

[0178] FIG. 2D depicts a gel image and a graph showing the existence of non-productive mature *NSD1* mRNA transcript in human cortex.

[0179] FIGs. 3A-3D depict confirmation of inclusion or exclusion an ASCE of mouse *NSD1* (mouse exon 7, corresponding to human exon 8) in *NSD1* mRNA products processed from *NSD1* pre-mRNA in mouse brains via *in vivo* or *ex vivo* cycloheximide treatment.

[0180] FIG. 3A depicts gel images showing that, in *ex vivo* cycloheximide-treated mouse brains, exclusion of an ASCE of mouse *NSD1* (mouse exon 7, corresponding to human exon 8) leads to formation of a processed mRNA containing a premature termination codon rendering the transcript a target of NMD.

[0181] FIG. 3B depicts graphs of the percentage of NMD (top) and fold-change (bottom) of the NMD event of the non-productive *NSD1* mRNA product relative to *NSD1* productive *NSD1* mRNA product from the gel images of **FIG. 3A**.

[0182] FIG. 3C depicts gel images showing that, in *in vivo* cycloheximide-treated mouse brains, exclusion of an ASCE of mouse *NSD1* (mouse exon 7, corresponding to human exon 8) leads to formation of a processed mRNA containing a premature termination codon rendering the transcript a target of NMD.

[0183] FIG. 3D depicts graphs of the percentage of NMD (left) and fold-change (right) of the NMD event of the non-productive *NSD1* mRNA product relative to *NSD1* productive *NSD1* mRNA product from the gel images of **FIG. 3C**.

[0184] FIGs. 4A-4B depict confirmation of inclusion or exclusion an ASCE of mouse *NSD1* (mouse exon 7, corresponding to human exon 8) in *NSD1* mRNA products processed from *NSD1* pre-mRNA in mouse brains via *in vivo* cycloheximide treatment.

[0185] FIG. 4A depicts a gel image showing that, in *in vivo* cycloheximide-treated mouse brains, exclusion of an ASCE of mouse *NSD1* (mouse exon 7, corresponding to human exon 8) leads to formation of a processed mRNA containing a premature termination codon rendering the transcript a target of NMD.

[0186] FIG. 4B depicts graphs of the percentage of NMD (left) and fold-change (right) of the NMD event of the non-productive *NSD1* mRNA product relative to *NSD1* productive *NSD1* mRNA product from the gel images of **FIG. 4A**.

[0187] FIG. 5 depicts an exemplary ASO walk around the human *NSD1* exon 8 (GRCh38/hg38: chr5 177238237:177238507) region. The underlined nucleotides correspond to the exon skipping event and arrows point to canonical 5' or 3' splice sites. Figure discloses SEQ ID NO: 1775-1780, respectively, in order of appearance.

[0188] FIGs. 6A-6B show graphs summarizing the changes in the level of productive *NSD1* mRNA (**FIG. 6A**) and non-productive *NSD1* mRNA (**FIG. 6B**) in one ASO walk around exon 8 in HEK293 cells.

[0189] FIGs. 7A-7B show graphs summarizing the changes in the level of productive *NSD1* mRNA (**FIG. 7A**) and non-productive *NSD1* mRNA (**FIG. 7B**) in one ASO walk around exon 8.

[0190] FIG. 8 depicts an exemplary ASO walk around the human *NSD1* exon 8 (GRCh38/hg38: chr5 177238237:177238507) region for an ASO vectorization approach using U7 snRNA.

[0191] FIG. 9 depicts an exemplary ASO walk around the human *NSD1* exon 8 (GRCh38/hg38: chr5 177238237:177238507) region for an ASO vectorization approach using U1 snRNA.

[0192] FIG. 10 shows representative histograms of non-productive *NSD1* mRNA levels when different cell lines are treated with alternative NMD inhibitors. SH-SY5Y, U-87 MG, HEK293, and SK-N-AS cell lines were each treated with one of three conditions: a mock control (vehicle only), NMD inhibitor cycloheximide (CHX), or NMD inhibitor SMG1i. Treatment with SMG1i resulted in ~28% *NSD1* non-productive mRNA (percentage of the level of non-productive *NSD1* mRNA transcript in the total level of all *NSD1* mRNA transcripts) in U-87 MG cells, ~19% *NSD1* non-productive mRNA levels in SH-SY5Y cells, and <~18% *NSD1* non-productive mRNA levels in HEK293 and SK-N-AS cells. Treatment with CHX resulted in ~23% *NSD1* non-productive mRNA in SH-SY5Y cells, ~15% *NSD1* non-productive mRNA in U-87 MG cells, and ~13% *NSD1* non-productive mRNA in HEK293 and SK-N-AS cells. In cells treated only with vehicle (mock), the percentage of non-productive RNA remained low.

[0193] FIGs. 11A-11C show data demonstrating that exemplary ASOs with alternative backbone modifications have similar effects on *NSD1* pre-mRNA splicing. **FIG. 11A** is a table showing the ASO names, their backbone chemistries, sequences, and lengths. Figure discloses SEQ ID NOS 1768-1774, respectively, in order of appearance. **FIG. 11B** is a scatterplot showing the fold change in productive and non-productive *NSD1* mRNA when various ASOs with either PMO or 2'MOE-PS backbone modifications were nucleofected into U-87 MG cells, relative to cells treated with mock control. **FIG. 11C** is a histogram of the *NSD1* protein levels present in U-87 MG cells after treatment with the ASOs of the various backbones (see **FIG. 11A**), relative to cells treated with mock control. Data from both **FIG. 11B** and **FIG. 11C** are normalized to the mock controls.

