MOErC/*/i2MOErC/*/32MOErA/
SEQ ID NO: 68	/52MOErC/*/i2MOErT/*/i2MOErT/*/i2MOErC/*A*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*T*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*/iMe-dC/T*/i2MOErC/*/i2MOErA/*/i2MOErG/*/32MOErT/
SEQ ID NO: 69	/52MOErT/*/i2MOErG/*/i2MOErG/*/i2MOErG/*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*T*G*T*G*T*/iMe-dC/*T*G*/i2MOErT/*/i2MOErC/*/i2MOErA/*/32MOErG/
SEQ ID NO: 70	/52MOErG/*/i2MOErC/*/i2MOErC/*/i2MOErC/*T*/iMe-dC/*/iMe-dC/*A*G*T*G*A*/iMe-dC/*/iMe-dC/*/iMe-dC/T*/i2MOErG/*/i2MOErC/*/i2MOErC/*/32MOErA/
SEQ ID NO: 71	/52MOErG/*/i2MOErT/*/i2MOErC/*/i2MOErC/*A*G*G*A*G*T*/iMe-dC/*T*T*T*/iMe-dC/*A*/i2MOErG/*/i2MOErC/*/i2MOErT/*/32MOErT/
SEQ ID NO: 72	/52MOErC/*/i2MOErT/*/i2MOErG/*/i2MOErC/*A*T*T*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*T*G*T*G*/iMe-dC/*/i2MOErC/*/i2MOErA/*/i2MOErG/*/32MOErC/
SEQ ID NO: 73	/52MOErG/*/i2MOErG/*/i2MOErG/*/i2MOErT/*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*T*A*G*T*T*T*G*/i2MOErT/*/i2MOErT/*/i2MOErC/*/32MOErC/
SEQ ID NO: 74	/52MOErG/*/i2MOErT/*/i2MOErT/*/i2MOErT/*/iMe-dC/*/iMe-dC/*T*T*A*T*G*/iMe-dC/*/iMe-dC/*A*G*T*/i2MOErT/*/i2MOErC/*/i2MOErC/*/32MOErC/
SEQ ID NO: 75	/52MOErA/*/i2MOErT/*/i2MOErG/*/i2MOErA/*G*/iMe-dC/*A*G*G*G*T*/iMe-dC/*/iMe-dC/*A*G*/iMe-dC/*/i2MOErA/*/i2MOErG/*/i2MOErG/*/32MOErA/
SEQ ID NO: 76	/52MOErT/*/i2MOErT/*/i2MOErG/*/i2MOErC/*/iMe-dC/*A*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*/i2MOErC/*/i2MOErT/*/i2MOErG/*/32MOErC/

SEQ ID NO: 77	/52MOErG/*/i2MOErA/*/i2MOErC/*/i2MOErT/*/iMe-dC/*T*A*/iMe-dC/*A*/iMe-dC/*T*G*T*/iMe-dC/*/iMe-dC/*A*/i2MOErG/*/i2MOErC/*/i2MOErC/*/32MOErA/
SEQ ID NO: 78	/52MOErC/*/i2MOErT/*/i2MOErC/*/i2MOErC/*A*T*T*A*G*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*A*/i2MOErG/*/i2MOErA/*/i2MOErG/*/32MOErT/
SEQ ID NO: 79	/52MOErT/*/i2MOErC/*/i2MOErC/*/i2MOErT/*/iMe-dC/*/iMe-dC/*T*A*A*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*T*T*/iMe-dC/*/i2MOErC/*/i2MOErA/*/i2MOErG/*/32MOErA/
SEQ ID NO: 80	/52MOErC/*/i2MOErC/*/i2MOErA/*/i2MOErC/*A*T*/iMe-dC/*T*/iMe-dC/*A*G*/iMe-dC/*/iMe-dC/*A*T*T*/i2MOErC/*/i2MOErC/*/i2MOErT/*/32MOErT/
SEQ ID NO: 101	/52MOErG/*/i2MOErG/*/i2MOErA/*/i2MOErG/*G*G*A*T*G*A*G*G*A*T*/iMe-dC/*A*/i2MOErC/*/i2MOErA/*/i2MOErG/*/32MOErA/
SEQ ID NO: 102	/52MOErG/*/i2MOErC/*/i2MOErT/*/i2MOErT/*G*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*T*G*/i2MOErG/*/i2MOErA/*/i2MOErG/*/32MOErG/
SEQ ID NO: 103	/52MOErT/*/i2MOErA/*/i2MOErT/*/i2MOErC/*T*/iMe-dC/*A*G*A*G*/iMe-dC/*A*G*G*A*G*/i2MOErT/*/i2MOErT/*/i2MOErG/*/32MOErT/
SEQ ID NO: 104	/52MOErG/*/i2MOErC/*/i2MOErT/*/i2MOErC/*T*G*T*A*/iMe-dC/*/iMe-dC/*A*A*T*G*/iMe-dC/*/iMe-dC/*/i2MOErT/*/i2MOErC/*/i2MOErA/*/32MOErG/
SEQ ID NO: 105	/52MOErC/*/i2MOErA/*/i2MOErG/*/i2MOErA/*A*/iMe-dC/*A*T*G*/iMe-dC/*A*G*/iMe-dC/*T*T*T*/i2MOErT/*/i2MOErT/*/i2MOErC/*/32MOErC/
SEQ ID NO: 106	/52MOErG/*/i2MOErC/*/i2MOErC/*/i2MOErA/*T*T*T*/iMe-dC/*/iMe-dC/*A*G*A*T*A*T*T*/i2MOErC/*/i2MOErA/*/i2MOErG/*/32MOErG/
SEQ ID NO: 107	/52MOErT/*/i2MOErC/*/i2MOErA/*/i2MOErG/*T*T*T*T*/iMe-dC/*/iMe-dC/*T*T*G*G*G*/iMe-dC/*/i2MOErT/*/i2MOErG/*/i2MOErC/*/32MOErA/
SEQ ID NO: 108	/52MOErG/*/i2MOErT/*/i2MOErT/*/i2MOErG/*/iMe-dC/*T*G*A*A*A*T*G*T*/iMe-dC/*T*/iMe-dC/*/i2MOErC/*/i2MOErA/*/i2MOErT/*/32MOErC/
SEQ ID NO: 109	/52MOErC/*/i2MOErC/*/i2MOErC/*/i2MOErT/*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*T*A*/iMe-dC/*A*T*T*T*/i2MOErG/*/i2MOErC/*/i2MOErA/*/32MOErT/
SEQ ID NO: 110	/52MOErC/*/i2MOErT/*/i2MOErA/*/i2MOErG/*A*A*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*A*T*T*/iMe-dC/*A*G*/i2MOErT/*/i2MOErG/*/i2MOErG/*/32MOErT/
SEQ ID NO: 111	/52MOErG/*/i2MOErA/*/i2MOErT/*/i2MOErT/*/iMe-dC/*A*A*/iMe-dC/*T*G*/iMe-dC/*T*G*T*/iMe-dC/*/iMe-dC/*/i2MOErT/*/i2MOErT/*/i2MOErG/*/32MOErA/

SEQ ID NO: 112	/52MOErC/*/i2MOErC/*/i2MOErA/*/i2MOErC/*A*T*A*/iMe-dC/*A*A*/iMe-dC/*T*G*/iMe-dC/*T*T*/i2MOErC/*/i2MOErT/*/i2MOErT/*/32MOErC/
SEQ ID NO: 113	/52MOErC/*/i2MOErC/*/i2MOErA/*/i2MOErG/*A*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*G*T*A*/iMe-dC/*T*A*T*/i2MOErG/*/i2MOErC/*/i2MOErC/*/32MOErA/
SEQ ID NO: 114	/52MOErT/*/i2MOErT/*/i2MOErC/*/i2MOErC/*/iMe-dC/*A*G*A*A*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*T*A*/i2MOErA/*/i2MOErT/*/i2MOErC/*/32MOErA/
SEQ ID NO: 115	/52MOErG/*/i2MOErG/*/i2MOErT/*/i2MOErA/*A*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*G*T*G*T*/i2MOErC/*/i2MOErT/*/i2MOErG/*/32MOErG/
SEQ ID NO: 116	/52MOErG/*/i2MOErG/*/i2MOErC/*/i2MOErC/*T*T*/iMe-dC/*A*A*/iMe-dC/*A*A*T*/iMe-dC/*T*/iMe-dC/*/i2MOErT/*/i2MOErC/*/i2MOErT/*/32MOErT/
SEQ ID NO: 117	/52MOErG/*/i2MOErC/*/i2MOErC/*/i2MOErT/*T*T*T*T*G*T*A*/iMe-dC/*T*G*G*G*/i2MOErA/*/i2MOErC/*/i2MOErA/*/32MOErC/
SEQ ID NO: 118	/52MOErT/*/i2MOErC/*/i2MOErT/*/i2MOErG/*/iMe-dC/*T*G*T*T*/iMe-dC/*/iMe-dC/*A*G*/iMe-dC/*/iMe-dC/*/iMe-dC/*/i2MOErA/*/i2MOErC/*/i2MOErA/*/32MOErT/
SEQ ID NO: 119	/52MOErA/*/i2MOErT/*/i2MOErC/*/i2MOErT/*G*/iMe-dC/*T*G*T*T*/iMe-dC/*/iMe-dC/*A*G*/iMe-dC/*/iMe-dC/*/i2MOErC/*/i2MOErA/*/i2MOErC/*/32MOErA/
SEQ ID NO: 120	/52MOErC/*/i2MOErT/*/i2MOErA/*/i2MOErA/*A*G*T*T*/iMe-dC/*T*G*A*G*G*G*/iMe-dC/*/i2MOErT/*/i2MOErG/*/i2MOErC/*/32MOErA/
SEQ ID NO: 121	/52MOErC/*/i2MOErA/*/i2MOErT/*/i2MOErA/*/iMe-dC/*T*G*T*G*G*/iMe-dC/*A*T*G*A*G*/i2MOErT/*/i2MOErT/*/i2MOErG/*/32MOErT/
SEQ ID NO: 122	/52MOErG/*/i2MOErA/*/i2MOErC/*/i2MOErT/*A*/iMe-dC/*/iMe-dC/*A*T*T*T*/iMe-dC/*A*T*T*T*T*/i2MOErG/*/i2MOErG/*/i2MOErC/*/32MOErC/
SEQ ID NO: 123	/52MOErC/*/i2MOErA/*/i2MOErT/*/i2MOErT/*T*/iMe-dC/*/iMe-dC/*A*G*G*T*/iMe-dC/*A*G*/iMe-dC/*T*/i2MOErT/*/i2MOErA/*/i2MOErC/*/32MOErT/
SEQ ID NO: 124	/52MOErC/*/i2MOErA/*/i2MOErC/*/i2MOErC/*A*A*G*G*/iMe-dC/*A*/iMe-dC/*A*A*G*/iMe-dC/*T*/i2MOErC/*/i2MOErA/*/i2MOErG/*/32MOErC/
SEQ ID NO: 125	/52MOErA/*/i2MOErA/*/i2MOErA/*/i2MOErG/*/iMe-dC/*T*G*/iMe-dC/*A*T*T*T*T*T*/iMe-dC/*/iMe-dC/*/i2MOErT/*/i2MOErG/*/i2MOErC/*/32MOErC/
SEQ ID NO: 126	/52MOErA/*/i2MOErC/*/i2MOErA/*/i2MOErG/*T*G*T*T*/iMe-dC/*T*A*A*A*G*G*/iMe-dC/*/i2MOErT/*/i2MOErG/*/i2MOErG/*/32MOErC/

SEQ ID NO: 127	/52MOErC/*/i2MOErA/*/i2MOErG/*/i2MOErA/*/iMe-dC/*A*/iMe-dC/*A*T*/iMe-dC/*A*T*/iMe-dC/*A*G*G*/i2MOErG/*/i2MOErC/*/i2MOErC/*/32MOErT/
SEQ ID NO: 128	/52MOErA/*/i2MOErC/*/i2MOErA/*/i2MOErG/*A*/iMe-dC/*A*/iMe-dC/*A*T*/iMe-dC/*A*T*/iMe-dC/*A*G*/i2MOErG/*/i2MOErG/*/i2MOErC/*/32MOErC/
SEQ ID NO: 129	/52MOErC/*/i2MOErA/*/i2MOErC/*/i2MOErA/*G*A*/iMe-dC/*A*/iMe-dC/*A*T*/iMe-dC/*A*T*/iMe-dC/*A*/i2MOErG/*/i2MOErG/*/i2MOErG/*/32MOErC/
SEQ ID NO: 130	/52MOErG/*/i2MOErA/*/i2MOErC/*/i2MOErT/*/iMe-dC/*A*G*G*G*A*T*G*G*G*/iMe-dC/*T*/i2MOErC/*/i2MOErT/*/i2MOErT/*/32MOErC/
SEQ ID NO: 131	/52MOErG/*/i2MOErG/*/i2MOErA/*/i2MOErC/*T*/iMe-dC/*A*G*G*G*A*T*G*G*G*/iMe-dC/*/i2MOErT/*/i2MOErC/*/i2MOErT/*/32MOErT/
SEQ ID NO: 132	/52MOErT/*/i2MOErG/*/i2MOErG/*/i2MOErA/*/iMe-dC/*T*/iMe-dC/*A*G*G*G*A*T*G*G*G*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErT/
SEQ ID NO: 133	/52MOErT/*/i2MOErC/*/i2MOErC/*/i2MOErC/*T*T*/iMe-dC/*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*A*T*/iMe-dC/*T*/i2MOErT/*/i2MOErT/*/i2MOErC/*/32MOErT/
SEQ ID NO: 134	/52MOErC/*/i2MOErT/*/i2MOErC/*/i2MOErC/*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*T*T*/iMe-dC/*/iMe-dC/*A*T*/iMe-dC/*/i2MOErT/*/i2MOErT/*/i2MOErT/*/32MOErC/
SEQ ID NO: 135	/52MOErA/*/i2MOErC/*/i2MOErA/*/i2MOErT/*A*/iMe-dC/*T*G*T*G*G*/iMe-dC/*A*T*G*A*/i2MOErG/*/i2MOErT/*/i2MOErT/*/32MOErG/
SEQ ID NO: 136	/52MOErC/*/i2MOErA/*/i2MOErA/*/i2MOErT/*/iMe-dC/*A*G*A*G*T*A*A*A*/iMe-dC/*T*G*/i2MOErA/*/i2MOErC/*/i2MOErC/*/32MOErC/
SEQ ID NO: 137	/52MOErG/*/i2MOErA/*/i2MOErC/*/i2MOErA/*G*G*A*A*G*/iMe-dC/*A*/iMe-dC/*A*A*A*A*/i2MOErC/*/i2MOErT/*/i2MOErC/*/32MOErA/
SEQ ID NO: 138	/52MOErG/*/i2MOErG/*/i2MOErA/*/i2MOErC/*A*A*G*T*G*/iMe-dC/*A*T*/iMe-dC/*A*T*/iMe-dC/*/i2MOErT/*/i2MOErA/*/i2MOErT/*/32MOErG/
SEQ ID NO: 139	/52MOErT/*/i2MOErA/*/i2MOErA/*/i2MOErA/*T*A*G*/iMe-dC/*/iMe-dC/*A*G*A*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*/i2MOErG/*/i2MOErT/*/i2MOErA/*/32MOErC/
SEQ ID NO: 140	/52MOErG/*/i2MOErG//i2MOErA/ /i2MOErT/*T*/iMe-dC/*A*A*/iMe-dC/*T*G*/iMe-dC/*T*G*T*/iMe-dC/*/i2MOErC//i2MOErT/ /i2MOErT/*/32MOErG/
SEQ ID NO: 141	/52MOErG//i2MOErG/*/i2MOErA/ /i2MOErT/*T*/iMe-dC/*A*A*/iMe-dC/*T*G*/iMe-dC/*T*G*T*/iMe-dC/*/i2MOErC//i2MOErT/*/i2MOErT/*/32MOErG/

