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(54) Titre : COMPOSITIONS ET METHODES POUR L'EDITION DU GENE TTR ET LE TRAITEMENT DE L'AMYLOIDOSE ATTR

(54) Title: COMPOSITIONS AND METHODS FOR TTR GENE EDITING AND TREATING ATTR AMYLOIDOSIS

(57) Abrégé/Abstract:
Compositions and methods for editing, e.g., introducing double-stranded breaks, within the TTR gene are provided. Compositions and methods for treating subjects having amyloidosis associated with transthyretin (ATTR), are provided.

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(54) Title: COMPOSITIONS AND METHODS FOR TTR GENE EDITING AND TREATING ATTR AMYLOIDOSIS

(57) Abstract: Compositions and methods for editing, e.g., introducing double-stranded breaks, within the *TTR* gene are provided. Compositions and methods for treating subjects having amyloidosis associated with transthyretin (ATTR), are provided.

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COMPOSITIONS AND METHODS FOR TTR GENE EDITING AND TREATING ATTR AMYLOIDOSIS

[0001] This application claims the benefit of priority to United States Provisional Application No. 62/556,236, which was filed on September 29, 2017, and United States Provisional Application No. 62/671,902, which was filed on May 15, 2018, and which are incorporated by reference in their entirety.

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 27, 2018, is named 2018-09-27_01155-0013-PCT_ST25.txt and is 417,471 bytes in size.

[0003] Transthyretin (TTR) is a protein produced by the *TTR* gene that normally functions to transport retinol and thyroxine throughout the body. TTR is predominantly synthesized in the liver, with small fractions being produced in the choroid plexus and retina. TTR normally circulates as a soluble tetrameric protein in the blood.

[0004] Pathogenic variants of TTR, which may disrupt tetramer stability, can be encoded by mutant alleles of the *TTR* gene. Mutant TTR may result in misfolded TTR, which may generate amyloids (i.e., aggregates of misfolded TTR protein). In some cases, pathogenic variants of TTR can lead to amyloidosis, or disease resulting from build-up of amyloids. For example, misfolded TTR monomers can polymerize into amyloid fibrils within tissues, such as the peripheral nerves, heart, and gastrointestinal tract. Amyloid plaques can also comprise wild-type TTR that has deposited on misfolded TTR.

[0005] Misfolding and deposition of wild-type TTR has also been observed in males aged 60 or more and is associated with heart rhythm problems, heart failure, and carpal tunnel.

[0006] Amyloidosis characterized by deposition of TTR may be referred to as "ATTR," "TTR-related amyloidosis," "TTR amyloidosis," or "ATTR amyloidosis," "ATTR familial amyloidosis" (when associated with a genetic mutation in a family), or "ATTRwt" or "wild-type ATTR" (when arising from misfolding and deposition of wild-type TTR).

[0007] ATTR can present with a wide spectrum of symptoms, and patients with different classes of ATTR may have different characteristics and prognoses. Some classes of ATTR include familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), and wild-type TTR amyloidosis (wt-TTR amyloidosis). FAP commonly presents with sensorimotor neuropathy, while FAC and wt-TTR amyloidosis commonly present with congestive heart failure. FAP and FAC are usually associated with a genetic mutation in the

TTR gene, and more than 100 different mutations in the *TTR* gene have been associated with ATTR. In contrast, wt-TTR amyloidosis is associated with aging and not with a genetic mutation in *TTR*. It is estimated that approximately 50,000 patients worldwide may be affected by FAP and FAC.

[0008] While more than 100 mutations in *TTR* are associated with ATTR, certain mutations have been more closely associated with neuropathy and/or cardiomyopathy. For example, mutations at T60 of TTR are associated with both cardiomyopathy and neuropathy; mutations at V30 are more associated with neuropathy; and mutations at V122 are more associated with cardiomyopathy.

[0009] A range of treatment approaches have been studied for treatment of ATTR, but there are no approved drugs that stop disease progression and improve quality of life. While liver transplant has been studied for treatment of ATTR, its use is declining as it involves significant risk and disease progression sometimes continues after transplantation. Small molecule stabilizers, such as diflunisal and tafamidis, appear to slow ATTR progression, but these agents do not halt disease progression.