[0194] FIGs. 12A-12B depict representative data illustrating the effects of exemplary ASOs on *NSD1* protein expression and H3K36me2 levels in U-87 MG cells. **FIG. 12A** is a histogram showing the fold change of *NSD1* protein in U-87 MG cells treated with various ASOs, relative to cells treated with water only. **FIG. 12B** is a histogram showing the fold change in cellular H3K36me2 levels in U-87 MG cells treated with various ASOs, relative to cells treated with water only. U-87 cells were nucleofected with 1 μ M of each ASO and cells were harvested 72 hours after nucleofection. *NSD1* protein levels were measured by immuno-capillary electrophoresis (JESS), and H3K36me2 levels were measured by AlphaLISA®. The data present in **FIGs. 12A-12B** are the sum of 2-3 independent experiments; mean $\pm$ SEM.

complex are likely to be targeted for degradation by the nonsense-mediated mRNA decay (NMD) pathway. Mutations in traditional (BPS/PPT/3'ss/5'ss) and auxiliary splicing motifs can cause aberrant splicing, such as exon skipping or cryptic (or pseudo-) exon inclusion or splice-site activation and contribute significantly to human morbidity and mortality. Both aberrant and alternative splicing patterns can be influenced by natural DNA variants in exons and introns.

[0215] Given that exon-intron boundaries can occur at any of the three positions of a codon, it is clear that only a subset of alternative splicing events can maintain the canonical open reading frame. For example, only exons that are evenly divisible by 3 can be skipped or included in the mRNA without any alteration of reading frame. Splicing events that do not have compatible phases will induce a frameshift. Unless reversed by downstream events, frameshifts can certainly lead to one or more PTCs, probably resulting in subsequent degradation by NMD. NMD is a translation-coupled mechanism that eliminates mRNAs containing PTCs. NMD can function as a surveillance pathway that exists in all eukaryotes. NMD can reduce errors in gene expression by eliminating mRNA transcripts that contain premature stop codons or PTCs. Translation of these aberrant mRNAs could, in some cases, lead to deleterious gain-of-function or dominant-negative activity of the resulting proteins. NMD targets not only transcripts with PTCs but also a broad array of mRNA isoforms expressed from many endogenous genes, suggesting that NMD is a master regulator that drives both fine and coarse adjustments in steady-state RNA levels in the cell.

[0216] In some cases, a therapeutic agent comprises a modified snRNA, such as a modified human or murine snRNA. In some cases, a therapeutic agent comprises a vector, such as a viral vector, that encodes a modified snRNA. In some embodiments, the modified snRNA is a modified U1 snRNA (see, *e.g.*, Alanis et al., Human Molecular Genetics, 2012, Vol. 21, No. 11 2389–2398). In some embodiments, the modified snRNA is a modified U7 snRNA (see, *e.g.*, Gadgil et al., J Gene Med. 2021;23:e3321). Modified U7 snRNAs can be made by any method known in the art including the methods described in Meyer, K.; Schümperli, Daniel (2012), Antisense Derivatives of U7 Small Nuclear RNA as Modulators of Pre-mRNA Splicing. In: Stamm, Stefan; Smith, Christopher W. J.; Lührmann, Reinhard (eds.) Alternative pre-mRNA Splicing: Theory and Protocols (pp. 481-494), Chichester: John Wiley & Sons 10.1002/9783527636778.ch45, incorporated by reference herein in its entirety. In some embodiments, a modified U7 (smOPT) does not compete with WT U7 (Stefanovic et al., 1995).

[0217] In some embodiments, the modified snRNA comprises an smOPT modification. For example, the modified snRNA can comprise a sequence AAUUUUUGGAG (SEQ ID NO: 1749). For example, the sequence AAUUUUUGGAG (SEQ ID NO: 1749) can replace a sequence AAUUUGUCUAG (SEQ ID NO: 1750) in a wild-type U7 snRNA to generate the modified U7 snRNA (smOPT). In some embodiments, a smOPT

Gene Symbol	Gene ID No.	SEQ ID NO.	Disease	OMIM	Genetics	ASCE Sequences
NSD1	64324	chr5: 177131835-177300213 (GRCh38/hg38) Size: 168,379 bases Orientation: Plus strand	Sotos syndrome	606681	Autosomal dominant	chr5 177238237 177238507

TABLE 3. SEQUENCES OF EXEMPLARY TARGET SEQUENCES IN HUMAN PRE-MRNA TRANSCRIPTS.

Gene/pre-mRNA symbol	Gene	Pre-mRNA	Sequence of ASCE
PKD1	ENSG00000008710 (SEQ ID NO: 1)	ENST00000262304.8	chr16 2092954 2093093
ABCA4	ENSG00000198691 (SEQ ID NO: 2)	ENST00000370225.4	chr1 94111438 94111579
FUS	ENSG00000089280 (SEQ ID NO: 3)	ENST00000254108.11	chr16 31186802 31186836
CEL	ENSG00000170835 (SEQ ID NO: 4)	ENST00000372080.6	chr9 133066530 133066660
NSD1	ENSG00000165671 (SEQ ID NO: 5)	ENST00000354179.8	chr5 177238237 177238507

TABLE 4. EXEMPLARY ASO SEQUENCES

Event	Sequence	Chr	Strand	Oligo Start (Chr coordinate)	Oligo End Chr coordinate)	Oligo Names	ASO SEQ ID NO:
Exon skipping	ACAAAGGCTACAAAAGT	chr5	+	177238123	177238141	NSD1:NM_172349:IVS7:-96	16
Exon skipping	TTCTGACAAAGGCTACAA	chr5	+	177238128	177238146	NSD1:NM_172349:IVS7:-91	17