SEQ ID NO: 142	/52MOErG/*/i2MOErG//i2MOErA/ /i2MOErT/T*/iMe-dC/*A*A*/iMe-dC/*T*G*/iMe-dC/*T*G*T*/iMe-dC/*/i2MOErC/*/i2MOErT/ /i2MOErT/*/32MOErG/
SEQ ID NO: 143	/52MOErA/*/i2MOErA//i2MOErC/ /i2MOErC/*T*T*T*/iMe-dC/*T*G*T*G*T*/iMe-dC/*T*G*/i2MOErG//i2MOErG/ /i2MOErC/*/32MOErC/
SEQ ID NO: 144	/52MOErA//i2MOErA//i2MOErC/*/i2MOErC/*T*T*T*/iMe-dC/*T*G*T*G*T*/iMe-dC/*T*G*/i2MOErG//i2MOErG/ /i2MOErC/*/32MOErC/
SEQ ID NO: 145	/52MOErA/*/i2MOErA//i2MOErC/ /i2MOErC/T*T*T*/iMe-dC/*T*G*T*G*T*/iMe-dC/*T*G*/i2MOErG//i2MOErG/ /i2MOErC/*/32MOErC/
SEQ ID NO: 146	/52MOErG/*/i2MOErC//i2MOErT/ /i2MOErT/*G*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*T*G*/i2MOErG//i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 147	/52MOErG//i2MOErC//i2MOErT/ /i2MOErT/*G*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*T*G*/i2MOErG/*/i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 148	/52MOErG/*/i2MOErC//i2MOErT/ /i2MOErT/G*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*T*G*/i2MOErG/*/i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 149	/52MOErG//i2MOErG//i2MOErT/*/i2MOErA/*A*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*G*T*G*T*/i2MOErC//i2MOErT/ /i2MOErG/*/32MOErG/
SEQ ID NO: 150	/52MOErG/*/i2MOErG//i2MOErT/ /i2MOErA/*A*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*G*T*G*T*/i2MOErC/*/i2MOErT/ /i2MOErG/*/32MOErG/
SEQ ID NO: 151	/52MOErG/*/i2MOErG//i2MOErT/ /i2MOErA/A*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*G*T*G*T*/i2MOErC//i2MOErT/ /i2MOErG/*/32MOErG/
SEQ ID NO: 152	/52MOErG/*/i2MOErG//i2MOErC/ /i2MOErC/*T*T*/iMe-dC/*A*A*/iMe-dC/*A*A*T*/iMe-dC/*T*/iMe-dC/*/i2MOErT/*/i2MOErC/ /i2MOErT/*/32MOErT/
SEQ ID NO: 153	/52MOErG/*/i2MOErG//i2MOErC/ /i2MOErC/*T*T*/iMe-dC/*A*A*/iMe-dC/*A*A*T*/iMe-dC/*T*/iMe-dC/*/i2MOErT//i2MOErC/ /i2MOErT/*/32MOErT/
SEQ ID NO: 154	/52MOErG/*/i2MOErG//i2MOErC/ /i2MOErC/T*T*/iMe-dC/*A*A*/iMe-dC/*A*A*T*/iMe-dC/*T*/iMe-dC/*/i2MOErT/*/i2MOErC/ /i2MOErT/*/32MOErT/
SEQ ID NO: 155	52MOErG/*/i2MOErC//i2MOErA/ /i2MOErA/*T*/iMe-dC/*T*G*G*T*G*T*A*G*A*/iMe-dC/*/i2MOErC/*/i2MOErC/ /i2MOErT/*/32MOErT/
SEQ ID NO: 156	/52MOErG/*/i2MOErC//i2MOErA/ /i2MOErA/*T*/iMe-dC/*T*G*G*T*G*T*A*G*A*/iMe-dC/*/i2MOErC//i2MOErC/ /i2MOErT/*/32MOErT/

SEQ ID NO: 157	/52MOErG//i2MOErC//i2MOErA/ /i2MOErA/*T*/iMe-dC/*T*G*G*T*G*T*A*G*A*/iMe-dC/*/i2MOErC/*/i2MOErC/ /i2MOErT/*/32MOErT/
SEQ ID NO: 158	/52MOErG/*/i2MOErG//i2MOErG/ /i2MOErA/*T*G*G*G*/iMe-dC/*T*/iMe-dC/*T*T*/iMe-dC/*A*T*/i2MOErC/*/i2MOErA/ /i2MOErT/*/32MOErC/
SEQ ID NO: 159	/52MOErG/*/i2MOErG//i2MOErG/ /i2MOErA/*T*G*G*G*/iMe-dC/*T*/iMe-dC/*T*T*/iMe-dC/*A*T*/i2MOErC//i2MOErA/ /i2MOErT/*/32MOErC/
SEQ ID NO: 160	/52MOErG//i2MOErG/*/i2MOErG/ /i2MOErA/*T*G*G*G*/iMe-dC/*T*/iMe-dC/*T*T*/iMe-dC/*A*T*/i2MOErC/*/i2MOErA/ /i2MOErT/*/32MOErC/
SEQ ID NO: 161	/52MOErA/*/i2MOErC//i2MOErC/ /i2MOErA/*A*G*T*T*/iMe-dC/*A*G*T*T*T*/iMe-dC/*/iMe-dC/*/i2MOErA/*/i2MOErG/ /i2MOErG/*/32MOErG/
SEQ ID NO: 162	/52MOErA/*/i2MOErC//i2MOErC/ /i2MOErA/*A*G*T*T*/iMe-dC/*A*G*T*T*T*/iMe-dC/*/iMe-dC/*/i2MOErA//i2MOErG/ /i2MOErG/*/32MOErG/
SEQ ID NO: 163	/52MOErA//i2MOErC//i2MOErC/*/i2MOErA/*A*G*T*T*/iMe-dC/*A*G*T*T*T*/iMe-dC/*/iMe-dC/*/i2MOErA/*/i2MOErG/ /i2MOErG/*/32MOErG/
SEQ ID NO: 164	/52MOErG/*/i2MOErG//i2MOErA/ /i2MOErT/*T*/iMe-dC/*A*A*/iMe-dC/*T*G*/iMe-dC/*T*G*T*/iMe-dC/*/i2MOErC/*/i2MOErT/ /i2MOErT/*/32MOErG/
SEQ ID NO: 165	/52MOErG//i2MOErG//i2MOErA/*/i2MOErT/*T*/iMe-dC/*A*A*/iMe-dC/*T*G*/iMe-dC/*T*G*T*/iMe-dC/*/i2MOErC//i2MOErT/ /i2MOErT/*/32MOErG/
SEQ ID NO: 166	/52MOErA/*/i2MOErT//i2MOErT/ /i2MOErT/*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*A*A*/iMe-dC/*/iMe-dC/*A*/i2MOErG/*/i2MOErC/ /i2MOErT/*/32MOErG/
SEQ ID NO: 167	/52MOErA/*/i2MOErT//i2MOErT/ /i2MOErT/*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*A*A*/iMe-dC/*/iMe-dC/*A*/i2MOErG//i2MOErC/ /i2MOErT/*/32MOErG/
SEQ ID NO: 168	/52MOErA//i2MOErT//i2MOErT/*/i2MOErT/*/iMe-dC/*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*A*/iMe-dC/*A*A*/iMe-dC/*/iMe-dC/*A*/i2MOErG/*/i2MOErC/ /i2MOErT/*/32MOErG/
SEQ ID NO: 169	/52MOErC/*/i2MOErA//i2MOErG/ /i2MOErC/*/iMe-dC/*T*T*T*T*T*G*T*A*/iMe-dC/*T*G*/i2MOErG/*/i2MOErG/ /i2MOErA/*/32MOErC/
SEQ ID NO: 170	/52MOErC//i2MOErA//i2MOErG/*/i2MOErC/*/iMe-dC/*T*T*T*T*T*G*T*A*/iMe-dC/*T*G*/i2MOErG//i2MOErG/ /i2MOErA/*/32MOErC/
SEQ ID NO: 171	/52MOErC/*/i2MOErA//i2MOErG/ /i2MOErC/*/iMe-dC/*T*T*T*T*T*G*T*A*/iMe-dC/*T*G*/i2MOErG//i2MOErG/ /i2MOErA/*/32MOErC/

SEQ ID NO: 172	/52MOErG/*/i2MOErC//i2MOErT/ /i2MOErT/*G*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*T*G*/i2MOErG/*/i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 173	/52MOErG//i2MOErC/*/i2MOErT/ /i2MOErT/*G*/iMe-dC/*T*/iMe-dC/*/iMe-dC/*T*T*T*/iMe-dC/*T*T*G*/i2MOErG/*/i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 174	/52MOErG/*/i2MOErC//i2MOErC/ /i2MOErA/*T*T*T*/iMe-dC/*/iMe-dC/*A*G*A*T*A*T*T*/i2MOErC/*/i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 175	/52MOErG//i2MOErC//i2MOErC/*/i2MOErA/*T*T*T*/iMe-dC/*/iMe-dC/*A*G*A*T*A*T*T*/i2MOErC/*/i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 176	/52MOErG/*/i2MOErC//i2MOErC/ /i2MOErA/*T*T*T*/iMe-dC/*/iMe-dC/*A*G*A*T*A*T*T*/i2MOErC//i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 177	/52MOErG//i2MOErG/*/i2MOErC/ /i2MOErC/*T*T*/iMe-dC/*A*A*/iMe-dC/*A*A*T*/iMe-dC/*T*/iMe-dC/*/i2MOErT/*/i2MOErC/ /i2MOErT/*/32MOErT/
SEQ ID NO: 178	/52MOErG/*/i2MOErC//i2MOErC/ /i2MOErT/*T*T*T*T*T*G*T*A*C*T*G*G*G*/i2MOErA/*/i2MOErC/ /i2MOErA/*/32MOErC/
SEQ ID NO: 179	/52MOErG//i2MOErC/*/i2MOErC/ /i2MOErT/*T*T*T*T*T*G*T*A*C*T*G*G*G*/i2MOErA/*/i2MOErC/ /i2MOErA/*/32MOErC/
SEQ ID NO: 180	/52MOErG/*/i2MOErC//i2MOErC/ /i2MOErT/*T*T*T*T*T*G*T*A*C*T*G*G*G*/i2MOErA//i2MOErC/ /i2MOErA/*/32MOErC/
SEQ ID NO: 181	/52MOErG/*/i2MOErA//i2MOErC/ /i2MOErT/*A*/iMe-dC/*/iMe-dC/*A*T*T*T*/iMe-dC/*A*T*T*T*T*/i2MOErG/*/i2MOErG/ /i2MOErC/*/32MOErC/
SEQ ID NO: 182	/52MOErG//i2MOErA/*/i2MOErC/ /i2MOErT/*A*/iMe-dC/*/iMe-dC/*A*T*T*T*T*/iMe-dC/*A*T*T*T*T*/i2MOErG/*/i2MOErG/ /i2MOErC/*/32MOErC/
SEQ ID NO: 183	/52MOErG/*/i2MOErA//i2MOErC/ /i2MOErT/*A*/iMe-dC/*/iMe-dC/*A*T*T*T*T*/iMe-dC/*A*T*T*T*T*/i2MOErG//i2MOErG/ /i2MOErC/*/32MOErC/
SEQ ID NO: 204	52MOErC/*/i2MOErC//i2MOErT/ /i2MOErT/*T*/iMe-dC/*T*T*G*G*A*G*G*G*A*T*/i2MOErG/*/i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 205	/52MOErC/*/i2MOErC//i2MOErT/ /i2MOErT/*T*/iMe-dC/*T*T*G*G*A*G*G*G*A*T*/i2MOErG//i2MOErA/ /i2MOErG/*/32MOErG/
SEQ ID NO: 206	/52MOErC/*/i2MOErC//i2MOErT/ /i2MOErT/T*/iMe-dC/*T*T*G*G*A*G*G*G*A*T*/i2MOErG/*/i2MOErA/ /i2MOErG/*/32MOErG/

SEQ ID NO: 207	/52MOErA/*/i2MOErC//i2MOErA/ /i2MOErG/*G*T*G*/iMe-dC/*T*/iMe-dC/*T*G*T*/iMe-dC/*T*G*/i2MOErT/*/i2MOErG/ /i2MOErC/*/32MOErC/
SEQ ID NO: 208	52MOErA/*/i2MOErC//i2MOErA/ /i2MOErG/*G*T*G*/iMe-dC/*T*/iMe-dC/*T*G*T*/iMe-dC/*T*G*/i2MOErT//i2MOErG/ /i2MOErC/*/32MOErC/
SEQ ID NO: 209	/52MOErA/*/i2MOErC//i2MOErA/ /i2MOErG/G*T*G*/iMe-dC/*T*/iMe-dC/*T*G*T*/iMe-dC/*T*G*/i2MOErT/*/i2MOErG/ /i2MOErC/*/32MOErC
SEQ ID NO: 210	/52MOErA/*/i2MOErC//i2MOErC/ /i2MOErT/*T*T*/iMe-dC/*T*G*T*G*T*/iMe-dC/*T*G*G*/i2MOErG//i2MOErC/ /i2MOErC/*/32MOErA/
SEQ ID NO: 211	/52MOErA/*/i2MOErC//i2MOErC/ /i2MOErT/*T*T*/iMe-dC/*T*G*T*G*T*/iMe-dC/*T*G*G*/i2MOErG/*/i2MOErC/ /i2MOErC/*/32MOErA/
SEQ ID NO: 212	/52MOErA/*/i2MOErC//i2MOErC/ /i2MOErT/T*T*/iMe-dC/*T*G*T*G*T*/iMe-dC/*T*G*G*/i2MOErG/*/i2MOErC/ /i2MOErC/*/32MOErA/
SEQ ID NO: 213	/52MOErA/*/i2MOErC//i2MOErA/ /i2MOErG/*/iMe-dC/*/iMe-dC/*T*T*T*T*T*G*T*A*/iMe-dC/*T*/i2MOErG//i2MOErG/ /i2MOErG/*/32MOErA/
SEQ ID NO: 214	/52MOErA/*/i2MOErC//i2MOErA/ /i2MOErG/*/iMe-dC/*/iMe-dC/*T*T*T*T*T*G*T*A*/iMe-dC/*T*/i2MOErG/*/i2MOErG/ /i2MOErG/*/32MOErA/
SEQ ID NO: 215	/52MOErA/*/i2MOErC//i2MOErA/ /i2MOErG//iMe-dC/*/iMe-dC/*T*T*T*T*T*G*T*A*/iMe-dC/*T*/i2MOErG/*/i2MOErG/ /i2MOErG/*/32MOErA/
SEQ ID NO: 216	/52MOErG/*/i2MOErC//i2MOErA/ /i2MOErC/*T*T*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*G*T*A*A*/i2MOErA//i2MOErC/ /i2MOErT/*/32MOErT/
SEQ ID NO: 217	/52MOErG/*/i2MOErC//i2MOErA/ /i2MOErC/*T*T*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*G*T*A*A*/i2MOErA/*/i2MOErC/ /i2MOErT/*/32MOErT/
SEQ ID NO: 218	/52MOErG/*/i2MOErC//i2MOErA/ /i2MOErC/T*T*T*/iMe-dC/*/iMe-dC/*/iMe-dC/*/iMe-dC/*A*G*T*A*A*/i2MOErA/*/i2MOErC/ /i2MOErT/*/32MOErT/
SEQ ID NO: 219	/52MOErA/*/i2MOErC//i2MOErA/*/i2MOErG/*/iMe-dC/*/iMe-dC/*T*T*T*T*T*G*T*A*/iMe-dC/*T*/i2MOErG/*/i2MOErG/ /i2MOErG/*/32MOErA/
Monomer Abbreviations 52MOEr =5' 2'-O-methoxyethyl RNA 32MOEr = 3' 2'-O-methoxyethyl RNA i2MOEr = internal 2'-O-methoxyethyl RNA iMe-dC = 5-methyl deoxycytidine *= PS linkage / / = PO linkage (non-PS linkage)	

Conjugation of the oligonucleotide 107 to one or more non-nucleotide moieties may improve the pharmacology of the oligonucleotide, e.g., by affecting the activity, cellular distribution, cellular uptake or stability of the oligonucleotide. In some embodiments the conjugate moiety can modify or enhance the pharmacokinetic properties of the oligonucleotide by improving cellular distribution, bioavailability, metabolism, excretion, permeability, and/or cellular uptake of the oligonucleotide. In particular, the conjugate may target the oligonucleotide to a specific organ, tissue or cell type and thereby enhance the effectiveness of the oligonucleotide in that organ, tissue or cell type. The conjugate may also serve to reduce activity of the oligonucleotide in non-target cell types, tissues or organs, e.g., off target activity or activity in non-target cell types, tissues or organs.

In an embodiment, the non-nucleotide moiety (conjugate moiety) is selected from the group consisting of carbohydrates, cell surface receptor ligands, drug substances, hormones, lipophilic substances, polymers, proteins, peptides, toxins (e.g., bacterial toxins), vitamins, viral proteins (e.g., capsids) or combinations thereof.

Oligonucleotides 107 of the disclosure may be provided in pharmaceutical compositions that include any of the aforementioned oligonucleotides and/or oligonucleotide conjugates or salts thereof and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant. A pharmaceutically acceptable diluent includes ACSF (artificial cerebrospinal fluid) and pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts. In some embodiments the pharmaceutically acceptable diluent is sterile phosphate buffered saline or sterile sodium carbonate buffer. In some preferred embodiments, diluents for clinical application include Elliotts B solution and/or ACSF (artificial cerebrospinal fluid).

In some embodiments the oligonucleotide of the invention is in the form of a solution in the pharmaceutically acceptable diluent, for example dissolved in PBS or sodium carbonate buffer. The oligonucleotide may be pre-formulated in the solution or in some embodiments may be in the form of a dry powder (e.g., a lyophilized powder) which may be dissolved in the pharmaceutically acceptable diluent prior to administration. Suitably, for example the oligonucleotide may be dissolved in a concentration of 0.1-100 mg/mL, such as 1-10 mg/mL.