[0010] Approaches using small interfering RNA (siRNA) knockdown, antisense knockdown, or a monoclonal antibody targeting amyloid fibrils for destruction are also currently being investigated, but while results on short-term suppression of TTR expression show encouraging preliminary data, a need exists for treatments that can produce long-lasting suppression of TTR.

[0011] Accordingly, the following embodiments are provided. In some embodiments, the present invention provides compositions and methods using a guide RNA with an RNA-guided DNA binding agent such as the CRISPR/Cas system to substantially reduce or knockout expression of the *TTR* gene, thereby substantially reducing or eliminating the production of TTR protein associated with ATTR. The substantial reduction or elimination of the production of TTR protein associated with ATTR through alteration of the *TTR* gene can be a long-term reduction or elimination.

SUMMARY

[0012] Embodiment 1 is a method of inducing a double-stranded break (DSB) within the TTR gene, comprising delivering a composition to a cell, wherein the composition comprises

a. a guide RNA comprising a guide sequence selected from SEQ ID NOs: 5-82;

b. a guide RNA comprising at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide RNA comprising a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

[0013] Embodiment 2 is a method of modifying the TTR gene comprising delivering a composition to a cell, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

[0014] Embodiment 3 is a method of treating amyloidosis associated with TTR (ATTR), comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82,

thereby treating ATTR.

[0015] Embodiment 4 is a method of reducing TTR serum concentration, comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82,

thereby reducing TTR serum concentration.

[0016] Embodiment 5 is a method for reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in a subject, comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding

agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82,

thereby reducing accumulation of amyloids or amyloid fibrils.

[0017] Embodiment 6 is a composition comprising a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

[0018] Embodiment 7 is a composition comprising a vector encoding a guide RNA, wherein the guide RNA comprises:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

[0019] Embodiment 8 is the composition of embodiment 6 or 7, for use in inducing a double-stranded break (DSB) within the TTR gene in a cell or subject.

[0020] Embodiment 9 is the composition of embodiment 6 or 7, for use in modifying the TTR gene in a cell or subject.

[0021] Embodiment 10 is the composition of embodiment 6 or 7, for use in treating amyloidosis associated with TTR (ATTR) in a subject.

[0022] Embodiment 11 is the composition of embodiment 6 or 7, for use in reducing TTR serum concentration in a subject.

[0023] Embodiment 12 is the composition of embodiment 6 or 7, for use in reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

[0024] Embodiment 13 is the method of any one of embodiments 1-5 or the composition for use of any one of embodiments 8-12, wherein the composition reduces serum TTR levels.

[0025] Embodiment 14 is the method or composition for use of embodiment 13, wherein the serum TTR levels are reduced by at least 50% as compared to serum TTR levels before administration of the composition.

[0026] Embodiment 15 is the method or composition for use of embodiment 13, wherein the serum TTR levels are reduced by 50-60%, 60-70%, 70-80%, 80-90%, 90-95%, 95-98%, 98-99%, or 99-100% as compared to serum TTR levels before administration of the composition.

[0027] Embodiment 16 is the method or composition for use of any one of embodiments 1-5 or 8-15, wherein the composition results in editing of the TTR gene.

[0028] Embodiment 17 is the method or composition for use of embodiment 16, wherein the editing is calculated as a percentage of the population that is edited (percent editing).

[0029] Embodiment 18 is the method or composition for use of embodiment 17, wherein the percent editing is between 30 and 99% of the population.

[0030] Embodiment 19 is the method or composition for use of embodiment 17, wherein the percent editing is between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the population.

[0031] Embodiment 20 is the method of any one of embodiments 1-5 or the composition for use of any one of embodiments 8-19, wherein the composition reduces amyloid deposition in at least one tissue.

[0032] Embodiment 21 is the method or composition for use of embodiment 20, wherein the at least one tissue comprises one or more of stomach, colon, sciatic nerve, or dorsal root ganglion.

[0033] Embodiment 22 is the method or composition for use of embodiment 20 or 21, wherein amyloid deposition is measured 8 weeks after administration of the composition.

[0034] Embodiment 23 is the method or composition for use of any one of embodiments 20-22, wherein amyloid deposition is compared to a negative control or a level measured before administration of the composition.

[0035] Embodiment 24 is the method or composition for use of any one of embodiments 20-23, wherein amyloid deposition is measured in a biopsy sample and/or by immunostaining.