Event	Sequence	Chr	Strand	Oligo Start (Chr coordinate)	Oligo End Chr coordinate)	Oligo Names	ASO SEQ ID NO:
Exon skippin g	TGAAATT CTGACAA AGGC	chr5	+	177238133	177238151	NSD1:NM_1723 49:IVS7:-86	18
Exon skippin g	AGGAATG AAATTCT GACA	chr5	+	177238138	177238156	NSD1:NM_1723 49:IVS7:-81	19
Exon skippin g	TTAAAAG GAATGAA ATTC	chr5	+	177238143	177238161	NSD1:NM_1723 49:IVS7:-76	20
Exon skippin g	ACACTTT AAAAGGA ATGA	chr5	+	177238148	177238166	NSD1:NM_1723 49:IVS7:-71	21
Exon skippin g	ATAACAC ACTTTAA AAGG	chr5	+	177238153	177238171	NSD1:NM_1723 49:IVS7:-66	22
Exon skippin g	AAAGAAT AACACAC TTTA	chr5	+	177238158	177238176	NSD1:NM_1723 49:IVS7:-61	23
Exon skippin g	GTCAAAA AGAATAA CACA	chr5	+	177238163	177238181	NSD1:NM_1723 49:IVS7:-56	24
Exon skippin g	TAAGTGT CAAAAAG AATA	chr5	+	177238168	177238186	NSD1:NM_1723 49:IVS7:-51	25
Exon skippin g	TAATTTA AGTGTCA AAAA	chr5	+	177238173	177238191	NSD1:NM_1723 49:IVS7:-46	26
Exon skippin g	TGTTGTA ATTTAAG TGTC	chr5	+	177238178	177238196	NSD1:NM_1723 49:IVS7:-41	27
Exon skippin g	AAAATTG TTGTAAT TTAA	chr5	+	177238183	177238201	NSD1:NM_1723 49:IVS7:-36	28
Exon skippin g	AGGCCAA AATTGTT GTAA	chr5	+	177238188	177238206	NSD1:NM_1723 49:IVS7:-31	29
Exon skippin g	TCCACAG GCCAAAA TTGT	chr5	+	177238193	177238211	NSD1:NM_1723 49:IVS7:-26	30
Exon skippin g	TAGAGTC CACAGGC CAAA	chr5	+	177238198	177238216	NSD1:NM_1723 49:IVS7:-21	31
Exon skippin g	AAAAATA GAGTCCA CAGG	chr5	+	177238203	177238221	NSD1:NM_1723 49:IVS7:-16	32
Exon skippin g	CTTCACA GCGGGAA CTTA	chr5	+	177238237	177238255	NSD1:NM_1723 49:EX8:+2	33

Event	Sequence	Chr	Strand	Oligo Start (Chr coordinate)	Oligo End Chr coordinate)	Oligo Names	ASO SEQ ID NO:
Exon skippin g	TTCCTCTT CACAGCG GGA	chr5	+	177238242	177238260	NSD1:NM_1723 49:EX8:+7	34
Exon skippin g	AGGCTTT CCTCTTC ACAG	chr5	+	177238247	177238265	NSD1:NM_1723 49:EX8:+12	35
Exon skippin g	CTAGAAG GCTTTCC TCTT	chr5	+	177238252	177238270	NSD1:NM_1723 49:EX8:+17	36
Exon skippin g	TCGGGCT AGAAGGC TTTC	chr5	+	177238257	177238275	NSD1:NM_1723 49:EX8:+22	37
Exon skippin g	CGACCTC GGGCTAG AAGG	chr5	+	177238262	177238280	NSD1:NM_1723 49:EX8:+27	38
Exon skippin g	TAGATCG ACCTCGG GCTA	chr5	+	177238267	177238285	NSD1:NM_1723 49:EX8:+32	39
Exon skippin g	AGCACTA GATCGAC CTCG	chr5	+	177238272	177238290	NSD1:NM_1723 49:EX8:+37	40
Exon skippin g	TTCTGAG CACTAGA TCGA	chr5	+	177238277	177238295	NSD1:NM_1723 49:EX8:+42	41
Exon skippin g	GCTTGTT CTGAGCA CTAG	chr5	+	177238282	177238300	NSD1:NM_1723 49:EX8:+47	42
Exon skippin g	CACCTGC TTGTTCT GAGC	chr5	+	177238287	177238305	NSD1:NM_1723 49:EX8:+52	43
Exon skippin g	TCGTCCA CCTGCTT GTTC	chr5	+	177238292	177238310	NSD1:NM_1723 49:EX8:+57	44
Exon skippin g	AATTCTC GTCCACC TGCT	chr5	+	177238297	177238315	NSD1:NM_1723 49:EX8:+62	45
Exon skippin g	CAAAGAA TTCTCGT CCAC	chr5	+	177238302	177238320	NSD1:NM_1723 49:EX8:+67	46
Exon skippin g	GAAATCA AAGAATT CTCG	chr5	+	177238307	177238325	NSD1:NM_1723 49:EX8:+72	47
Exon skippin g	TGGTTGA AATCAAA GAAT	chr5	+	177238312	177238330	NSD1:NM_1723 49:EX8:+77	48
Exon skippin g	TTCTTTG GTTGAAA TCAA	chr5	+	177238317	177238335	NSD1:NM_1723 49:EX8:+82	49