EXAMPLES

The following examples provide exemplary methods for screening ASOs of the invention. In the examples, a series of ASOs were screened. Based on the resulting data, ASOs corresponding to SEQ ID NOS: 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214 were identified as lead candidate ASOs based on dose-response efficacy, sequence motif liabilities, and off-target alignment analyses. Those ASOs showed the greatest *in vitro* efficacy, lowest off-target alignments, and limited sequence motif concerns. However, other ASOs as described herein also work as described to knock down UBE3A for the treatment of various conditions.

Example 1 – Single dose screening of UBE3A ASOs

Forty UBE3A-targeting ASOs (SEQ ID NOS: 1-40) were screened *in vitro* by treating primary fibroblasts, plated 10k per well of a 96-well plate, with 200nM of ASO. ASOs were delivered by transfection using RNAi Max at 0.5uL per well of a 96-well plate.

The data shown in FIG. 3 are qPCR data of normalized relative UBE3A transcript expression of ASO-treated fibroblasts versus a vehicle. All samples were normalized to a second vehicle condition. Cell only conditions (white) show no change in UBE3A expression. UBE3A siRNA was used as a positive control and shows ~80% knockdown of UBE3A transcript. A non-targeting siRNA was used as a negative control and shows no knockdown of UBE3A. The top graph shows data for UBE3A ASOs 001-020 (SEQ ID NOS: 1-20). Bottom graph shows data for UBE3A ASOs 021-040 (SEQ ID NOS: 21-40).

All cells were transfected with ASOs 48-hours after plating. Cells were harvested for qPCR an additional 48 hours after ASO transfection. Actin was used as the normalizing gene for UBE3A. Each bar represents 3 technical replicates and 1 biological replicate. The dots above certain bars indicate preferred ASOs identified within this set of 40 ASOs, and correspond to SEQ ID NOS: 4, 7, 8, 14, 17, 18, 21, 26, 34, and 35.

FIG. 4 provides results showing the dose-response of ten ASO candidates (SEQ ID NOS.: 14, 17, 4, 7, 8, 18, 21, 26, 34, and 35) at 6 concentrations each, designed according to embodiments of the disclosure (about 20 bases in length with an about 10-12 base DNA central region flanked by RNA wings with 2'-O modified RNA and phosphorothioate linkages throughout the ASO). All ten ASOs decreased UBE3A expression, relative to controls in a dose-dependent manner (vehicle-only treated cells and untreated "cells only" conditions).

Example 2 – Single dose screening of all UBE3A ASOs

Using the methods of Example 1, UBE3A-targeting ASOs (SEQ ID NOS: 1-80, 101-139, and 184-203) were screened *in vitro* by treating primary fibroblasts, plated 10k per well of a 96-well plate, with 200nM of ASO. ASOs were delivered by transfection using RNAi Max at 0.5uL per well of a 96-well plate.

All screened ASOs were designed according to embodiments of the disclosure, i.e., about 20 bases in length with an about 10-12 base DNA central region flanked by RNA wings with 2'-O modified RNA and phosphorothioate linkages throughout the ASO.

The data shown in FIGS. 6-9 are presented as summary tables of qPCR readouts of UBE3A knockdown (expressed as percent of UBE3A knockdown) for all 139 ASOs screened. All samples were normalized to either a vehicle condition or cell only condition. The tables of ASOs are broken down into UBE3A exon-targeting ASOs (FIGS. 6-7), UBE3A intron-targeting ASOs (FIG. 8), and UBE3A ASOs with 100% homology to both human and mouse UBE3A transcript (FIG. 9), for downstream rodent proof-of-concept *in vivo* studies.

All cells were transfected with ASOs 48-hours after plating. Cells were harvested for qPCR an additional 48 hours after ASO transfection. Actin was used as the normalizing gene for UBE3A. Where appropriate, ASOs were screened in both control fibroblasts and fibroblasts from a Dup15q patient (FIGS. 6-8). In FIG. 9, for the ASOs with mouse UBE3A homology, data is shown for 2 rounds.

Example 3 – Dose-response screening of UBE3A lead ASO candidates

Based on the data from Examples 1 and 2, candidate lead UBE3A-targeting ASOs were selected based on greater than 80-85% transcript knockdown in the primary single-dose screenings. For each candidate lead, new ASOs with identical sequences, were synthesized with 1 to 3 phosphodiester (PO) backbone modifications each in the 3' and 5', 2'-MOE RNA-like wings, with total of 4-5 PO modifications (i.e., a PS linkage replaced with a PO linkage) per ASO. These modifications replace the corresponding PS linkages in the original lead ASOs. The PO-modified ASOs are referred to in FIG. 10 as daughter ASOs.

These candidate leads were then tested for dose-response modulation of UBE3A transcript expression. For these experiments either primary fibroblasts, plated 10k per well of a

96-well plate, or mouse embryonic fibroblasts plated at 15k per well, were plated onto a 96-well plate. ASOs were screened at 6 doses: 6.25, 12.5, 25, 50, 100, and 200 nM. ASOs were delivered by transfection using RNAi Max at 0.5uL per well of a 96-well plate.

FIG. 10 displays example data of UBE3A ASO dose-response modulation of target expression for 2 lead candidate examples and their PO-modified daughter molecules in Dup15q patient fibroblasts (top) or mouse embryonic fibroblasts (bottom). All samples were normalized to vehicle conditions.

FIG. 11 plots the dose-response and indicates EC50 for the same 2 example lead candidates from FIG. 10. All cells were transfected with ASOs 48-hours after plating. Cells were harvested for qPCR an additional 48 hours after ASO transfection. Actin was used as the normalizing gene for UBE3A. Each data point represents 2 technical replicates and from 1 biological replicate.

Example 4 – Dose-response screening of UBE3A lead ASO candidates

Candidate lead UBE3A-targeting ASOs were selected based on greater than 80-85% transcript knockdown in the primary single-dose screening from Examples 1 and 2. For each candidate lead, new ASOs with identical sequences, were synthesized with 1 to 3 PO backbone modifications each in the 3' and 5', 2'-MOE RNA-like wings (total of 4-5 PO modifications per ASO), as described in Example 3. All candidate leads were then tested for dose-response modulation of UBE3A transcript expression.

For these experiments either primary fibroblasts, plated 10k per well of a 96-well plate, or mouse embryonic fibroblasts plated at 15k per well, were plated onto a 96-well plate. ASOs were screened at 6 doses: 6.25, 12.5, 25, 50, 100, and 200 nM, unless otherwise indicated. ASOs were delivered by transfection using RNAi Max at 0.5uL per well of a 96-well plate.

All samples were normalized to either vehicle or control conditions within each experiment. All cells were transfected with ASOs 48-hours after plating. Cells were harvested for qPCR an additional 48 hours after ASO transfection. Actin was used as the normalizing gene for UBE3A.

FIG. 12 shows the resulting dose-response data for the lead all-PS backbone candidates targeting UBE3A exons.

FIG. 13 shows the resulting dose-response data for the lead all-PS backbone candidates targeting UBE3A introns.

FIG. 14 shows the resulting dose-response data for the lead all-PS backbone candidates with 100% mouse homology for rodent *in vivo* efficacy studies.

FIG. 15 shows the resulting dose-response data for the PO-modified daughter leads with 100% mouse homology for rodent *in vivo* efficacy studies.

FIG. 16 shows the resulting dose-response data for the PO-modified daughter leads for human clinical candidate studies.

Example 5 – Protein knockdown of UBE3A using UBE3A ASOs

ASO-treated Dup15q patient fibroblasts were screened for UBE3A protein knockdown to help determine efficacy and rank ASOs for downstream experiments.

Fibroblasts were plated at 10k per well of a 96-well plate. ASO treatment occurred 48-hours post-plating. To allow for accumulation of protein knockdown, fibroblasts were harvested ~4.5 days post-ASO treatment for Western Blot analysis.

FIG. 17 shows a western blot for a certain candidate lead UBE3A ASOand 3 PO-modified daughter molecules with identical ASO sequences. A GFP-targeting ASO was used as a negative control. UBE3A expression was normalized to the house keeping gene ACTIN and then normalized to a vehicle condition.

FIG. 18 show a quantification of the UBE3A protein knockdown for the abovementioned samples. For the UBE3A blot, exposure was 600s. For GAPDH, exposure was 15s. 5µg of protein were loaded per lane and a high molecular weight transfer was used. UBE3A Antibody: Rb- E6AP Antibody (Bethyl) -A300-351A (1:1000). Actin Antibody: Ms ß-Actin – (Cell Signaling) – 8H10D10 (1:2000).

Example 6 – Protein knockdown of UBE3A *using UBE3A-targeting ASOs*

ASO-treated Dup15q patient fibroblasts were screened for UBE3A protein knockdown to help determine efficacy and rank ASOs for downstream experiments.

FIGS. 19-22 provide summary tables for UBE3A protein knockdown for candidate leads.

ASO-treated Dup15q patient fibroblasts were screened for UBE3A protein knockdown to help determine efficacy and rank ASOs for downstream experiments. Fibroblasts were plated at

10k per well of a 96-well plate. ASO treatment occurred 48-hours post-plating. To allow for accumulation of protein knockdown, fibroblasts were harvested ~4.5 days post-ASO treatment for Western Blot analysis. In all experiments, a GFP-targeting ASO was used as a negative control. UBE3A expression was normalized to the house keeping gene ACTIN and then normalized to a vehicle condition. For UBE3A blots, exposure was 600s. For GAPDH, exposure was 15s. 5µg of protein were loaded per lane and a high molecular weight transfer was used. UBE3A Antibody: Rb- E6AP Antibody (Bethyl) -A300-351A (1:1000). Actin Antibody: Ms ß-Actin – (Cell Signaling) – 8H10D10 (1:2000).

FIG. 19 provides a table summarizing UBE3A protein knockdown results for lead all-PS backbone candidates targeting UBE3A.

FIG. 20 provides a table summarizing UBE3A protein knockdown results for lead all-PS backbone candidates with 100% mouse homology for rodent in vivo efficacy studies. (C)

FIG. 21 provides a table summarizing UBE3A protein knockdown results for PO-modified daughter leads with 100% mouse homology for rodent in vivo efficacy studies.

FIG. 22 provides a table summarizing UBE3A protein knockdown results for PO-modified daughter leads for human clinical candidates.

Example 7 - Knockdown of UBE3A transcript in human NGN2 stem cell-derived neurons using UBE3A lead candidates

UBE3A is imprinted in neurons, and this cell type is critical for the pathogenesis of Dup15q. To show that the ASOs of the invention are effective in a disease-relevant human cell type, in this Example, human induced pluripotent stem cell-derived neurons (differentiated via overexpression of the transcription factor NGN2 and small molecule inhibition of SMAD signaling) were treated with *UBE3A*-targeting ASOs of the invention.

Neurons were plated at a density of 80,000 cells per well on a 96-well plate and treated with 100nM of *UBE3A*-targeting ASO. ASOs were delivered into the cultured neurons with Endoporter reagent at DIV (day *in vitro*) 21. Cells were harvested for qPCR 10 days after treatment at DIV31. UBE3A lead candidate ASOs and optimized lead candidate ASOs were screened.

FIG. 23 provides the data summarizing this screening. As shown, many ASOs showed >80% knockdown of *UBE3A* transcript in human neurons. *UBE3A* expression levels were

normalized to beta tubulin transcript levels (a housekeeping gene used as a reference). All normalized expression was then quantified relative to the first vehicle condition. Each bar represents 3 technical replicates and 1 biological replicate.

Example 8 - Knockdown of UBE3A transcript in human primary neurons using UBE3A lead candidate ASOs

UBE3A is imprinted in neurons, and that cell type is critical for the pathogenesis of Dup15q. To show that the ASOs of the invention are effective in a relevant human cell type, human primary neurons (derived from a 19-week-old male fetus; acquired from Sciencell) were treated with *UBE3A*-targeting ASOs. Neurons were plated at a density of 30,000 cells per well on a 96-well plate and treated with 500nM of *UBE3A*-targeting ASOs. ASOs were delivered gymnotically (no transfection reagent) on DIV 1. Cells were harvested for qPCR 6 days after ASO treatment. A subset of UBE3A lead candidate ASOs and optimized lead candidate ASOs were screened.

FIG. 24 provides the results summarizing this screen. As shown, many ASOs show >60% knockdown of *UBE3A* transcript in human primary neurons with gymnotic delivery. *UBE3A* expression levels were normalized to beta tubulin transcript levels (a housekeeping gene used as a reference). All normalized expression was then quantified relative to the first vehicle condition. Each bar represents 3 technical replicates and 1 biological replicate.

Example 9 - Knockdown of UBE3A transcript in non- human primate primary fibroblast cultures using UBE3A lead candidate ASOs.

UBE3A ASOs that have 100% homology to the corresponding sequence in cynomolgus non-human primates (NHP) were selected for this assay. Lead ASO candidates are screened *in vivo* in NHP to test for in vivo tolerability, toxicology, PK and PD.

To show that the ASOs of the invention are effective in a relevant NHP cell type, NHP primary fibroblasts (Coriell) were transduced with UBE3A-targeting ASOs. Fibroblasts were plated at a density of 10,000 cells per well on a 96-well plate and treated with 200nM UBE3A ASO. ASOs were transfected into NHP fibroblasts using RNAi Max on DIV 2. Cells were harvested for qPCR 48 hours after ASO treatment. UBE3A lead candidate ASOs and optimized lead candidates were screened.

FIG. 25 provides results summarizing this screening. As shown, many ASOs show 80-90% knockdown of *UBE3A* transcript. *UBE3A* expression levels were normalized to *GAPDH* (a housekeeping gene used as a reference). All normalized expression was then quantified relative to the first cells only condition. Each bar represents 2 technical replicates and 1 biological replicate.

Example 10 - Knockdown of UBE3A transcript in mouse primary cortical neurons using UBE3A lead candidates

UBE3A is imprinted in neurons, and this cell type is critical for the pathogenesis of Dup15q. Lead ASOs are screened *in vivo* in mice to test for in vivo tolerability, toxicology, PK and PD.

Mouse models of Dup15q are useful for showing proof-of-concept and efficacy in disease model systems in vivo. To show that the ASOs of the invention are effective in a relevant mouse cell type, mouse primary cortical neurons (Brainbits) were treated with UBE3A ASOs. Neurons were plated at 9k per well on a 96-well plate and treated with 1uM UBE3A ASO. ASOs were delivered gymnotically on DIV 3. Cells were harvested for qPCR 8 days after ASO treatment (DIV11). UBE3A lead candidates and optimized lead candidates were screened. The resulting data from these screens are presented in FIG. 26. As shown, many ASOs show >60% knockdown of UBE3A transcript with gymnotic delivery, especially ASOs with 100% rat homology. UBE3A expression levels were normalized to beta tubulin (used as a housekeeping gene). All normalized expression was then quantified relative to the second cells only condition. Each bar represents 2 technical replicates and 1 biological replicate.

Example 11 - Knockdown of UBE3A transcript in rat primary cortical neurons using UBE3A lead candidates

UBE3A is imprinted in neurons, and this cell type is critical for the pathogenesis of Dup15q. Lead ASOs are screened *in vivo* in rats to test for *in vivo* tolerability, toxicology, PK and PD.

To show that the ASOs of the invention are effective in a relevant rat cell type, rat primary cortical neurons (Brainbits) were treated with UBE3A ASOs as described herein.

Neurons were plated at 9k per well on a 96-well plate and treated with 3uM UBE3A ASO. ASOs were delivered gymnotically on DIV 3.

Cells were harvested for qPCR 4 days and 8 days after ASO treatment (DIV7 and DIV11, respectively). UBE3A lead candidates and optimized lead candidates were screened.

FIG. 27 provides the results summarizing the screens after cells were harvested for qPCR after four days.

FIG. 28 provides the results summarizing the screens after cells were harvested for qPCR after eight days.

As shown in FIGS. 27-28, many ASOs show >60% knockdown of UBE3A transcript with gymnotic delivery, especially ASOs with 100% rat homology. UBE3A expression levels were normalized to beta tubulin (used as a housekeeping gene). All normalized expression was then quantified relative to the second cells only condition. Each bar represents 2 technical replicates and 1 biological replicate.

CLAIMS

What is claimed is:

1. A composition comprising:

a synthetic antisense oligonucleotide (ASO) that inhibits expression of a ubiquitin ligase protein.

2. The composition of claim 1, wherein the protein is ubiquitin protein ligase E3A.

3. The composition of claim 1, wherein the ASO hybridizes to a complementary target in a transcript from the *UBE3A* gene.

4. The composition of claim 1, wherein a sequence of bases in the ASO has at least 80% identity to one of SEQ ID NOS: 1-219.

5. The composition of claim 1, wherein a sequence of bases in the ASO is at least 90% identical to one of SEQ ID NOS: 1-219, wherein the oligonucleotide can hybridize to, and induce RNaseH-mediated cleavage of, *UBE3A* pre-mRNA or mRNA.