[0036] Embodiment 25 is the method or composition for use of any one of embodiments 20-24, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and

80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen in a negative control.

[0037] Embodiment 26 is the method or composition for use of any one of embodiments 20-25, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen before administration of the composition.

[0038] Embodiment 27 is the method or composition for use of any one of embodiments 1-5 or 8-26, wherein the composition is administered or delivered at least two times.

[0039] Embodiment 28 is the method or composition for use of embodiment 27, wherein the composition is administered or delivered at least three times.

[0040] Embodiment 29 is the method or composition for use of embodiment 27, wherein the composition is administered or delivered at least four times.

[0041] Embodiment 30 is the method or composition for use of embodiment 27, wherein the composition is administered or delivered up to five, six, seven, eight, nine, or ten times.

[0042] Embodiment 31 is the method or composition for use of any one of embodiments 27-30, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days.

[0043] Embodiment 32 is the method or composition for use of any one of embodiments 27-30, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks.

[0044] Embodiment 33 is the method or composition for use of any one of embodiments 27-30, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 months.

[0045] Embodiment 34 is the method or composition of any one of the preceding embodiments, wherein the guide sequence is selected from SEQ ID NOs: 5-82.

[0046] Embodiment 35 is the method or composition of any one of the preceding embodiments, wherein the guide RNA is at least partially complementary to a target sequence present in the human TTR gene.

[0047] Embodiment 36 is the method or composition of embodiment 35, wherein the target sequence is in exon 1, 2, 3, or 4 of the human TTR gene.

[0048] Embodiment 37 is the method or composition of embodiment 35, wherein the target sequence is in exon 1 of the human TTR gene.

[0049] Embodiment 38 is the method or composition of embodiment 35, wherein the target sequence is in exon 2 of the human TTR gene.

[0050] Embodiment 39 is the method or composition of embodiment 35, wherein the target sequence is in exon 3 of the human TTR gene.

[0051] Embodiment 40 is the method or composition of embodiment 35, wherein the target sequence is in exon 4 of the human TTR gene.

[0052] Embodiment 41 is the method or composition of any one of embodiments 1-40, wherein the guide sequence is complementary to a target sequence in the positive strand of TTR.

[0053] Embodiment 42 is the method or composition of any one of embodiments 1-40, wherein the guide sequence is complementary to a target sequence in the negative strand of TTR.

[0054] Embodiment 43 is the method or composition of any one of embodiments 1-40, wherein the first guide sequence is complementary to a first target sequence in the positive strand of the TTR gene, and wherein the composition further comprises a second guide sequence that is complementary to a second target sequence in the negative strand of the TTR gene.

[0055] Embodiment 44 is the method or composition of any one of the preceding embodiments, wherein the guide RNA comprises a crRNA that comprises the guide sequence and further comprises a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end.

[0056] Embodiment 45 is the method or composition of any one of the preceding embodiments, wherein the guide RNA is a dual guide (dgRNA).

[0057] Embodiment 46 is the method or composition of embodiment 45, wherein the dual guide RNA comprises a crRNA comprising a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end, and a trRNA.

[0058] Embodiment 47 is the method or composition of any one of embodiments 1-43, wherein the guide RNA is a single guide (sgRNA).

[0059] Embodiment 48 is the method or composition of embodiment 47, wherein the sgRNA comprises a guide sequence that has the pattern of SEQ ID NO: 3.

[0060] Embodiment 49 is the method or composition of embodiment 47, wherein the sgRNA comprises the sequence of SEQ ID NO: 3.

[0061] Embodiment 50 is the method or composition of embodiment 48 or 49, wherein each N in SEQ ID NO: 3 is any natural or non-natural nucleotide, wherein the N's form the guide sequence, and the guide sequence targets Cas9 to the TTR gene.

[0062] Embodiment 51 is the method or composition of any one of embodiments 47-50, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-82 and the nucleotides of SEQ ID NO: 126.

[0063] Embodiment 52 is the method or composition of any one of embodiments 47-51, wherein the sgRNA comprises a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID Nos: 87-124.

[0064] Embodiment 53 is the method or composition of embodiment 47, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 87-124.

[0065] Embodiment 54 is the method or composition of any one of the preceding embodiments, wherein the guide RNA comprises at least one modification.