Event	Sequence	Chr	Strand	Oligo Start (Chr coordinate)	Oligo End Chr coordinate)	Oligo Names	ASO SEQ ID NO:
Exon skippin g	GGCTCTT CTTTGGT TGAA	chr5	+	177238322	177238340	NSD1:NM_1723 49:EX8:+87	50
Exon skippin g	CTGGAGG CTCTTCTT TGG	chr5	+	177238327	177238345	NSD1:NM_1723 49:EX8:+92	51
Exon skippin g	AAGAACT GGAGGCT CTTC	chr5	+	177238332	177238350	NSD1:NM_1723 49:EX8:+97	52
Exon skippin g	CTTTCAA GAACTGG AGGC	chr5	+	177238337	177238355	NSD1:NM_1723 49:EX8:+102	53
Exon skippin g	CCTCCCT TTCAAGA ACTG	chr5	+	177238342	177238360	NSD1:NM_1723 49:EX8:+107	54
Exon skippin g	CGGAGCC TCCCTTTC AAG	chr5	+	177238347	177238365	NSD1:NM_1723 49:EX8:+112	55
Exon skippin g	AAAAACG GAGCCTC CCTT	chr5	+	177238352	177238370	NSD1:NM_1723 49:EX8:+117	56
Exon skippin g	CCTCCAA AAACGGA GCCT	chr5	+	177238357	177238375	NSD1:NM_1723 49:EX8:+122	57
Exon skippin g	GGGGCCC TCCAAAA ACGG	chr5	+	177238362	177238380	NSD1:NM_1723 49:EX8:+127	58
Exon skippin g	GCCAAGG GGCCCTC CAAA	chr5	+	177238367	177238385	NSD1:NM_1723 49:EX8:+132	59
Exon skippin g	ACTGAGC CAAGGGG CCCT	chr5	+	177238372	177238390	NSD1:NM_1723 49:EX8:-118	60
Exon skippin g	TTCTGAC TGAGCCA AGGG	chr5	+	177238377	177238395	NSD1:NM_1723 49:EX8:-113	61
Exon skippin g	CCAAGTT CTGACTG AGCC	chr5	+	177238382	177238400	NSD1:NM_1723 49:EX8:-108	62
Exon skippin g	CACCTCC AAGTTCT GACT	chr5	+	177238387	177238405	NSD1:NM_1723 49:EX8:-103	63
Exon skippin g	ATGTCCA CCTCCAA GTTC	chr5	+	177238392	177238410	NSD1:NM_1723 49:EX8:-98	64
Exon skippin g	TCAGCAT GTCCACC TCCA	chr5	+	177238397	177238415	NSD1:NM_1723 49:EX8:-93	65

Event	Sequence	Chr	Strand	Oligo Start (Chr coordinate)	Oligo End Chr coordinate)	Oligo Names	ASO SEQ ID NO:
Exon skippin g	GCAACTC AGCATGT CCAC	chr5	+	177238402	177238420	NSD1:NM_1723 49:EX8:-88	66
Exon skippin g	CTGCGGC AACTCAG CATG	chr5	+	177238407	177238425	NSD1:NM_1723 49:EX8:-83	67
Exon skippin g	GTCAGCT GCGGCAA CTCA	chr5	+	177238412	177238430	NSD1:NM_1723 49:EX8:-78	68
Exon skippin g	ACAAGGT CAGCTGC GGCA	chr5	+	177238417	177238435	NSD1:NM_1723 49:EX8:-73	69
Exon skippin g	CACAGAC AAGGTCA GCTG	chr5	+	177238422	177238440	NSD1:NM_1723 49:EX8:-68	70
Exon skippin g	ACAGGCA CAGACAA GGTC	chr5	+	177238427	177238445	NSD1:NM_1723 49:EX8:-63	71
Exon skippin g	GAGCCAC AGGCACA GACA	chr5	+	177238432	177238450	NSD1:NM_1723 49:EX8:-58	72
Exon skippin g	TTCCGGA GCCACAG GCAC	chr5	+	177238437	177238455	NSD1:NM_1723 49:EX8:-53	73
Exon skippin g	GAGACTT CCGGAGC CACA	chr5	+	177238442	177238460	NSD1:NM_1723 49:EX8:-48	74
Exon skippin g	GTGGAGA GACTTCC GGAG	chr5	+	177238447	177238465	NSD1:NM_1723 49:EX8:-43	75
Exon skippin g	AGGCCGT GGAGAGA CTTC	chr5	+	177238452	177238470	NSD1:NM_1723 49:EX8:-38	76
Exon skippin g	AGGGCAG GCCGTGG AGAG	chr5	+	177238457	177238475	NSD1:NM_1723 49:EX8:-33	77
Exon skippin g	ACTCAAG GGCAGGC CGTG	chr5	+	177238462	177238480	NSD1:NM_1723 49:EX8:-28	78
Exon skippin g	CTCAGAC TCAAGGG CAGG	chr5	+	177238467	177238485	NSD1:NM_1723 49:EX8:-23	79
Exon skippin g	AATTCCT CAGACTC AAGG	chr5	+	177238472	177238490	NSD1:NM_1723 49:EX8:-18	80
Exon skippin g	CTAGCAA TTCCTCA GACT	chr5	+	177238477	177238495	NSD1:NM_1723 49:EX8:-13	81

Event	Sequence	Chr	Strand	Oligo Start (Chr coordinate)	Oligo End Chr coordinate)	Oligo Names	ASO SEQ ID NO:
Exon skippin g	TTTAACT AGCAATT CCTC	chr5	+	177238482	177238500	NSD1:NM_1723 49:EX8:-8	82
Exon skippin g	GGCGTTT TAACTAG CAAT	chr5	+	177238487	177238505	NSD1:NM_1723 49:EX8:-3	83
Exon skippin g	CTGAGAC CCCAACC CCAC	chr5	+	177238512	177238530	NSD1:NM_1723 49:IVS8:+6	84
Exon skippin g	AAATACT GAGACCC CAAC	chr5	+	177238517	177238535	NSD1:NM_1723 49:IVS8:+11	85
Exon skippin g	TGCTCAA ATACTGA GACC	chr5	+	177238522	177238540	NSD1:NM_1723 49:IVS8:+16	86
Exon skippin g	ATATCTG CTCAAAT ACTG	chr5	+	177238527	177238545	NSD1:NM_1723 49:IVS8:+21	87
Exon skippin g	TAATCAT ATCTGCT CAAA	chr5	+	177238532	177238550	NSD1:NM_1723 49:IVS8:+26	88
Exon skippin g	TCCTCTA ATCATAT CTGC	chr5	+	177238537	177238555	NSD1:NM_1723 49:IVS8:+31	89
Exon skippin g	CTGCTTC CTCTAAT CATA	chr5	+	177238542	177238560	NSD1:NM_1723 49:IVS8:+36	90
Exon skippin g	ATCTCCT GCTTCCT CTAA	chr5	+	177238547	177238565	NSD1:NM_1723 49:IVS8:+41	91
Exon skippin g	CTAAAAT CTCCTGC TTCC	chr5	+	177238552	177238570	NSD1:NM_1723 49:IVS8:+46	92
Exon skippin g	ACATACT AAAATCT CCTG	chr5	+	177238557	177238575	NSD1:NM_1723 49:IVS8:+51	93
Exon skippin g	TCAAAAC ATACTAA AATC	chr5	+	177238562	177238580	NSD1:NM_1723 49:IVS8:+56	94
Exon skippin g	TTACATC AAAACAT ACTA	chr5	+	177238567	177238585	NSD1:NM_1723 49:IVS8:+61	95
Exon skippin g	TGGCTTT ACATCAA AACA	chr5	+	177238572	177238590	NSD1:NM_1723 49:IVS8:+66	96
Exon skippin g	AATGTTG GCTTTAC ATCA	chr5	+	177238577	177238595	NSD1:NM_1723 49:IVS8:+71	97