6. The composition of claim 1, wherein the oligonucleotide comprises two wings flanking a central region of at least 10 DNA bases.

7. The composition of claim 6, wherein at least one wing of the ASO comprises modified RNA bases.

8. The composition of claim 7, wherein each modified RNA base is selected from the group consisting of 2'-O-methoxyethyl RNA and 2'-O-methyl RNA.

9. The composition of claim 1, wherein the ASO comprises at least about 15 bases.

10. The composition of claim 1, wherein the ASO comprises between about 15 about 25 bases.

11. The composition of claim 1, wherein the ASO has a backbone comprising a plurality of phosphorothioate bonds.

12. The composition of claim 1, wherein the ASO has a base sequence that has been screened and determined to not meet a threshold match for any non-target transcripts in humans.

13. The composition of claim 1, wherein the ASO has a base sequence with 0 mismatches to a homologous segment in a non-human primate genome and no more than about 5 mismatches in a homologous segment in a rodent genome.

14. The composition of claim 1, wherein the composition comprises a plurality of ASOs each having a base sequence at least 80% identical to one of SEQ ID NOS: 1-40, 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214 wherein each of the ASOs has a gapmer structure that comprises a central DNA segment flanked by RNA wings.

15. The composition of claim 2, wherein the oligonucleotide has a base sequence with at least a 90% match to one of SEQ ID NO: 1-219, with bases linked only by phosphorothioate linkages, the oligonucleotide further comprising a central 12 DNA bases flanked by a 5' wing and a 3' wing, the 5' wing and the 3' wing each comprising four consecutive 2' modified RNA bases.

16. The composition of claim 2, wherein the oligonucleotide has a base sequence matching one of SEQ ID NO: 1-40, 146, 155, 156, 158, 159, 161, 164, 169, 174, 175, 178, 179, 213, and 214, with at least a majority of inter-base linkages comprising phosphorothioate linkages, the oligonucleotide further comprising a central 12 DNA bases flanked by a 5' wing and a 3' wing, the 5' wing and the 3' wing each comprising four consecutive 2'-MOE RNA bases.

17. The composition of claim 1, wherein the ASO is conjugated to a member selected from the group consisting of carbohydrates, cell surface receptor ligands, drug substances, hormones, lipophilic substances, polymers, proteins, peptides, toxins, vitamins, viral proteins, and combinations thereof.

18. A method comprising:

administering to a subject with Dup15q syndrome a composition of any one of claims 1-16 to thereby knock down expression of the *UBE3A* gene.

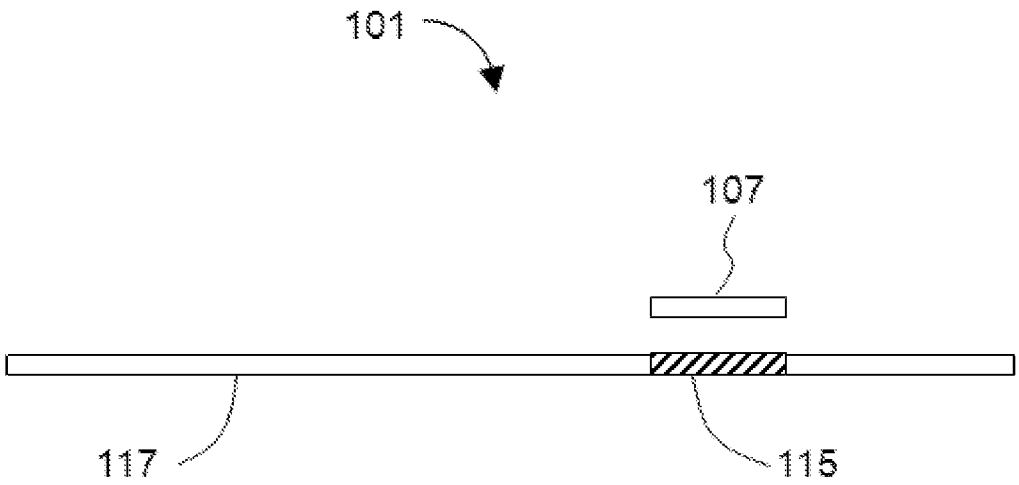

FIG. 1

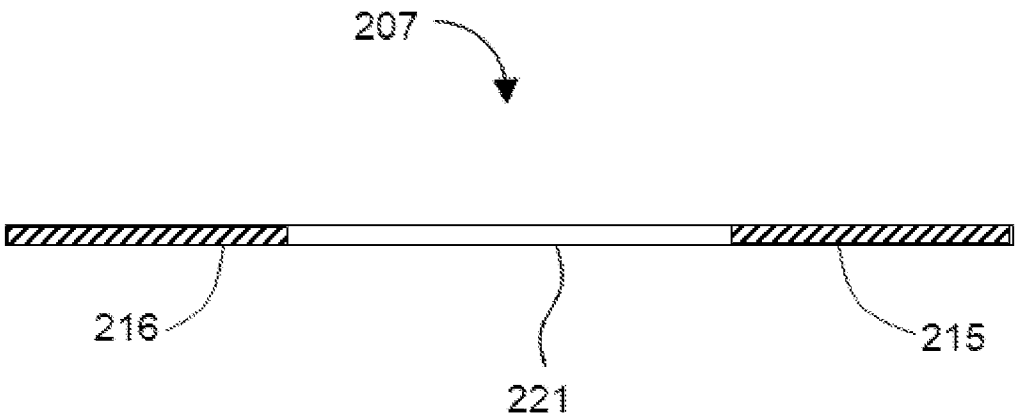

FIG. 2

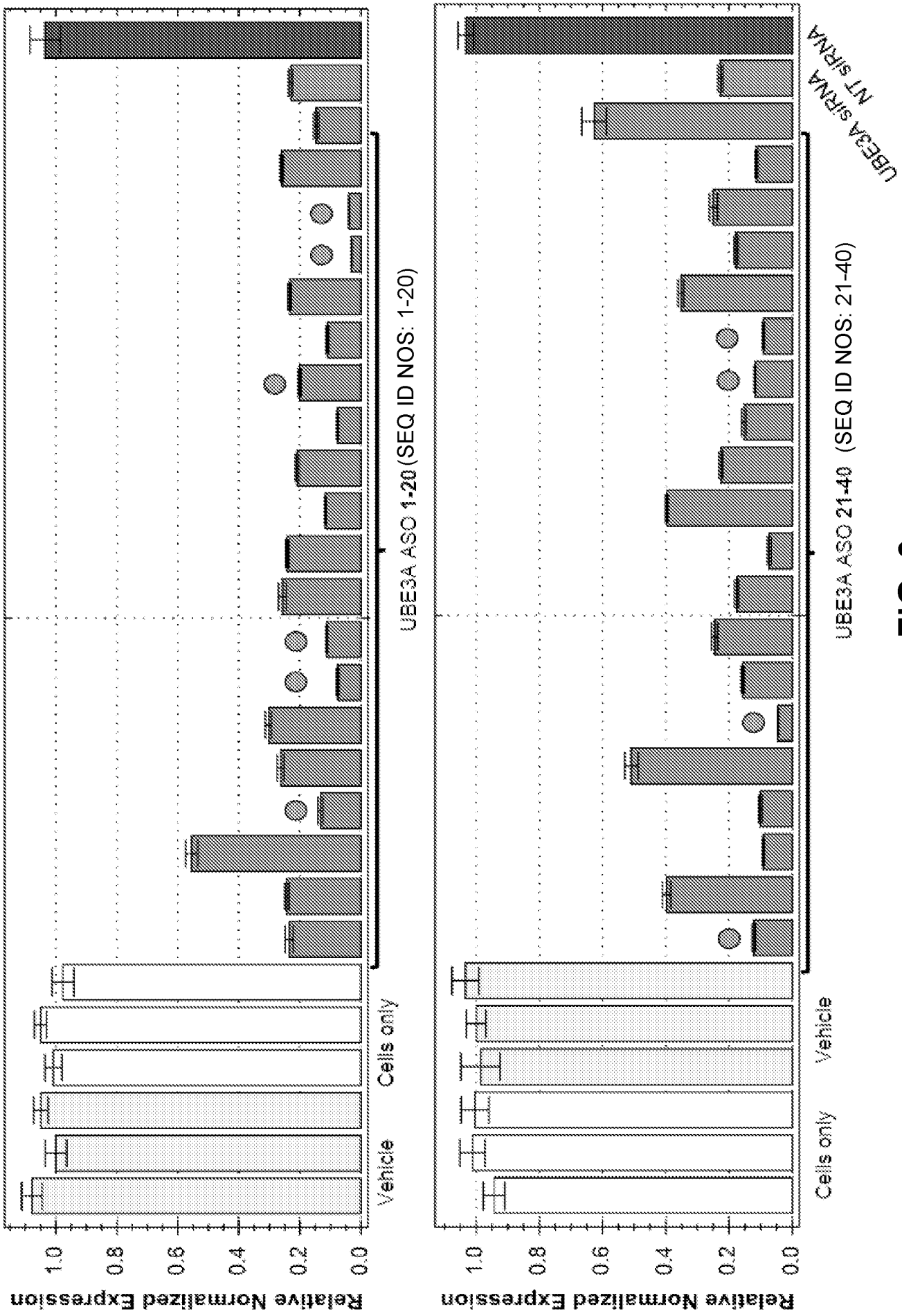
Relative Normalized Expression
0.0
0.2
0.4
0.6
0.8
1.0
Vehicle
Cells only
UBE3A ASO 1-20 (SEQ ID NOS: 1-20)
Relative Normalized Expression
0.0
0.2
0.4
0.6
0.8
1.0
Cells only
Vehicle
UBE3A ASO 21-40 (SEQ ID NOS: 21-40)
UBE3A siRNA
NT siRNA

FIG. 3

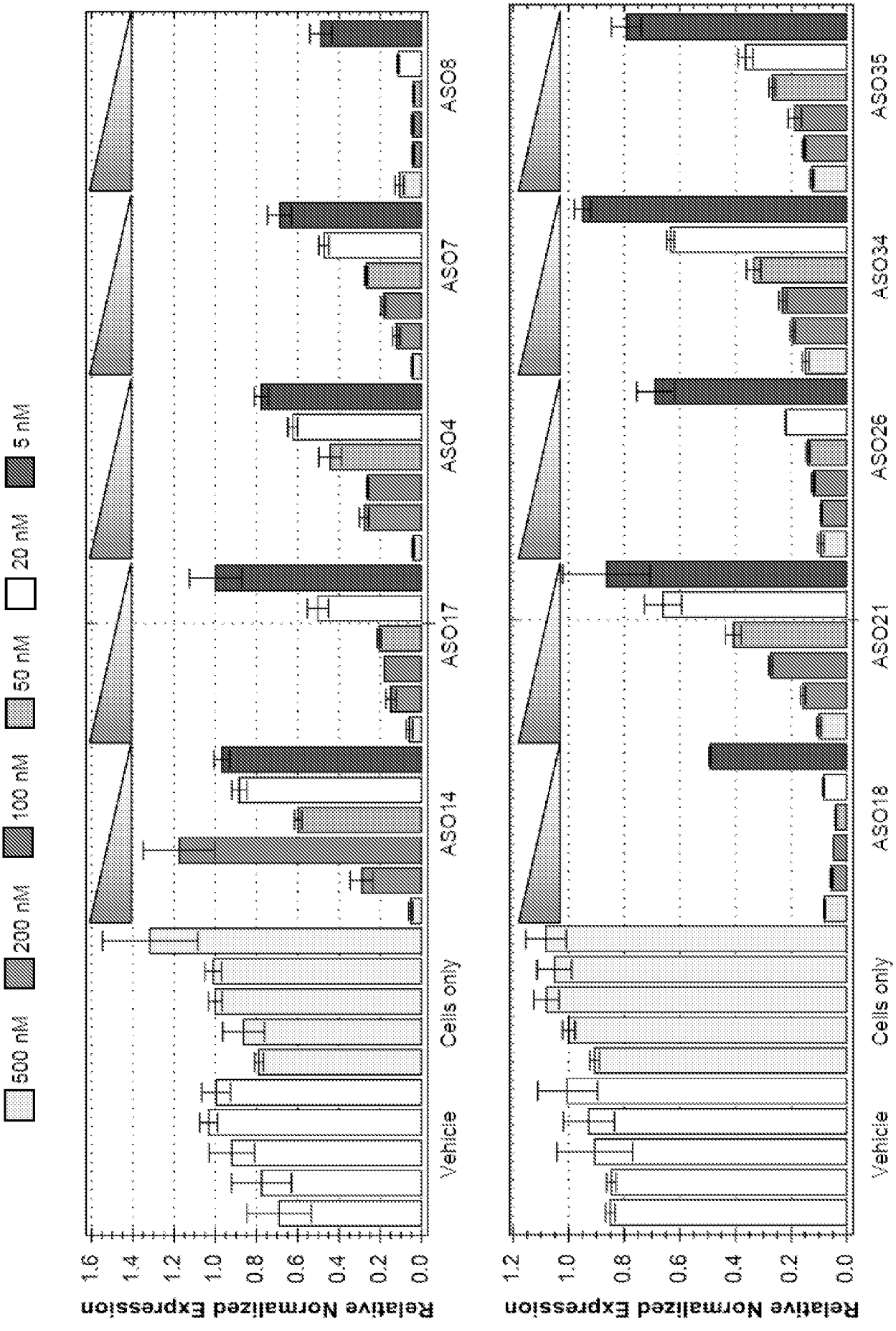
500 nM
200 nM
100 nM
50 nM
20 nM
5 nM
Relative Normalized Expression
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Vehicle
Cells only
ASO14
ASO17
ASO4
ASO7
ASO8
Relative Normalized Expression
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Vehicle
Cells only
ASO18
ASO21
ASO26
ASO34
ASO35

FIG. 4

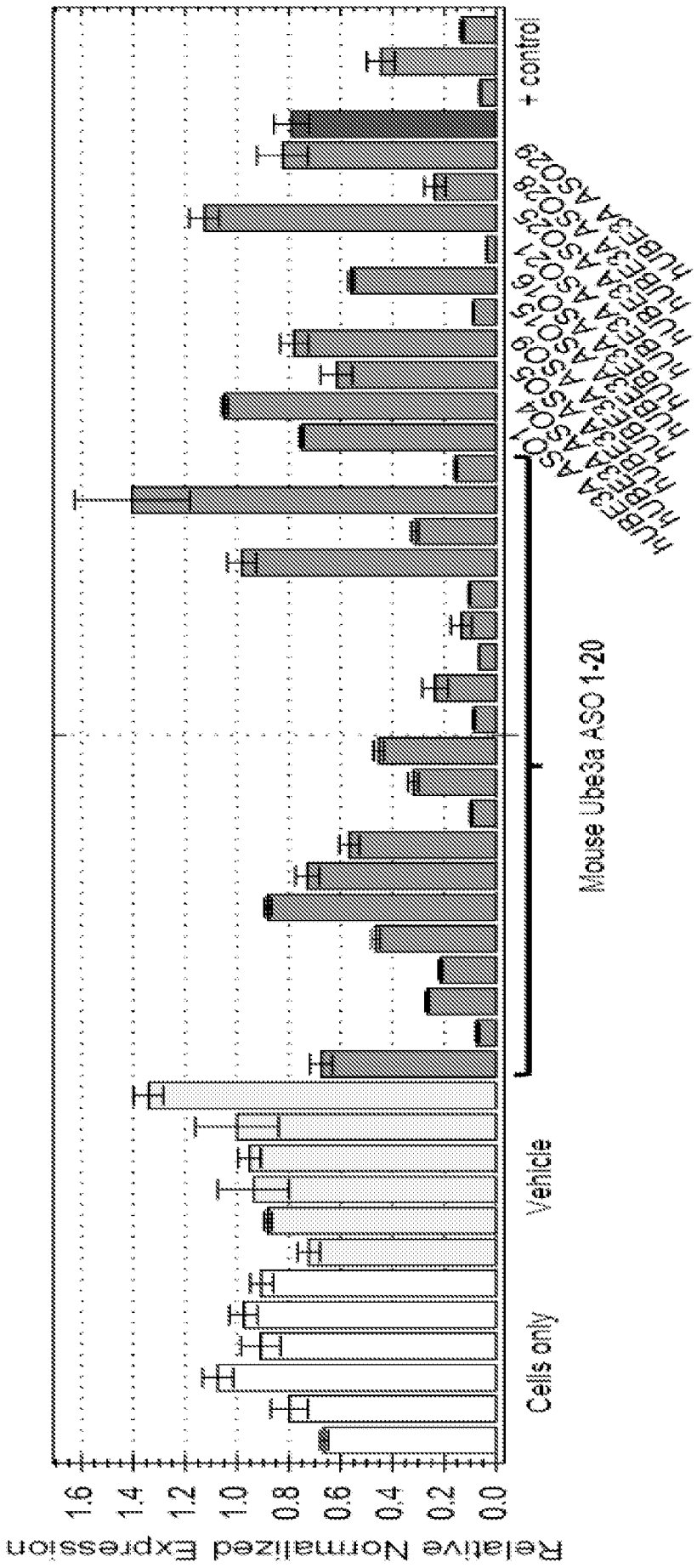
Relative Normalized Expression
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Cells only
Vehicle
Mouse Ube3a ASO 1-20
+ control

FIG. 5

SEQ ID NO:	ASO ID	CTR	DUP
1	QS-Tg-15-ASO-001	82%	77%
2	QS-Tg-15-ASO-002	88%	88%
3	QS-Tg-15-ASO-003	83%	90%
4	QS-Tg-15-ASO-004	86%	81%
5	QS-Tg-15-ASO-005	85%	86%
6	QS-Tg-15-ASO-006	95%	95%
7	QS-Tg-15-ASO-007	89%	95%
8	QS-Tg-15-ASO-008	93%	96%
9	QS-Tg-15-ASO-009	74%	54%
10	QS-Tg-15-ASO-010	74%	59%
11	QS-Tg-15-ASO-011	93%	91%
12	QS-Tg-15-ASO-012	78%	90%
13	QS-Tg-15-ASO-013	87%	92%
14	QS-Tg-15-ASO-014	78%	77%
15	QS-Tg-15-ASO-015	91%	95%
16	QS-Tg-15-ASO-016	65%	43%
17	QS-Tg-15-ASO-017	91%	92%
18	QS-Tg-15-ASO-018	91%	93%
19	QS-Tg-15-ASO-019	69%	37%
20	QS-Tg-15-ASO-020	81%	84%
21	QS-Tg-15-ASO-021	84%	74%
22	QS-Tg-15-ASO-022	35%	63%
23	QS-Tg-15-ASO-023	80%	81%
24	QS-Tg-15-ASO-024	81%	80%
25	QS-Tg-15-ASO-025	75%	91%
26	QS-Tg-15-ASO-026	87%	88%
27	QS-Tg-15-ASO-027	78%	40%
28	QS-Tg-15-ASO-028	70%	66%
29	QS-Tg-15-ASO-029	77%	83%
30	QS-Tg-15-ASO-030	86%	86%
31	QS-Tg-15-ASO-031	54%	22%
32	QS-Tg-15-ASO-032	73%	42%
33	QS-Tg-15-ASO-033	60%	83%
34	QS-Tg-15-ASO-034	80%	71%
35	QS-Tg-15-ASO-035	86%	61%
36	QS-Tg-15-ASO-036	58%	57%
37	QS-Tg-15-ASO-037	80%	64%
38	QS-Tg-15-ASO-038	71%	22%
39	QS-Tg-15-ASO-039	82%	63%
40	QS-Tg-15-ASO-040	30%	68%

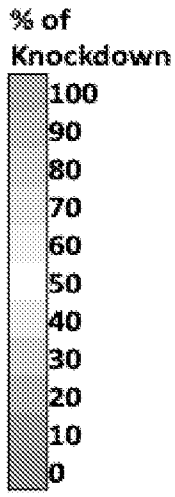

FIG. 6

SEQ ID NO:	ASO ID	CTR	DUP
101	QS-Tg-15-ASO-101	99%	98%
102	QS-Tg-15-ASO-102	96%	96%
103	QS-Tg-15-ASO-103	82%	89%
104	QS-Tg-15-ASO-104	95%	94%
105	QS-Tg-15-ASO-105	86%	82%
106	QS-Tg-15-ASO-106	97%	94%
107	QS-Tg-15-ASO-107	93%	94%
108	QS-Tg-15-ASO-108	92%	90%
109	QS-Tg-15-ASO-109	85%	62%
110	QS-Tg-15-ASO-110	92%	82%
111	QS-Tg-15-ASO-111	79%	70%
112	QS-Tg-15-ASO-112	35%	46%
113	QS-Tg-15-ASO-113	64%	37%
114	QS-Tg-15-ASO-114	20%	7%
115	QS-Tg-15-ASO-115	89%	88%
116	QS-Tg-15-ASO-116	93%	93%
117	QS-Tg-15-ASO-117	87%	86%
118	QS-Tg-15-ASO-118	45%	29%
119	QS-Tg-15-ASO-119	31%	27%
120	QS-Tg-15-ASO-120	69%	60%
121	QS-Tg-15-ASO-121	82%	90%
122	QS-Tg-15-ASO-122	88%	94%
123	QS-Tg-15-ASO-123	88%	91%
124	QS-Tg-15-ASO-124	64%	83%
125	QS-Tg-15-ASO-125	87%	91%
126	QS-Tg-15-ASO-126	77%	87%
127	QS-Tg-15-ASO-127	97%	98%
128	QS-Tg-15-ASO-128	97%	99%
129	QS-Tg-15-ASO-129	97%	96%
130	QS-Tg-15-ASO-130	90%	89%
131	QS-Tg-15-ASO-131	90%	91%
132	QS-Tg-15-ASO-132	89%	89%
133	QS-Tg-15-ASO-133	56%	64%
134	QS-Tg-15-ASO-134	57%	60%
135	QS-Tg-15-ASO-135	80%	89%
136	QS-Tg-15-ASO-136	60%	65%
137	QS-Tg-15-ASO-137	79%	89%
138	QS-Tg-15-ASO-138	92%	95%
139	QS-Tg-15-ASO-139	87%	88%

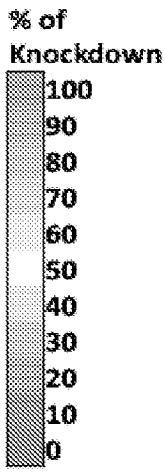

FIG. 7

SEQ ID NO:	ASO ID	CTR	DUP
41	QS-Tg-15-ASO-041	93%	91%
42	QS-Tg-15-ASO-042	89%	89%
43	QS-Tg-15-ASO-043	51%	56%
44	QS-Tg-15-ASO-044	57%	63%
45	QS-Tg-15-ASO-045	51%	54%
46	QS-Tg-15-ASO-046	90%	94%
47	QS-Tg-15-ASO-047	79%	77%
48	QS-Tg-15-ASO-048	87%	87%
49	QS-Tg-15-ASO-049	33%	37%
50	QS-Tg-15-ASO-050	66%	71%
51	QS-Tg-15-ASO-051	31%	33%
52	QS-Tg-15-ASO-052	57%	70%
53	QS-Tg-15-ASO-053	82%	81%
54	QS-Tg-15-ASO-054	46%	52%
55	QS-Tg-15-ASO-055	69%	79%
56	QS-Tg-15-ASO-056	35%	46%
57	QS-Tg-15-ASO-057	40%	43%
58	QS-Tg-15-ASO-058	39%	44%
59	QS-Tg-15-ASO-059	26%	32%
60	QS-Tg-15-ASO-060	74%	68%
61	QS-Tg-15-ASO-061	55%	55%
62	QS-Tg-15-ASO-062	48%	55%
63	QS-Tg-15-ASO-063	47%	58%
64	QS-Tg-15-ASO-064	N/A	80%
65	QS-Tg-15-ASO-065	32%	41%
66	QS-Tg-15-ASO-066	54%	70%
67	QS-Tg-15-ASO-067	61%	76%
68	QS-Tg-15-ASO-068	40%	51%
69	QS-Tg-15-ASO-069	90%	95%
70	QS-Tg-15-ASO-070	52%	71%
71	QS-Tg-15-ASO-071	58%	80%
72	QS-Tg-15-ASO-072	76%	87%
73	QS-Tg-15-ASO-073	69%	89%
74	QS-Tg-15-ASO-074	78%	78%
75	QS-Tg-15-ASO-075	69%	77%
76	QS-Tg-15-ASO-076	54%	52%
77	QS-Tg-15-ASO-077	64%	65%
78	QS-Tg-15-ASO-078	33%	58%
79	QS-Tg-15-ASO-079	26%	18%
80	QS-Tg-15-ASO-080	18%	29%

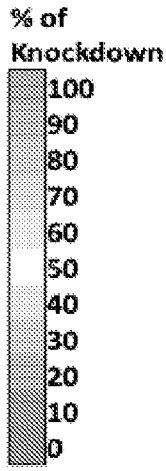

FIG. 8

SEQ ID NO:	ASO ID	Rd 1	Rd 2
184	QS-Tg-17-ASO-001	32%	11%
185	QS-Tg-17-ASO-002	93%	82%
186	QS-Tg-17-ASO-003	73%	43%
187	QS-Tg-17-ASO-004	79%	59%
188	QS-Tg-17-ASO-005	53%	43%
189	QS-Tg-17-ASO-006	12%	22%
190	QS-Tg-17-ASO-007	27%	2%
191	QS-Tg-17-ASO-008	43%	25%
192	QS-Tg-17-ASO-009	90%	69%
193	QS-Tg-17-ASO-010	68%	45%
194	QS-Tg-17-ASO-011	55%	42%
195	QS-Tg-17-ASO-012	91%	76%
196	QS-Tg-17-ASO-013	77%	54%
197	QS-Tg-17-ASO-014	93%	79%
198	QS-Tg-17-ASO-015	87%	74%
199	QS-Tg-17-ASO-016	90%	76%
200	QS-Tg-17-ASO-017	2%	N/A
201	QS-Tg-17-ASO-018	69%	48%
202	QS-Tg-17-ASO-019	N/A	4%
203	QS-Tg-17-ASO-020	84%	64%
1	QS-Tg-15-ASO-001	25%	22%
4	QS-Tg-15-ASO-004	N/A	22%
5	QS-Tg-15-ASO-005	38%	43%
9	QS-Tg-15-ASO-009	22%	41%
15	QS-Tg-15-ASO-015	91%	70%
21	QS-Tg-15-ASO-021	44%	55%
25	QS-Tg-15-ASO-025	96%	92%
28	QS-Tg-15-ASO-028	N/A	20%
29	QS-Tg-15-ASO-029	76%	59%
32	QS-Tg-15-ASO-032	18%	8%
101	QS-Tg-15-ASO-101	36%	N/A
102	QS-Tg-15-ASO-102	88%	N/A
105	QS-Tg-15-ASO-105	18%	N/A
106	QS-Tg-15-ASO-106	61%	N/A
109	QS-Tg-15-ASO-109	29%	N/A
111	QS-Tg-15-ASO-111	20%	N/A
112	QS-Tg-15-ASO-112	37%	N/A
114	QS-Tg-15-ASO-114	53%	N/A
115	QS-Tg-15-ASO-115	90%	N/A
116	QS-Tg-15-ASO-116	82%	N/A
119	QS-Tg-15-ASO-119	27%	N/A
121	QS-Tg-15-ASO-121	61%	N/A

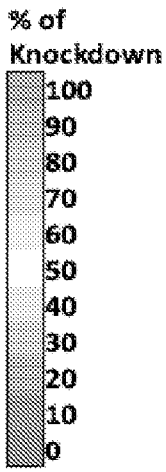

FIG. 9

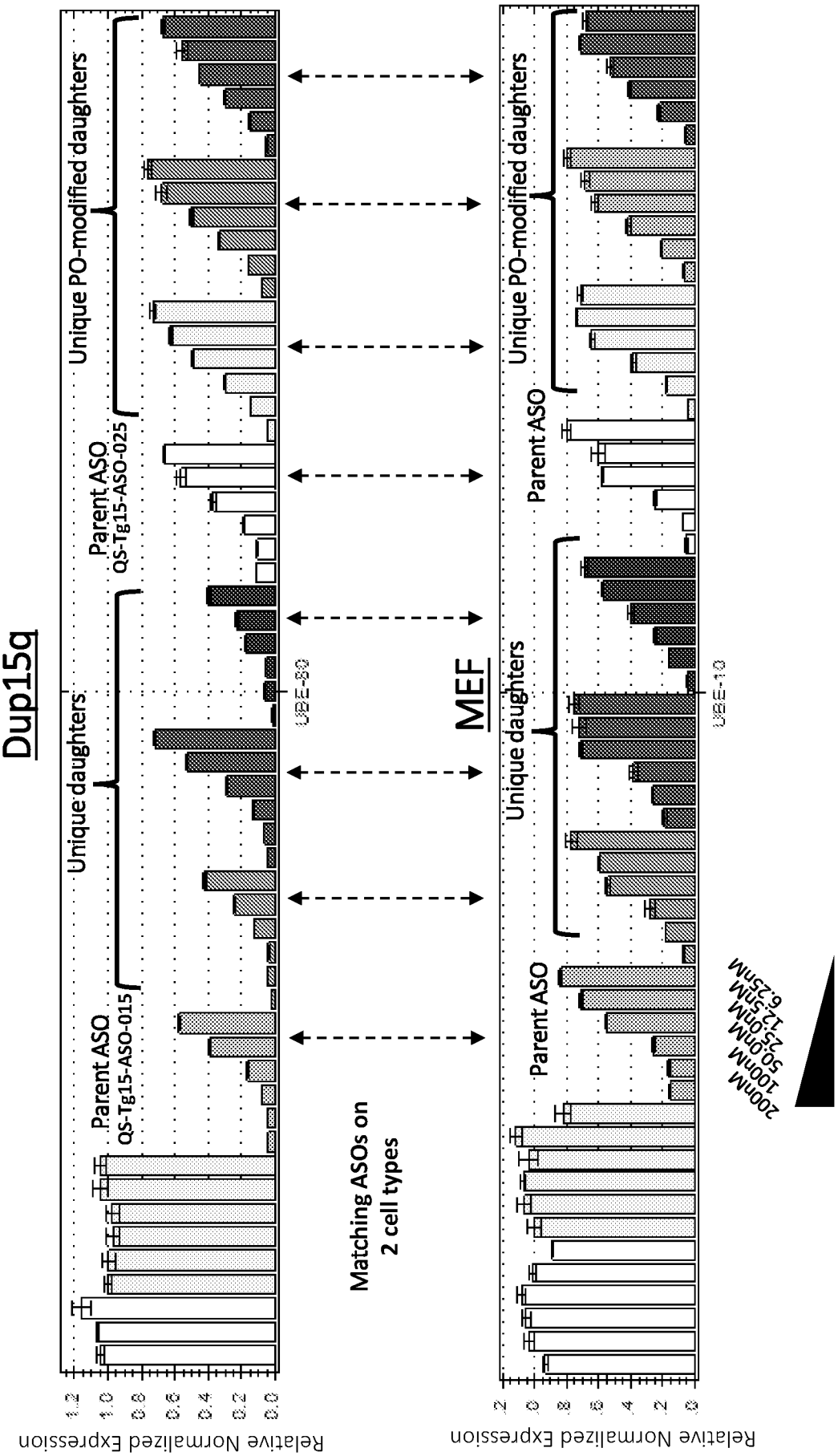
Dup15q
Unique daughters
Parent ASO QS-Tg15-ASO-015
Parent ASO QS-Tg15-ASO-025
Unique PO-modified daughters
UBE-3a
Relative Normalized Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Matching ASOs on 2 cell types
MEF
Unique daughters
Parent ASO
Unique PO-modified daughters
Parent ASO
UBE-1b
Relative Normalized Expression
200nM
100nM
50.0nM
25.0nM
12.5nM
6.25nM