Event	Sequence	Chr	Strand	Oligo Start (Chr coordinate)	Oligo End Chr coordinate)	Oligo Names	ASO SEQ ID NO:
Exon skippin g	GATACAA TGTGGC TTTA	chr5	+	177238582	177238600	NSD1:NM_1723 49:IVS8:+76	98
Exon skippin g	ATATAGA TACAATG TTGG	chr5	+	177238587	177238605	NSD1:NM_1723 49:IVS8:+81	99
Exon skippin g	ATTGTAT ATAGATA CAAT	chr5	+	177238592	177238610	NSD1:NM_1723 49:IVS8:+86	100
Exon skippin g	AGTTTAT TGTATAT AGAT	chr5	+	177238597	177238615	NSD1:NM_1723 49:IVS8:+91	101
Exon skippin g	GGGGTAG TTTATTGT ATA	chr5	+	177238602	177238620	NSD1:NM_1723 49:IVS8:+96	102
Exon skippin g	AGTCCAC AGGCCAA AATT	chr5	+	177238195	177238213	NSD1:NM_1723 49:IVS7:-24	103
Exon skippin g	GAGTCCA CAGGCCA AAAT	chr5	+	177238196	177238214	NSD1:NM_1723 49:IVS7:-23	104
Exon skippin g	AGAGTCC ACAGGCC AAAA	chr5	+	177238197	177238215	NSD1:NM_1723 49:IVS7:-22	105
Exon skippin g	ATAGAGT CCACAGG CCAA	chr5	+	177238199	177238217	NSD1:NM_1723 49:IVS7:-20	106
Exon skippin g	AATAGAG TCCACAG GCCA	chr5	+	177238200	177238218	NSD1:NM_1723 49:IVS7:-19	107
Exon skippin g	AAATAGA GTCCACA GGCC	chr5	+	177238201	177238219	NSD1:NM_1723 49:IVS7:-18	108
Exon skippin g	AAAATAG AGTCCAC AGGC	chr5	+	177238202	177238220	NSD1:NM_1723 49:IVS7:-17	109

TABLE 5A: EXEMPLARY ASO SEQUENCES

chr #	Start	End	Name	Strand	SEQ ID NO	ASO sequence
chr 5	177238119	177238137	NSD1:NM_001365 684:U7:IVS7:-100	+	322	AGGCTACAAAAAGTGGAT
chr 5	177238124	177238142	NSD1:NM_001365 684:U7:IVS7:-95	+	323	GACAAAGGCTACAAAAAG
chr 5	177238129	177238147	NSD1:NM_001365 684:U7:IVS7:-90	+	324	ATTCTGACAAAGGCTACA

chr	Start	End	Strand	SEQ ID NO	ASO sequence
chr5	177238593	177238611	+	1339	AATATTGTATATAGATACAA
chr5	177238594	177238612	+	1340	AATTATTGTATATAGATACA
chr5	177238595	177238613	+	1341	AATTTATTGTATATAGATAC
chr5	177238596	177238614	+	1342	AAGTTTATTGTATATAGATA
chr5	177238597	177238615	+	1343	AAGTTTATTGTATATAGAT
chr5	177238598	177238616	+	1344	AATAGTTTATTGTATATAGA
chr5	177238599	177238617	+	1345	AAGTAGTTTATTGTATATAG
chr5	177238600	177238618	+	1346	AAGGTAGTTTATTGTATATA
chr5	177238601	177238619	+	1347	AAGGGTAGTTTATTGTATAT
chr5	177238602	177238620	+	1348	AAGGGGTAGTTTATTGTATA
chr5	177238603	177238621	+	1349	AAGGGGGTAGTTTATTGTAT
chr5	177238604	177238622	+	1350	AAGGGGGTAGTTTATTGTA
chr5	177238605	177238623	+	1351	AAGGGGGTAGTTTATTGT
chr5	177238606	177238624	+	1352	AAAGGGGGTAGTTTATTG
chr5	177238607	177238625	+	1353	AAAAGGGGGTAGTTTATT
chr5	177238608	177238626	+	1354	AACAAAAGGGGGTAGTTTAT
chr5	177238609	177238627	+	1355	AACAAAAGGGGGTAGTTTA