FIG. 10

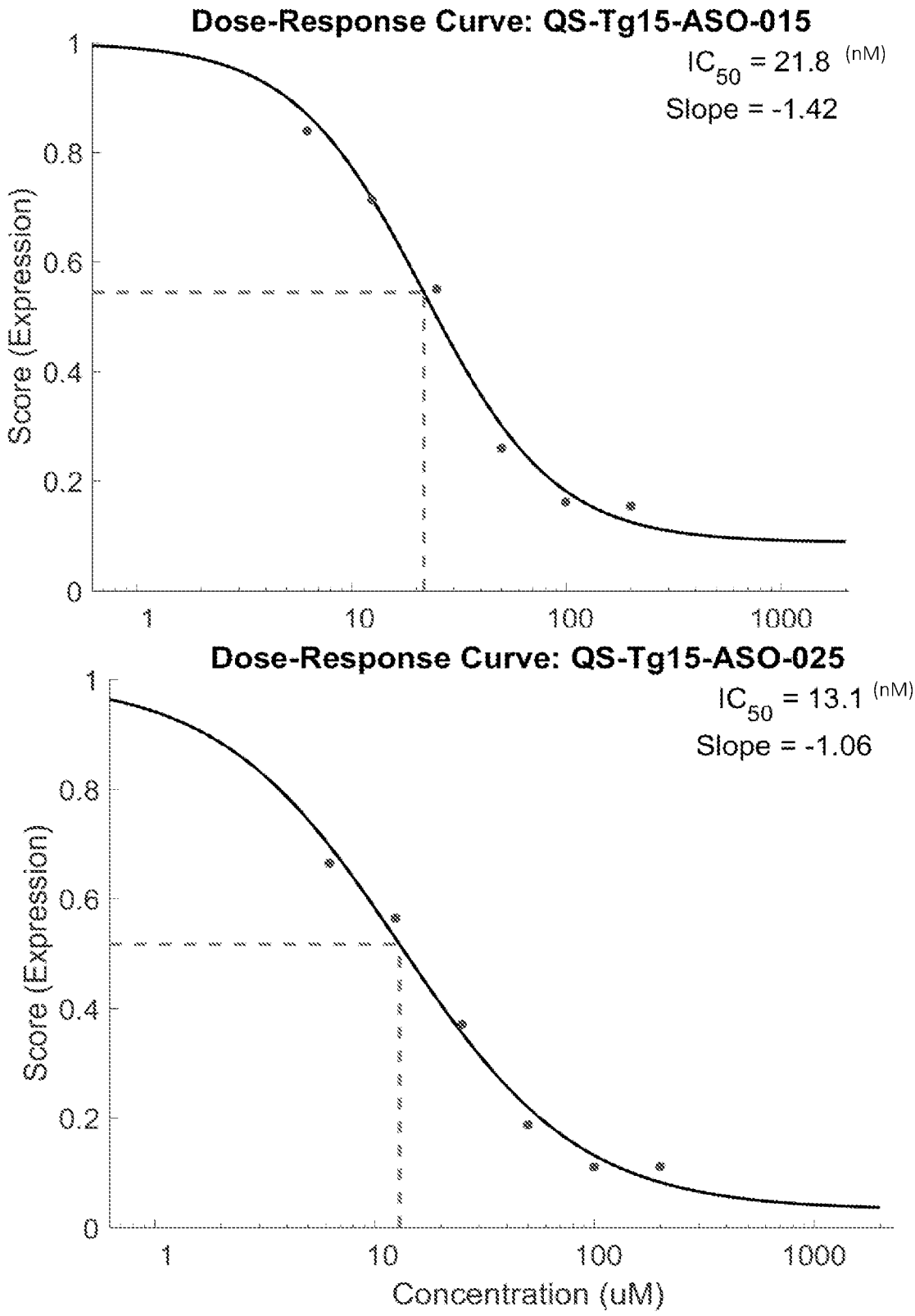
Dose-Response Curve: QS-Tg15-ASO-015
IC50 = 21.8 (nM)
Slope = -1.42
Score (Expression)
1
0.8
0.6
0.4
0.2
0
1
10
100
1000
Dose-Response Curve: QS-Tg15-ASO-025
IC50 = 13.1 (nM)
Slope = -1.06
Score (Expression)
1
0.8
0.6
0.4
0.2
0
1
10
100
1000
Concentration (uM)

FIG. 11

SEQ ID NO:	ASO ID	400nM	200nM	100nM	50nM	25nM	12.5nM	6.25nM
1	QS-Tg15-ASO-001	83	60	55	38	28	14	4
5	QS-Tg15-ASO-005	82	72	57	60	46	28	18
6	QS-Tg15-ASO-006	92	71	60	37	34	22	14
8	QS-Tg15-ASO-008	93	91	88	87	80	74	61
11	QS-Tg15-ASO-011	87	80	78	76	62	42	29
13	QS-Tg15-ASO-013	85	86	78	73	61	30	11
15	QS-Tg15-ASO-015	90	90	87	86	80	66	53
17	QS-Tg15-ASO-017	88	83	76	74	52	24	13
23	QS-Tg15-ASO-023	83	79	72	58	41	8	3
25	QS-Tg15-ASO-025	85	87	84	80	71	54	32
26	QS-Tg15-ASO-026	82	81	75	71	62	42	27
29	QS-Tg15-ASO-029	77	59	46	60	41	13	22
30	QS-Tg15-ASO-030	83	82	74	68	38	56	43
36	QS-Tg15-ASO-036	51	26	19	12	14	3	N/A
101	QS-Tg15-ASO-101	93	96	91	88	69	62	29
102	QS-Tg15-ASO-102	89	89	85	75	49	48	27
103	QS-Tg15-ASO-103	52	47	34	29	24	11	8
104	QS-Tg15-ASO-104	76	74	70	46	51	28	6
105	QS-Tg15-ASO-105	55	48	30	23	N/A	18	10
106	QS-Tg15-ASO-106	83	86	81	68	66	42	25
107	QS-Tg15-ASO-107	84	82	75	65	47	22	12
108	QS-Tg15-ASO-108	74	72	56	51	27	25	10
115	QS-Tg15-ASO-115	82	83	82	66	51	28	20
116	QS-Tg15-ASO-116	89	90	85	84	68	59	27
117	QS-Tg15-ASO-117	80	85	82	84	70	57	34
121	QS-Tg15-ASO-121	77	70	63	47	34	7	12
122	QS-Tg15-ASO-122	85	86	78	75	45	38	13
127	QS-Tg15-ASO-127	91	87	88	74	61	27	9

FIG. 12

SEQ ID NO:	ASO ID	400nM	200nM	100nM	50nM	25nM	12.5nM	6.25nM
41	QS-Tg-15-ASO-041	70	64	42	40	24	21	12
42	QS-Tg-15-ASO-042	68	59	63	42	16	0	5
46	QS-Tg-15-ASO-046	66	62	38	40	15	15	0
53	QS-Tg-15-ASO-053	72	75	68	46	25	16	4
64	QS-Tg-15-ASO-064	77	66	39	37	31	21	17
69	QS-Tg-15-ASO-069	93	93	85	74	46	17	0
71	QS-Tg-15-ASO-071	74	71	66	55	47	24	13
72	QS-Tg-15-ASO-072	76	73	57	35	18	9	5
73	QS-Tg-15-ASO-073	85	78	65	52	23	17	7

FIG. 13

SEQ ID NO:	ASO ID	400nM	200nM	100nM	50nM	25nM	12.5nM	6.25nM
185	QS-Tg-17-ASO-002	79	75	74	71	55	21	9
192	QS-Tg-17-ASO-009	88	87	82	75	66	39	19
195	QS-Tg-17-ASO-012	84	84	80	68	47	26	-4
197	QS-Tg-17-ASO-014	90	90	83	66	37	12	5
198	QS-Tg-17-ASO-015	79	82	72	61	36	21	9
199	QS-Tg-17-ASO-016	88	88	78	68	47	28	6
203	QS-Tg-17-ASO-020	85	72	57	54	41	42	23

FIG. 14

SEQ ID NO:	ASO ID	200nM	100nM	50nM	25nM	12.5nM	6.25nM
140	QS-Tg-15-ASO-140	95	96	96	88	76	58
141	QS-Tg-15-ASO-141	96	94	87	71	48	29
142	QS-Tg-15-ASO-142	99	94	95	83	77	60
143	QS-Tg-15-ASO-143	96	86	71	51	38	27
144	QS-Tg-15-ASO-144	92	85	67	51	33	24
145	QS-Tg-15-ASO-145	95	85	71	55	44	33
146	QS-Tg-15-ASO-146	97	93	83	68	57	52
147	QS-Tg-15-ASO-147	97	95	84	80	59	63
148	QS-Tg-15-ASO-148	98	92	87	66	52	55
149	QS-Tg-15-ASO-149	91	87	77	52	30	18
150	QS-Tg-15-ASO-150	89	87	72	59	31	20
151	QS-Tg-15-ASO-151	90	81	73	48	36	14
152	QS-Tg-15-ASO-152	94	95	86	86	66	59
153	QS-Tg-15-ASO-153	95	94	84	76	69	57
154	QS-Tg-15-ASO-154	94	93	77	80	64	61
204	QS-Tg-17-ASO-021	92	87	78	64	52	35
205	QS-Tg-17-ASO-022	83	78	57	51	30	23
206	QS-Tg-17-ASO-023	93	87	74	59	52	35
207	QS-Tg-17-ASO-024	82	75	65	45	40	26
208	QS-Tg-17-ASO-025	77	74	56	49	43	36
209	QS-Tg-17-ASO-026	78	69	62	42	40	27
210	QS-Tg-17-ASO-027	91	84	61	58	46	34
211	QS-Tg-17-ASO-028	88	85	53	56	33	37
212	QS-Tg-17-ASO-029	89	78	52	51	41	34
213	QS-Tg-17-ASO-030	76	56	40	26	27	9
214	QS-Tg-17-ASO-031	80	75	41	48	28	23
215	QS-Tg-17-ASO-032	78	73	42	40	32	13
216	QS-Tg-17-ASO-033	76	63	53	39	38	24
217	QS-Tg-17-ASO-034	68	63	37	38	23	30
218	QS-Tg-17-ASO-035	70	55	48	29	38	19

FIG. 15

SEQ ID NO:	ASO ID	200nM	100nM	50nM	25nM	12.5nM	6.25nM
140	QS-Tg-15-ASO-140	88	85	78	67	56	21
146	QS-Tg-15-ASO-146	94	92	92	80	73	57
152	QS-Tg-15-ASO-152	92	91	87	81	73	63
153	QS-Tg-15-ASO-153	92	91	83	81	72	68
155	QS-Tg-15-ASO-155	94	90	87	85	78	80
156	QS-Tg-15-ASO-156	93	90	85	83	78	81
157	QS-Tg-15-ASO-157	94	88	86	82	80	84
158	QS-Tg-15-ASO-158	88	84	79	60	42	42
159	QS-Tg-15-ASO-159	89	83	71	55	32	34
160	QS-Tg-15-ASO-160	84	80	73	50	29	23
161	QS-Tg-15-ASO-161	85	75	62	34	23	18
162	QS-Tg-15-ASO-162	82	69	49	28	12	10
163	QS-Tg-15-ASO-163	86	74	61	33	24	10
164	QS-Tg-15-ASO-164	91	86	82	63	61	41
165	QS-Tg-15-ASO-165	87	87	82	75	58	39
166	QS-Tg-15-ASO-166	80	77	67	54	29	7
167	QS-Tg-15-ASO-167	78	73	64	53	37	32
168	QS-Tg-15-ASO-168	83	74	64	44	33	5
169	QS-Tg-15-ASO-169	85	77	70	43	35	9
170	QS-Tg-15-ASO-170	82	70	54	37	20	9
171	QS-Tg-15-ASO-171	83	70	65	42	30	N/A
172	QS-Tg-15-ASO-172	96	94	95	87	78	65
173	QS-Tg-15-ASO-173	95	96	91	86	68	55
174	QS-Tg-15-ASO-174	90	82	71	36	27	12
175	QS-Tg-15-ASO-175	92	88	71	47	23	21
176	QS-Tg-15-ASO-176	90	79	66	32	19	8
177	QS-Tg-15-ASO-177	92	89	85	74	70	61
178	QS-Tg-15-ASO-178	92	85	85	58	45	39
179	QS-Tg-15-ASO-179	90	88	83	75	49	43
180	QS-Tg-15-ASO-180	90	84	86	70	55	52
181	QS-Tg-15-ASO-181	95	93	73	76	54	55
182	QS-Tg-15-ASO-182	95	90	84	71	55	50
183	QS-Tg-15-ASO-183	95	93	78	73	55	55
213	QS-Tg-17-ASO-030	95	87	79	54	38	30
214	QS-Tg-17-ASO-031	94	93	85	80	52	48
219	QS-Tg-17-ASO-036	95	93	90	78	58	47

FIG. 16

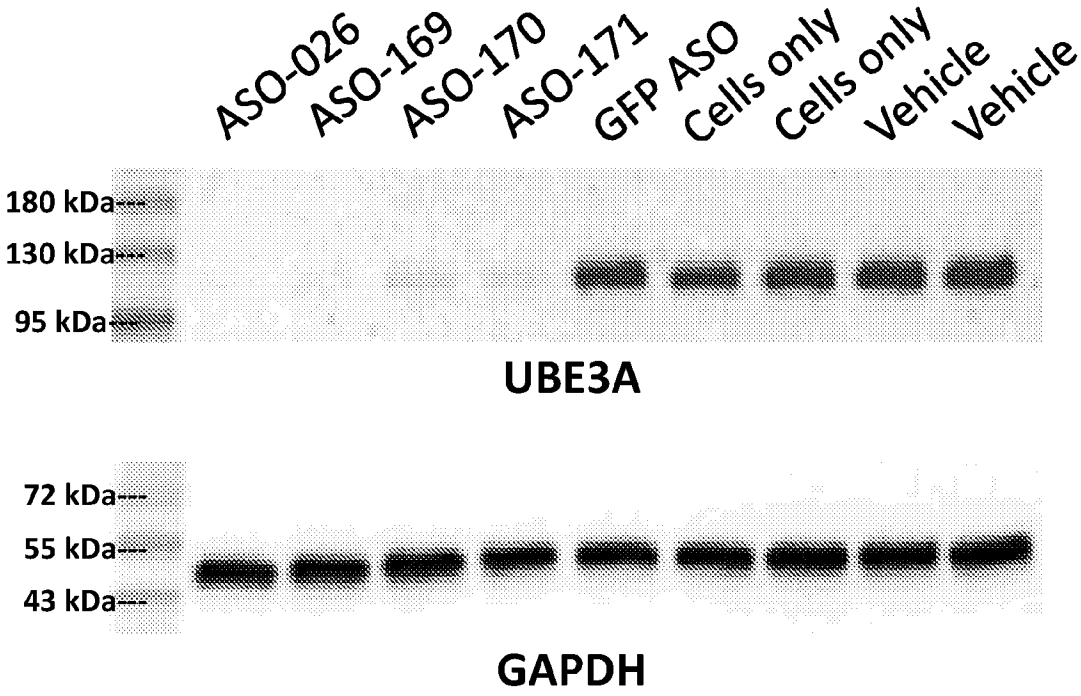
ASO-026
ASO-169
ASO-170
ASO-171
GFP ASO
Cells only
Cells only
Vehicle
Vehicle
180 kDa
130 kDa
95 kDa
UBE3A
72 kDa
55 kDa
43 kDa
GAPDH

FIG. 17

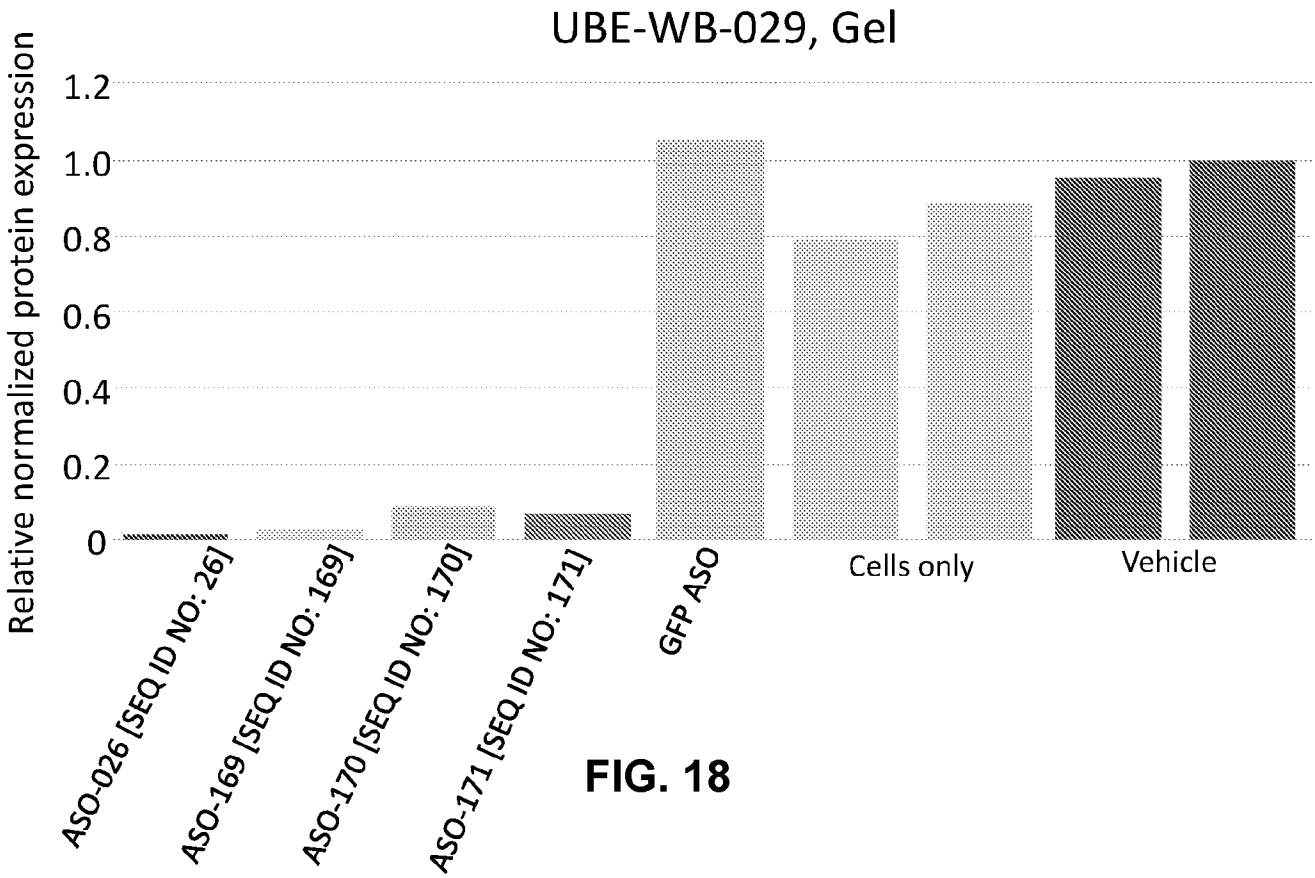
UBE-WB-029, Gel
Relative normalized protein expression
1.2
1.0
0.8
0.6
0.4
0.2
0
ASO-026 [SEQ ID NO: 26]
ASO-169 [SEQ ID NO: 169]
ASO-170 [SEQ ID NO: 170]
ASO-171 [SEQ ID NO: 171]
GFP ASO
Cells only
Vehicle

FIG. 18

SEQ ID NO:	ASO ID	200nM	20nM
1	QS-Tg15-ASO-001	79%	20%
5	QS-Tg15-ASO-005	80%	30%
6	QS-Tg15-ASO-006	92%	65%
8	QS-Tg15-ASO-008	95%	82%
11	QS-Tg15-ASO-011	86%	75%
13	QS-Tg15-ASO-013	96%	47%
15	QS-Tg15-ASO-015	91%	69%
17	QS-Tg15-ASO-017	76%	58%
23	QS-Tg15-ASO-023	94%	10%
25	QS-Tg15-ASO-025	95%	46%
26	QS-Tg15-ASO-026	90%	N/A
29	QS-Tg15-ASO-029	86%	59%
30	QS-Tg15-ASO-030	N/A	83%
36	QS-Tg15-ASO-036	57%	0%
101	QS-Tg15-ASO-101	95%	71%
102	QS-Tg15-ASO-102	94%	85%
103	QS-Tg15-ASO-103	74%	0%
104	QS-Tg15-ASO-104	96%	39%
105	QS-Tg15-ASO-105	82%	25%
106	QS-Tg15-ASO-106	99%	74%
107	QS-Tg15-ASO-107	98%	44%
108	QS-Tg15-ASO-108	80%	9%
115	QS-Tg15-ASO-115	97%	47%
116	QS-Tg15-ASO-116	100%	84%
117	QS-Tg15-ASO-117	98%	56%
151	QS-Tg15-ASO-121	95%	39%
122	QS-Tg15-ASO-122	98%	62%
127	QS-Tg15-ASO-127	99%	19%

FIG. 19

SEQ ID NO:	ASO ID	200nM	20nM
41	QS-Tg-15-ASO-041	N/A	0%
42	QS-Tg-15-ASO-042	99%	8%
46	QS-Tg-15-ASO-046	99%	53%
53	QS-Tg-15-ASO-053	99%	55%
64	QS-Tg-15-ASO-064	95%	49%
69	QS-Tg-17-ASO-069	95%	97%
71	QS-Tg-17-ASO-071	20%	0%
72	QS-Tg-17-ASO-072	89%	86%
73	QS-Tg-17-ASO-073	98%	0%
185	QS-Tg-17-ASO-002	97%	94%
192	QS-Tg-17-ASO-009	99%	76%
195	QS-Tg-17-ASO-012	99%	77%
197	QS-Tg-17-ASO-014	99%	82%
198	QS-Tg-17-ASO-015	91%	30%
199	QS-Tg-17-ASO-016	100%	87%
203	QS-Tg-17-ASO-020	100%	90%

FIG. 20

SEQ ID NO:	ASO ID	200nM
204	QS-Tg-17-ASO-021	96%
205	QS-Tg-17-ASO-022	93%
206	QS-Tg-17-ASO-023	94%
207	QS-Tg-17-ASO-024	71%
208	QS-Tg-17-ASO-025	77%
209	QS-Tg-17-ASO-026	74%
210	QS-Tg-17-ASO-027	78%
211	QS-Tg-17-ASO-028	68%
212	QS-Tg-17-ASO-029	69%
213	QS-Tg-17-ASO-030	55%
214	QS-Tg-17-ASO-031	62%
215	QS-Tg-17-ASO-032	75%
216	QS-Tg-17-ASO-033	85%
217	QS-Tg-17-ASO-034	81%
218	QS-Tg-17-ASO-035	85%

FIG. 21

SEQ ID NO:	ASO ID	200nM
155	QS-Tg-15-ASO-155	96%
156	QS-Tg-15-ASO-156	96%
157	QS-Tg-15-ASO-157	94%
158	QS-Tg-15-ASO-158	92%
159	QS-Tg-15-ASO-159	93%
160	QS-Tg-15-ASO-160	94%
161	QS-Tg-15-ASO-161	99%
162	QS-Tg-15-ASO-162	96%
163	QS-Tg-15-ASO-163	94%
164	QS-Tg-15-ASO-164	99%
165	QS-Tg-15-ASO-165	99%
166	QS-Tg-15-ASO-166	99%
167	QS-Tg-15-ASO-167	98%
168	QS-Tg-15-ASO-168	98%
169	QS-Tg-15-ASO-169	98%
170	QS-Tg-15-ASO-170	91%
171	QS-Tg-15-ASO-171	94%
172	QS-Tg-15-ASO-172	90%
173	QS-Tg-15-ASO-173	86%
174	QS-Tg-15-ASO-174	90%
175	QS-Tg-15-ASO-175	91%
176	QS-Tg-15-ASO-176	85%
177	QS-Tg-15-ASO-177	84%
178	QS-Tg-15-ASO-178	98%
179	QS-Tg-15-ASO-179	97%
180	QS-Tg-15-ASO-180	98%
181	QS-Tg-15-ASO-181	95%
182	QS-Tg-15-ASO-182	92%
183	QS-Tg-15-ASO-183	89%
219	QS-Tg-17-ASO-036	95%

FIG. 22

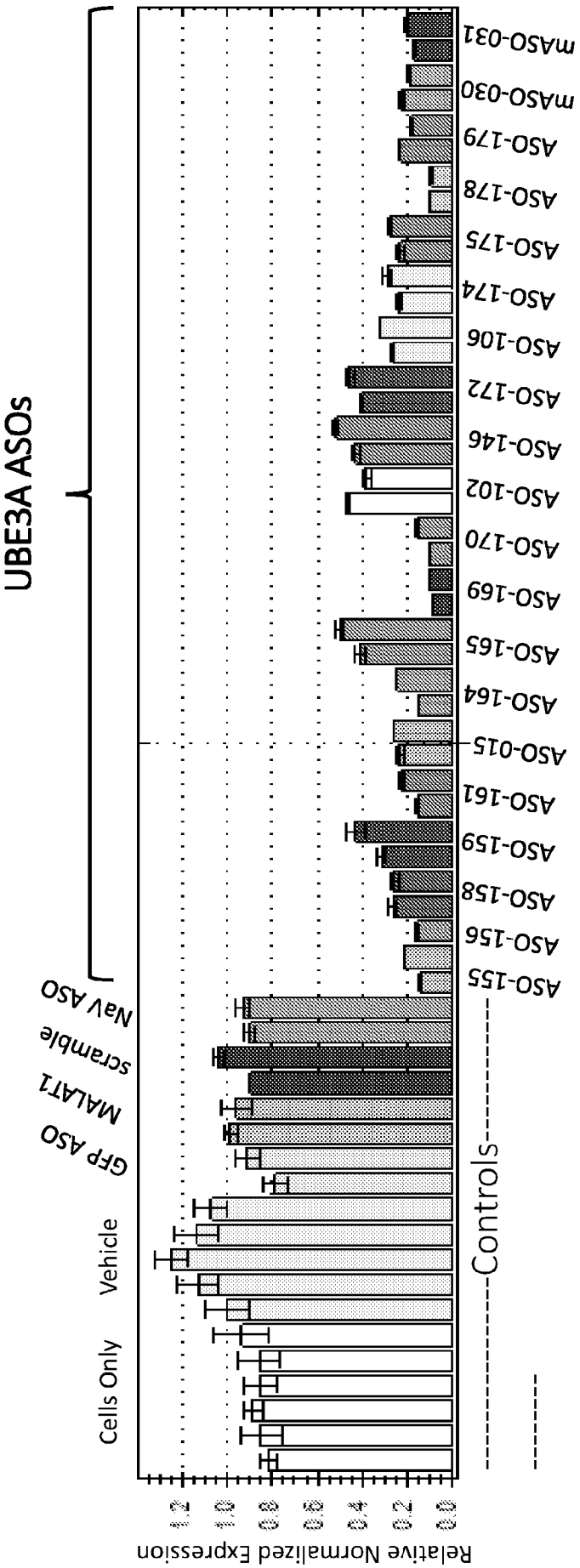
Relative Normalized Expression
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Cells Only
Vehicle
GFP ASO
MALAT1
scramble
NaV ASO
Controls
UBE3A ASOs
ASO-155
ASO-156
ASO-158
ASO-159
ASO-161
ASO-015
ASO-164
ASO-165
ASO-169
ASO-170
ASO-102
ASO-146
ASO-172
ASO-106
ASO-174
ASO-175
ASO-178
ASO-179
mASO-030
mASO-031

FIG. 23

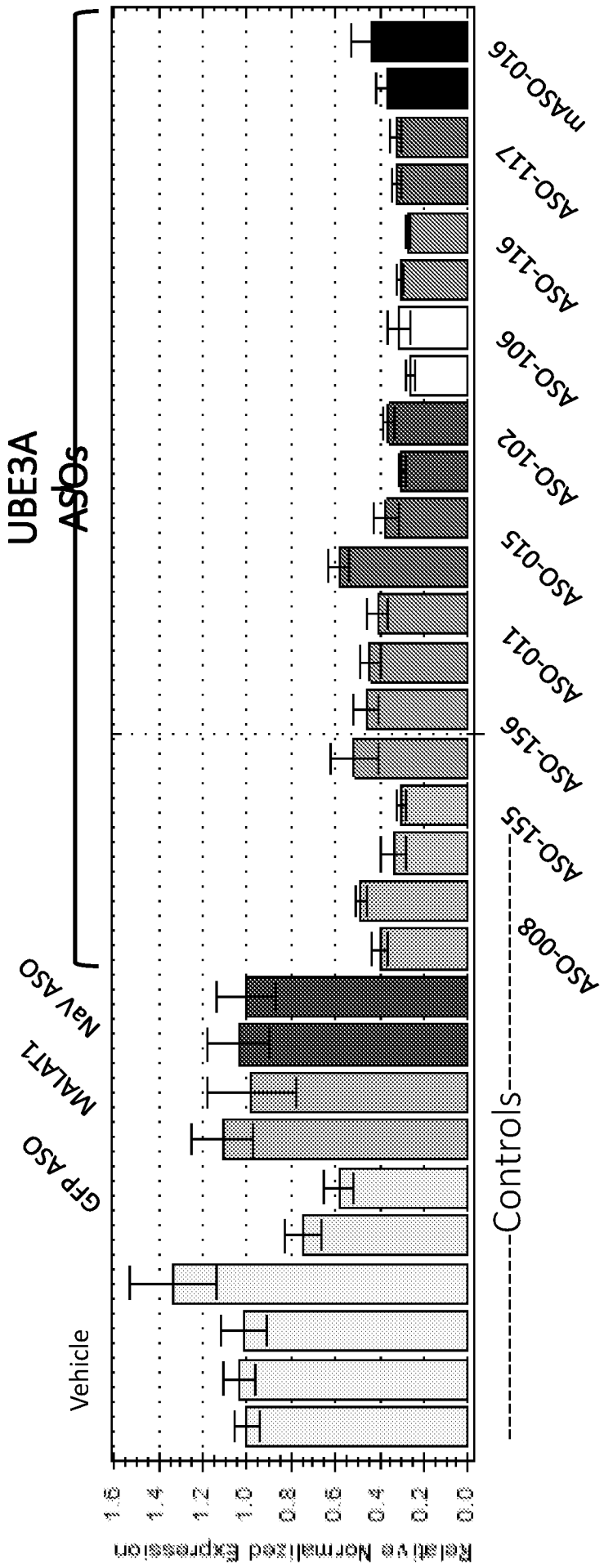
Relative Normalized Expression
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Vehicle
GFP ASO
MALAT1
NaV ASO
UBE3A ASOs
Controls
ASO-008
ASO-155
ASO-156
ASO-011
ASO-015
ASO-102
ASO-106
ASO-116
ASO-117
mASO-016

FIG. 24

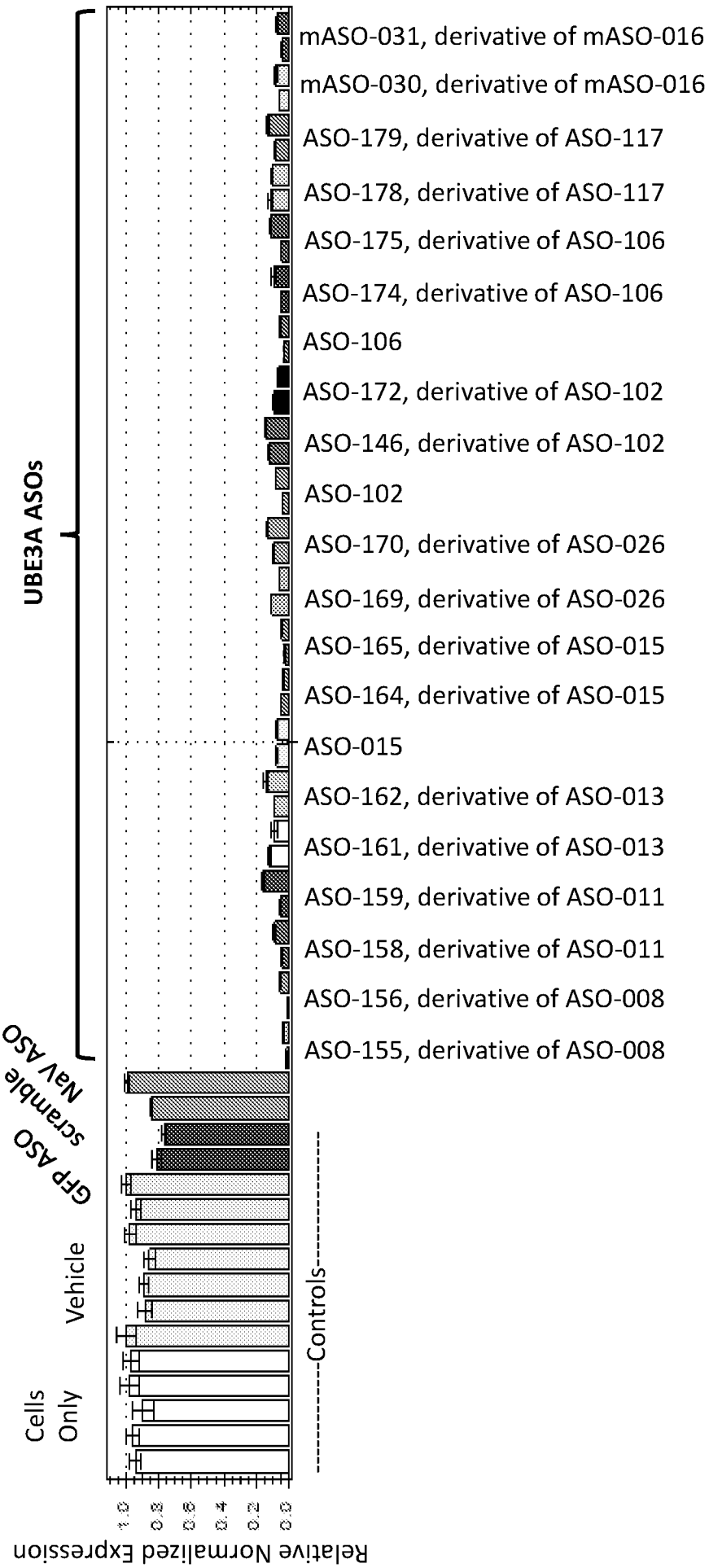
Relative Normalized Expression
1.0
0.8
0.6
0.4
0.2
0.0
Cells Only
Vehicle
GFP ASO
Scramble NaV ASO
Controls
UBE3A ASOs
mASO-031, derivative of mASO-016
mASO-030, derivative of mASO-016
ASO-179, derivative of ASO-117
ASO-178, derivative of ASO-117
ASO-175, derivative of ASO-106
ASO-174, derivative of ASO-106
ASO-106
ASO-172, derivative of ASO-102
ASO-146, derivative of ASO-102
ASO-102
ASO-170, derivative of ASO-026
ASO-169, derivative of ASO-026
ASO-165, derivative of ASO-015
ASO-164, derivative of ASO-015
ASO-015
ASO-162, derivative of ASO-013
ASO-161, derivative of ASO-013
ASO-159, derivative of ASO-011
ASO-158, derivative of ASO-011
ASO-156, derivative of ASO-008
ASO-155, derivative of ASO-008