TABLE 5C: EXEMPLARY U7 VECTOR SEQUENCE

Region	Sequence	SEQ ID NO:
Promoter sequence (Mouse U7 promoter)	cccacatcgctgccactacttaagtcgattcacttcggcttagctccaagccttaatctcggaagctctt tttttttttaacaacataggagctgtgattggctgttttcagccaatcagcactgactcattgcatagccttac aagcggtcacaaactcaagaaacgagcgggttaataagctctttagaatattgttatcgaaccgaataagga actgtgctttgtgattcacatatcagtgagggggtgtgaaatggcaccttgatctcacctcatcgaaagtg gagttgatgtccttcctggctcgctacagacgcacttcgc	1751
Wild-type U7 Antisense sequence	AAGTGTTACAGCTCTTTTAG	1752
Wild-type U7 non-coding RNA sequence	AAGTGTTACAGCTCTTTTAGAATTTGTCTAGCAGGTTTTCTGA CTTCGGTCGGAAAACCCCT	1753
ASO sequences replacing the Wild-type U7 Antisense sequence	Any of the ASO sequence from Table 4, Table 5A, Table 5A-1, Table 5B, Table 5B-1, Table 5G, and Table 5G-1	
Modified U7 snRNA (smOPT) non-coding RNA sequence	AAGTGTTACAGCTCTTTTAGAATTTTTGGAGCAGGTTTTCTGA CTTCGGTCGGAAAACCCCT	1754
smOPT sequence	AATTTTTGGAG	1755
3' regulatory sequence	cccaatttcactggctacaaatgaaagcaaacagttctctccccgctccccgggtgtgtgagaggggctttg atccttctctggttcctaggaacgcgtatgtgctagagccacgctctgagactccgcctcgtgcgggtccc gcttcttctgcctcctctgg	1756

Region	Sequence	SEQ ID NO:
Exemplary Full sequence of wild-type U7 snRNA	cccacatcgctgccactacttaagtccgattcacttcggcttagctccaagcctttaatctcggaagctctt tttttttttaacaacataggagctgtgattggctgttttcagccaatcagcactgactcattgcatagcctttac aagcgggcacaaactcaagaaacgagcgggtttaatagctctttagaatattgttatcgaaccgaataagga actgtgctttgtgattcacatatcagtgagggggtgtggaaatggcaccttgatctcacctcatcgaaagtg gagttgatgtccttcctggctcgctacagacgcacttccgc AAAGTGTTACAGCTCTTTT AGAATTTGTCTAGCAGGTTTCTGACTTCGGTCGGAAAACCCCT cccaatttcactggtctacaatgaaagcaaacagttctctccccgctccccgggtgtgtgagaggggctttg atccttctctggttcttaggaacgcgtatgtgctagagccacgctctgagacttccgctcgtgcgggtccc gcttctttctgctcctctgg	1757
Exemplary Full sequence of modified U7 snRNA (smOPT)	cccacatcgctgccactacttaagtccgattcacttcggcttagctccaagcctttaatctcggaagctctt tttttttttaacaacataggagctgtgattggctgttttcagccaatcagcactgactcattgcatagcctttac aagcgggcacaaactcaagaaacgagcgggtttaatagctctttagaatattgttatcgaaccgaataagga actgtgctttgtgattcacatatcagtgagggggtgtggaaatggcaccttgatctcacctcatcgaaagtg gagttgatgtccttcctggctcgctacagacgcacttccgc AAAGTGTTACAGCTCTTTT AGAATTTTGGAGCAGGTTTCTGACTTCGGTCGGAAAACCCCT cccaatttcactggtctacaatgaaagcaaacagttctctccccgctccccgggtgtgtgagaggggctttg atccttctctggttcttaggaacgcgtatgtgctagagccacgctctgagacttccgctcgtgcgggtccc gcttctttctgctcctctgg	1758
Full sequence of modified U7 snRNA (smOPT) containing an ASO sequence replacing the antisense sequence of U7 snRNA	cccacatcgctgccactacttaagtccgattcacttcggcttagctccaagcctttaatctcggaagctctt tttttttttaacaacataggagctgtgattggctgttttcagccaatcagcactgactcattgcatagcctttac aagcgggcacaaactcaagaaacgagcgggtttaatagctctttagaatattgttatcgaaccgaataagga actgtgctttgtgattcacatatcagtgagggggtgtggaaatggcaccttgatctcacctcatcgaaagtg gagttgatgtccttcctggctcgctacagacgcacttccgc [ASO sequence]AATTTTGGAGCAGGT TTTCTGACTTCGGTCGGAAAACCCCT cccaatttcactggtctacaatgaaagcaaa	1751
	acagttctctccccgctccccgggtgtgtgagaggggctttgatccttctctggttcttaggaacgcgtatg tctagagccacgctctgagacttccgctcgtgcgggtcccgttcttctgctcctctgg	1759
Exemplary full sequence of modified U7 snRNA (smOPT) containing an ASO sequence replacing the antisense sequence of U7 snRNA	cccacatcgctgccactacttaagtccgattcacttcggcttagctccaagcctttaatctcggaagctctt tttttttttaacaacataggagctgtgattggctgttttcagccaatcagcactgactcattgcatagcctttac aagcgggcacaaactcaagaaacgagcgggtttaatagctctttagaatattgttatcgaaccgaataagga actgtgctttgtgattcacatatcagtgagggggtgtggaaatggcaccttgatctcacctcatcgaaagtg gagttgatgtccttcctggctcgctacagacgcacttccgc AACAAAGGCTACAAAA GTAATTTTGGAGCAGGTTTCTGACTTCGGTCGGAAAACCCCT cccaatttcactggtctacaatgaaagcaaacagttctctccccgctccccgggtgtgtgagaggggctttg atccttctctggttcttaggaacgcgtatgtgctagagccacgctctgagacttccgctcgtgcgggtccc gcttctttctgctcctctgg	1760