FIG. 25

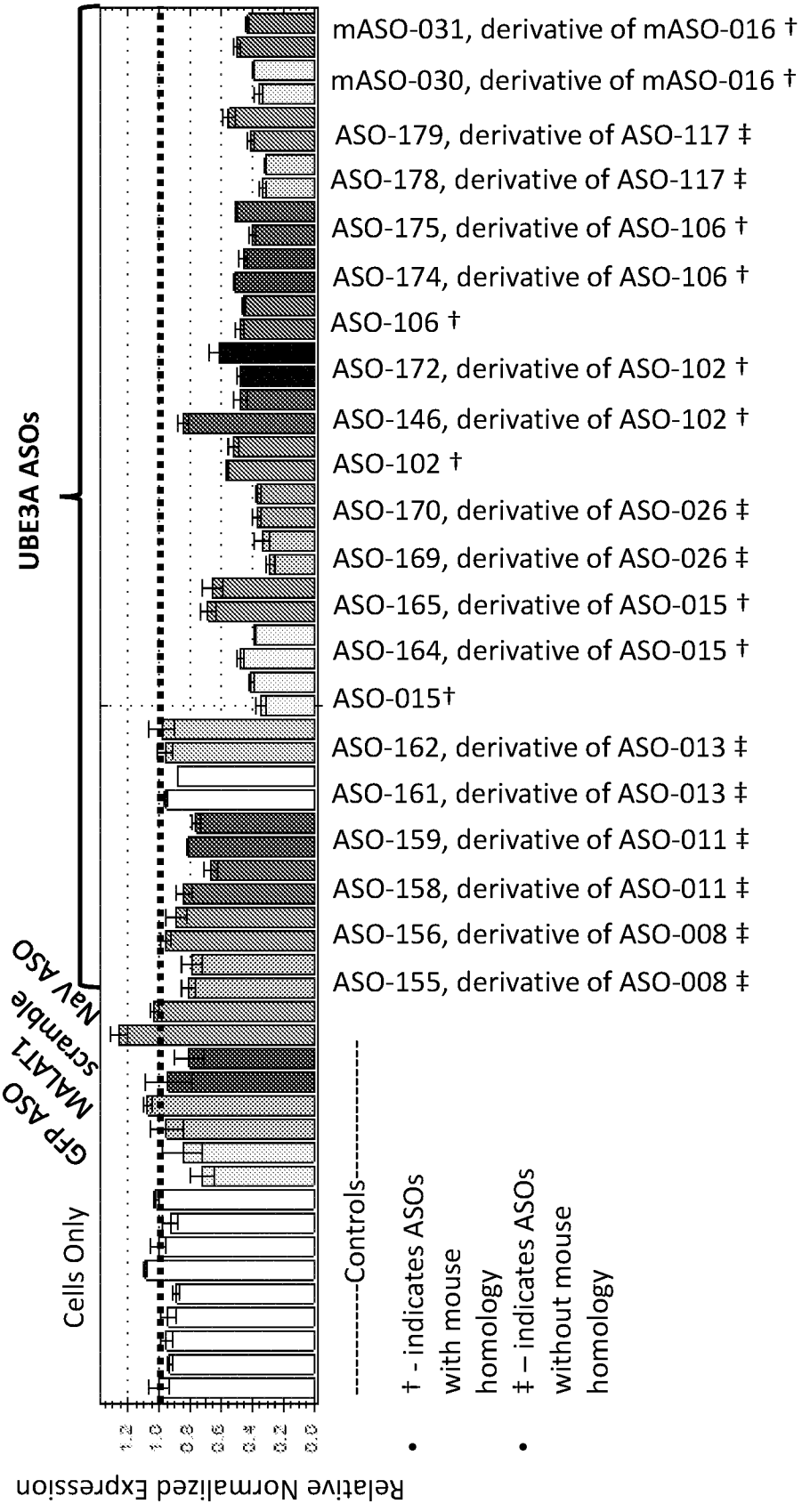
Relative Normalized Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Cells Only
GFP ASO
MALAT1 scramble NaV ASO
UBE3A ASOs
----------Controls----------
mASO-031, derivative of mASO-016 †
mASO-030, derivative of mASO-016 †
ASO-179, derivative of ASO-117 ‡
ASO-178, derivative of ASO-117 ‡
ASO-175, derivative of ASO-106 †
ASO-174, derivative of ASO-106 †
ASO-106 †
ASO-172, derivative of ASO-102 †
ASO-146, derivative of ASO-102 †
ASO-102 †
ASO-170, derivative of ASO-026 ‡
ASO-169, derivative of ASO-026 ‡
ASO-165, derivative of ASO-015 †
ASO-164, derivative of ASO-015 †
ASO-015†
ASO-162, derivative of ASO-013 ‡
ASO-161, derivative of ASO-013 ‡
ASO-159, derivative of ASO-011 ‡
ASO-158, derivative of ASO-011 ‡
ASO-156, derivative of ASO-008 ‡
ASO-155, derivative of ASO-008 ‡
• † - indicates ASOs with mouse homology
• ‡ – indicates ASOs without mouse homology

FIG. 26

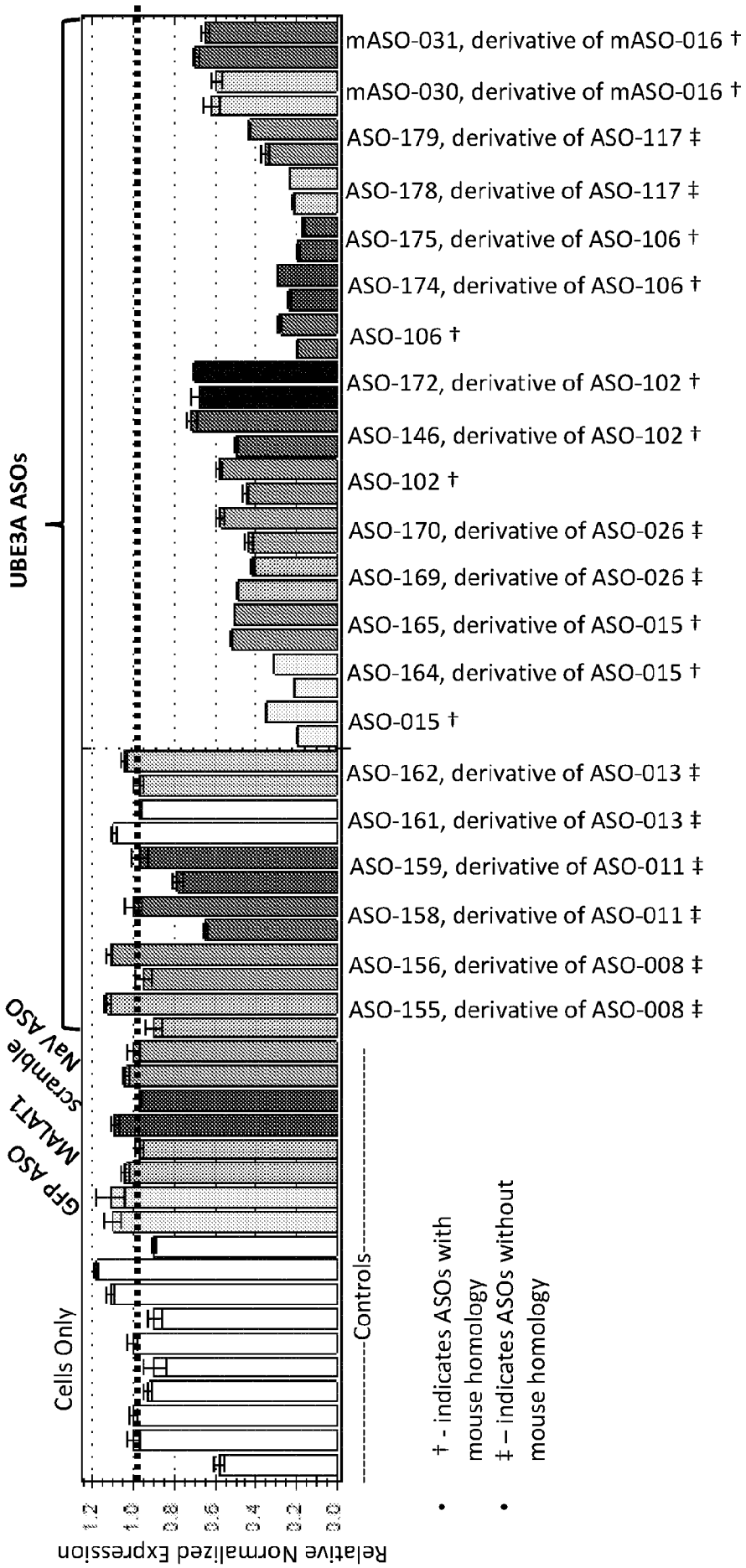
mASO-031, derivative of mASO-016 †
mASO-030, derivative of mASO-016 †
ASO-179, derivative of ASO-117 ‡
ASO-178, derivative of ASO-117 ‡
ASO-175, derivative of ASO-106 †
ASO-174, derivative of ASO-106 †
ASO-106 †
ASO-172, derivative of ASO-102 †
ASO-146, derivative of ASO-102 †
ASO-102 †
ASO-170, derivative of ASO-026 ‡
ASO-169, derivative of ASO-026 ‡
ASO-165, derivative of ASO-015 †
ASO-164, derivative of ASO-015 †
ASO-015 †
ASO-162, derivative of ASO-013 ‡
ASO-161, derivative of ASO-013 ‡
ASO-159, derivative of ASO-011 ‡
ASO-158, derivative of ASO-011 ‡
ASO-156, derivative of ASO-008 ‡
ASO-155, derivative of ASO-008 ‡
UBE3A ASOs
Scramble Nav ASO
MALAT1 ASO
GFP ASO
Cells Only
Controls
Relative Normalized Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
† - indicates ASOs with mouse homology
‡ – indicates ASOs without mouse homology

FIG. 27

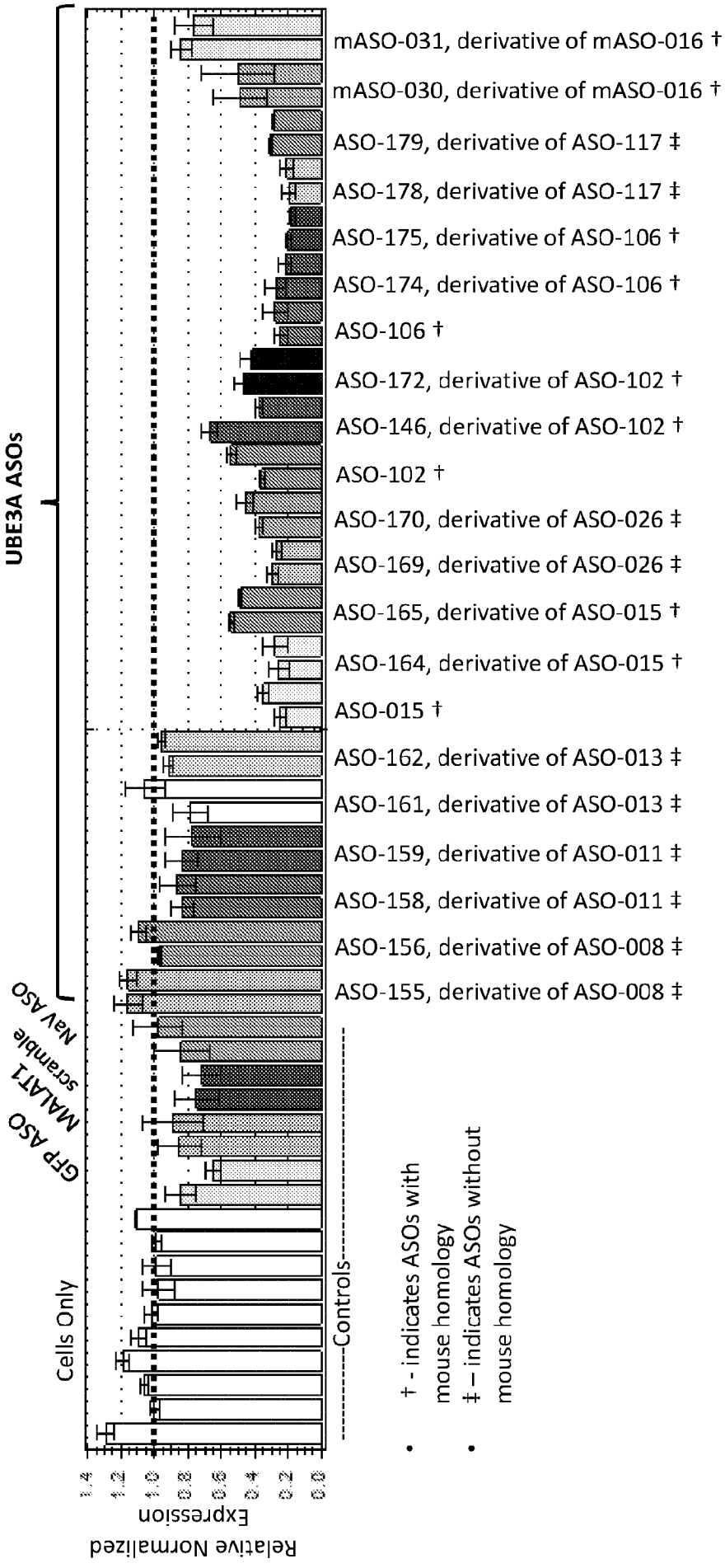
Relative Normalized Expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Cells Only
GFP ASO
MALAT1 scramble NaV ASO
UBE3A ASOs
Controls
mASO-031, derivative of mASO-016 †
mASO-030, derivative of mASO-016 †
ASO-179, derivative of ASO-117 ‡
ASO-178, derivative of ASO-117 ‡
ASO-175, derivative of ASO-106 †
ASO-174, derivative of ASO-106 †
ASO-106 †
ASO-172, derivative of ASO-102 †
ASO-146, derivative of ASO-102 †
ASO-102 †
ASO-170, derivative of ASO-026 ‡
ASO-169, derivative of ASO-026 ‡
ASO-165, derivative of ASO-015 †
ASO-164, derivative of ASO-015 †
ASO-015 †
ASO-162, derivative of ASO-013 ‡
ASO-161, derivative of ASO-013 ‡
ASO-159, derivative of ASO-011 ‡
ASO-158, derivative of ASO-011 ‡
ASO-156, derivative of ASO-008 ‡
ASO-155, derivative of ASO-008 ‡
† - indicates ASOs with mouse homology
‡ – indicates ASOs without mouse homology

FIG. 28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/16842

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/113; C07H 21/00; A61K 31/7088 (2022.01)

CPC - C12N 15/113; C12Y 603/02019; C12N 2310/14; C12N 2310/315; C12N 2310/321

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2010/0249052 A1 (BENSON et al.) 30 September 2010 (30.09.2010). Especially para [0039], [0069], [0084], SEQ ID NO: 719	1-4, 9-12, 17 --- 5-8, 13, 15, 16
Y	US 2018/0002695 A1 (IONIS PHARMACEUTICALS, INC.) 04 January 2018 (04.01.2018). Especially para [0235],[0307], [0310], [0339], [0370], [0377]	5-8, 13, 15, 16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 May 2022	JUL 29 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/16842

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-----Go to Extra Sheet for continuation-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13, 15-17

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box III: Lack of Unity of Invention

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-17, drawn to a composition comprising a synthetic antisense oligonucleotide (ASO) that inhibits expression of a ubiquitin ligase protein.
The composition will be searched to the extent that the antisense oligonucleotide (ASO) is the first named, SEQ ID NO: 1. It is believed that claims 1-13, 15-17 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1. Additional antisense oligonucleotides will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected target ASOs. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: SEQ ID NO: 155 (claims 1-17).

Group II: Claim 18, drawn to a method of treating Dup15q syndrome by administering an ASO composition that knocks down expression of the UBE3A gene.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Technical Features:

Group I+ has the special technical feature of composition comprising a synthetic antisense oligonucleotide (ASO) that inhibits expression of a ubiquitin ligase protein, not required by Group II.

Group II has the special technical feature of administering an ASO composition to knock-down UBE3A expression and treat Dup15q syndrome, not required by Group I+.

No technical features are shared between the antisense oligonucleotide sequences of Group I+ and accordingly, this group lacks unity a priori.

Additionally, even if Groups I+ inventions and Group II were considered to share the technical features of:

1. Group I+ inventions share claim 1.

2. Groups I+ and II share the technical feature of an antisense oligonucleotide that knocks-down expression of UBE3A.

However, said shared technical features do not represent a contribution over the prior art, and are disclosed by US 2010/0249052 A1 to Benson et al. (hereinafter "Benson").

As to shared technical feature #1 (claim 1), Benson discloses a composition comprising: a synthetic antisense oligonucleotide (ASO) that inhibits expression of a ubiquitin ligase protein (para [0039]; "As used herein, "E6AP" refers to the ubiquitin protein ligase E3A (ube3A, also referred to as E6-associated protein or E6AP) gene or protein"; claim 1; "A double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human E6AP gene in a cell, wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding E6AP, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said E6AP, inhibits expression of said E6AP gene by at least 40%.").

As to shared technical feature #2, Benson discloses an antisense oligonucleotide that knocks-down expression of UBE3A (para [0039], claim 1).

As the shared technical features were known in the art at the time of the invention, they cannot be considered shared special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I+ and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.