TABLE 5D: EXEMPLARY ASO SEQUENCES

chr #	Start	End	Name	Strand	SEQ ID NO	ASO Sequence
chr 5	177238265	177238283	NSD1:NM_001365684:U1:Ex8:30	+	1356	GATCGACCTCGGGCTAGA
chr 5	177238270	177238288	NSD1:NM_001365684:U1:Ex8:35	+	1357	CACTAGATCGACCTCGGG
chr 5	177238275	177238293	NSD1:NM_001365684:U1:Ex8:40	+	1358	CTGAGCACTAGATCGACC
chr 5	177238280	177238298	NSD1:NM_001365684:U1:Ex8:45	+	1359	TTGTTCTGAGCACTAGAT

chr	Start	End	Name	Strand	SEQ ID NO	ASO sequence
chr5	177238595	177238613	NSD1:NM_001365684:U1:IVS8:89	+	1737	TTTATTGTATATAGATAC
chr5	177238596	177238614	NSD1:NM_001365684:U1:IVS8:90	+	1738	GTTTATTGTATATAGATA
chr5	177238597	177238615	NSD1:NM_001365684:U1:IVS8:91	+	1739	AGTTTATTGTATATAGAT
chr5	177238598	177238616	NSD1:NM_001365684:U1:IVS8:92	+	1740	TAGTTTATTGTATATAGA
chr5	177238599	177238617	NSD1:NM_001365684:U1:IVS8:93	+	1741	GTAGTTTATTGTATATAG
chr5	177238600	177238618	NSD1:NM_001365684:U1:IVS8:94	+	1742	GGTAGTTTATTGTATATA
chr5	177238601	177238619	NSD1:NM_001365684:U1:IVS8:95	+	1743	GGGTAGTTTATTGTATAT
chr5	177238602	177238620	NSD1:NM_001365684:U1:IVS8:96	+	1744	GGGGTAGTTTATTGTATA
chr5	177238603	177238621	NSD1:NM_001365684:U1:IVS8:97	+	1745	GGGGGTAGTTTATTGTAT
chr5	177238604	177238622	NSD1:NM_001365684:U1:IVS8:98	+	1746	AGGGGGTAGTTTATTGTA
chr5	177238605	177238623	NSD1:NM_001365684:U1:IVS8:99	+	1747	AAGGGGGTAGTTTATTGT
chr5	177238606	177238624	NSD1:NM_001365684:U1:IVS8:100	+	1748	AAAGGGGGTAGTTTATTG

TABLE 5F: EXEMPLARY U1 VECTOR SEQUENCES

Region	Sequence	SEQ ID NO:
Promoter sequence (Human U1 promoter)	gctccatctggccaccgaaaggtgtccttaacacaggctaaggaccagcttcttgggagaga acagacgcagggcgaggagggaaaaagggagagagcagacgtcacttccccttggcggtctt ggcagcagattggctcggttagtggcagaaaggcagacggggactgggcaaggcactgtcg gtgacatcacggacagggcgacttctatgtagatgaggcagcgcagaggctgctgcttcgcca cttgctgcttcaccacgaaggaggtcccgtgcctgggagcgggttcaggaccgctgatcgga gtgagaatcccagctgtgtgtcagggctggaaagggctcgggagtcgcggggcaagtgacc gtgtgtgtaaagagtgaggcgatgaggctgtgtcggggcagaggcccaagatctc	1761
Wild-type U1 Antisense sequence	acttacctg	
Wild-type U1 non-coding RNA sequence	<u>atacttacctggcaggggagataccatgatcacgaaggtgggtttcccagggcgaggcttatcca</u> <u>ttgactccggatgtgctgaccctgcgatttcccaaagtgtgggaaactcgactgcataattgtg</u> <u>gtagtgggggactgcgttcgcgctttcccctg</u>	1762
ASO sequences replacing the Wild-type U1 Antisense sequence	Any of the ASO sequences from Table 4, Table 5A, Table 5B, Table 5D, Table 5E, and Table 5G	

Region	Sequence	SEQ ID NO:
3' regulatory sequence	actttctggagtttcaaaagtagactgtacgctaagggtcatatctttttgttttggttgtgtcttggtggcgctttaaagttaatacctacagtggagggtcggaataggaagtaacatgtcgctgcacgccataggagaaaaagcgagcatcagccgtatcggctttgtaacacaaattagctatcgtgaagtcgctcag	1763
Exemplary Full sequence of wild-type U1 snRNA	gctccatctggccaccgaaaggttgctccttaacacaggctaaggaccagcttctttgggagagaaacagacgaggggaggaggggaaaaaggagaggcagacgtcacttccccttggcggctctggcagcagattggtcggttgagtggcagaaaggcagacggggactgggcaaggcactgtcggtgacatcacggacagggcgacttctatgtagatgaggcagcgcagaggctgtgcttcgccacttgcgtcttcaccacgaaggagttcccgtgccctgggagcgggttcaggaccgctgatcgggaagtgagaatcccagctgtgtgtcagggctggaaagggctcgggagtgcgcggggcaagtgtgaccgtgtgtgttaaagagttaggcgtatgaggctgtgtcgggagaggcccaagatctcacttaccctggcaggggagataccatgatcacgaaggtggtttccagggagcaggttatccattgcactccggatgtgctgaccctgcgatttccccaaatgtgggaaactcgactgcataattgtggttagtgggggactgcgttcgcgctttcccctgactttctggagtttcaaaagtagactgtacgctaagggtcatatctttttgttttggttgtgtcttggtggcgctttaaagttaatacctacagtggagggtcggaataggaagtaacatgtcgctgcacgccataggagaaaaagcgagcatcagccgtatcggcttgaacacaaattagctatcgtgaagtcgctcag	1764
Full sequence of U1 snRNA containing an ASO sequence replacing the antisense sequence of U1 snRNA	Gtccatctggccaccgaaaggttgctccttaacacaggctaaggaccagcttctttgggagagaaacagacgaggggaggaggggaaaaaggagaggcagacgtcacttccccttggcggctctggcagcagattggtcggttgagtggcagaaaggcagacggggactgggcaaggcactgtcggtgacatcacggacagggcgacttctatgtagatgaggcagcgcagaggctgtgcttcgccacttgcgtcttcaccacgaaggagttcccgtgccctgggagcgggttcaggaccgctgatcgggaagtgagaatcccagctgtgtgtcagggctggaaagggctcgggagtgcgcggggcaagtgtgaccgtgtgtgttaaagagttaggcgtatgaggctgtgtcgggagaggcccaagatctcat[ASO sequence]gcaggggagataccatgatcacgaaggtggtttccaggggcaggttatccattgcactccggatgtgctgaccctgcgatttccccaaatgtgggaaactcgactgcataattgtggttagtgggggactgcgttcgcgctttcccctgactttctggagtttcaaaagtagactgtacgctaagggtcatatctttttgttttggttgtgtcttggtggcgctttaaagttaatacctacagtggagggtcggaataggaagtaacatgtcgctgcacgccataggagaaaaagcgagcatcagccgtatcggcttgaacacaaattagctatcgtgaagtcgctcag	1765 1766
Exemplary full sequence of U1 snRNA containing an ASO sequence replacing the antisense sequence of U1 snRNA	gctccatctggccaccgaaaggttgctccttaacacaggctaaggaccagcttctttgggagagaaacagacgaggggaggaggggaaaaaggagaggcagacgtcacttccccttggcggctctggcagcagattggtcggttgagtggcagaaaggcagacggggactgggcaaggcactgtcggtgacatcacggacagggtgacatcacggacagggcgacttctatgtagatgaggcagcgcagaggctgtgcttcgccacttgcgtcttcaccacgaaggagttcttgctgttcaccacgaaggagttcccgtgccctgggagcgggttcaggaccgctgatcggaaagtgaatcccagctgtgtgtcagggctggaaagggctcgggagtgcgcggggcaagtgtgaccgtgtgtgttaaagagttaggcgtatgaggctgtgtcgggagaggcccaagatctcat[ACAAAGGCTACAAAAAGT]gcaggggagataccatgatcacgaaggtggtttccaggggcaggttatccattgcactccggatgtgctgaccctgcgatttccccaaatgtgggaaactcgactgcataattgtggttagtgggggactgcgttcgcgctttcccctgactttctggagtttcaaaagtagactgtacgctaagggtcatatctttttgttttggttgtgtcttggtggcgctttaaagttaatacctacagtggagggtcggaataggaagtaacatgtcgctgcacgccataggagaaaaagcgagcatcagccgtatcggcttgaacacaaattagctatcgtgaagtcgctcag	1767

TABLE 5G: EXEMPLARY ASO SEQUENCES

chr	Start	End	SEQ ID NO	ASO Sequence
chr5	177238123	177238141	110	ACAAAGGCTACAAAAAGT
chr5	177238128	177238146	111	TTCTGACAAAGGCTACAA
chr5	177238133	177238151	112	TGAAATTCTGACAAAGGC
chr5	177238138	177238156	113	AGGAATGAAATTCTGACA
chr5	177238143	177238161	114	TTAAAAGGAATGAAATTC
chr5	177238148	177238166	115	ACACTTTAAAAGGAATGA

compared to mock controls (**FIG. 11C**). All results are normalized to the mock controls. 2'MOE-PS-containing ASO 1752 resulted in at least about a 1.3-fold increase in NSD1 protein compared to mock controls. PMO-containing ASO 1750 resulted in at least about a 1.1-fold increase in NSD1 protein compared to water-only mock controls. 2'MOE-PS-containing ASO 1754 resulted in at least about a 1.2-fold increase in NSD1 protein compared to mock controls. PMO-containing ASO 1751 resulted in at least about a 1.2-fold increase in NSD1 protein compared to mock controls. 2'MOE-PS-containing ASO 1755 resulted in at least about a 1.1-fold increase in NSD1 protein compared to mock controls. 2'MOE-PS-containing ASO 1753 resulted in at least about a 1.2-fold increase in NSD1 protein compared to mock controls. In general, NSD1 protein levels increased relative to mock controls when cells were treated with either 2 μ M ASOs with PMO modifications or 1 μ M ASOs with 2'MOE-PS modifications. As such, the effect of MOE-PS ASO hits translates to (*i.e.*, behaves similarly to) ASOs with alternative backbones such as those modified with PMO.

TABLE 6. Exemplary ASOs with Modified Backbone Chemistry

ASO Name	Chemistry	Sequence	SEQ ID NO:
ASO 1749	PMO	TTCCTCTAATCATATCTG	1768
ASO 1750	PMO	TTCCTCTAATCATATCTGCT	1769
ASO 1751	PMO	GCTTCCTCTAATCATATCTG	1770
ASO 1752	2'MOE PS	TTCCTCTAATCATATCTG	1771
ASO 1753	2'MOE PS	TTCCTCTAATCATATCTGC	1772
ASO 1754	2'MOE PS	TTCCTCTAATCATATCTGCT	1773
ASO 1755	2'MOE PS	GCTTCCTCTAATCATATCTG	1774

Example 9. Upregulation of NSD1 Protein by Exemplary ASO Resulted in Globally Elevated H3K36me2 Levels

[0341] H3K36me2 is an epigenetic modification on Histone H3, and NSD1 is a histone methyltransferase that can mediate the dimethylation of Histone H3 at residue K36 (H3K36me2). NSD1-mediated H3K36me2 can contribute to the recruitment of DNA methyltransferases and the maintenance of DNA methylation at intergenic regions. Thus, levels of H3K36me2 were examined to determine whether the upregulation of NSD1 protein by ASOs would facilitate the elevation of H3K36me2 levels in U-87 MG cells. All results in this Example comprise data drawn from 2-3 independent experiments and all results for each assay are normalized to the water-only controls, mean $\pm$ SEM.

[0342] ASOs were evaluated in U-87 MG cells to assess their potency in elevating NSD1 protein expression and H3K36me2 levels. U-87 MG cells were nucleofected with 1 μ M of one of four