[0066] Embodiment 55 is the method or composition of embodiment 54, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

[0067] Embodiment 56 is the method or composition of embodiment 54 or 55, wherein the at least one modification includes a phosphorothioate (PS) bond between nucleotides.

[0068] Embodiment 57 is the method or composition of any one of embodiments 54-56, wherein the at least one modification includes a 2'-fluoro (2'-F) modified nucleotide.

[0069] Embodiment 58 is the method or composition of any one of embodiments 54-57, wherein the at least one modification includes a modification at one or more of the first five nucleotides at the 5' end.

[0070] Embodiment 59 is the method or composition of any one of embodiments 54-58, wherein the at least one modification includes a modification at one or more of the last five nucleotides at the 3' end.

[0071] Embodiment 60 is the method or composition of any one of embodiments 54-59, wherein the at least one modification includes PS bonds between the first four nucleotides.

[0072] Embodiment 61 is the method or composition of any one of embodiments 54-60, wherein the at least one modification includes PS bonds between the last four nucleotides.

[0073] Embodiment 62 is the method or composition of any one of embodiments 54-61, wherein the at least one modification includes 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end.

[0074] Embodiment 63 is the method or composition of any one of embodiments 54-62, wherein the at least one modification includes 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

[0075] Embodiment 64 is the method or composition of any one of embodiments 54-63, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.

[0076] Embodiment 65 is the method or composition of any one of embodiments 1-64, wherein the composition further comprises a pharmaceutically acceptable excipient.

[0077] Embodiment 66 is the method or composition of any one of embodiments 1-65, wherein the guide RNA is associated with a lipid nanoparticle (LNP).

[0078] Embodiment 67 is the method or composition of embodiment 66, wherein the LNP comprises a CCD lipid.

[0079] Embodiment 68 is the method or composition of embodiment 67, wherein the CCD lipid is Lipid a or Lipid B.

[0080] Embodiment 69 is the method or composition of embodiment 66-68, wherein the LNP comprises a neutral lipid.

[0081] Embodiment 70 is the method or composition of embodiment 69, wherein the neutral lipid is DSPC

[0082] Embodiment 71 is the method or composition of any one of embodiments 66-70, wherein the LNP comprises a helper lipid.

[0083] Embodiment 72 is the method or composition of embodiment 71, wherein the helper lipid is cholesterol.

[0084] Embodiment 73 is the method or composition of any one of embodiments 66-72, wherein the LNP comprises a stealth lipid.

[0085] Embodiment 74 is the method or composition of embodiment 73, wherein the stealth lipid is PEG2k-DMG.

[0086] Embodiment 75 is the method or composition of any one of the preceding embodiments, wherein the composition further comprises an RNA-guided DNA binding agent.

[0087] Embodiment 76 is the method or composition of any one of the preceding embodiments, wherein the composition further comprises an mRNA that encodes an RNA-guided DNA binding agent.

[0088] Embodiment 77 is the method or composition of embodiment 75 or 76, wherein the RNA-guided DNA binding agent is a Cas cleavase.

[0089] Embodiment 78 is the method or composition of embodiment 77, wherein the RNA-guided DNA binding agent is Cas9.

[0090] Embodiment 79 is the method or composition of any one of embodiments 75-78, wherein the RNA-guided DNA binding agent is modified.

[0091] Embodiment 80 is the method or composition of any one of embodiments 75-79, wherein the RNA-guided DNA binding agent is a nickase.

[0092] Embodiment 81 is the method or composition of embodiment 79 or 80, wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).

[0093] Embodiment 82 is the method or composition of any one of embodiments 75-81, wherein the RNA-guided DNA binding agent is a Cas from a Type-II CRISPR/Cas system.

[0094] Embodiment 83 is the method or composition of any one of the preceding embodiments, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.

[0095] Embodiment 84 is the method or composition for use of any one of embodiments 1-5 or 8-83, wherein the composition reduces or prevents amyloids or amyloid fibrils comprising TTR.

[0096] Embodiment 85 is the method or composition for use of embodiment 84, wherein the amyloids or amyloid fibrils are in the nerves, heart, or gastrointestinal track.

[0097] Embodiment 86 is the method or composition for use of any one of embodiments 1-5 or 8-83, wherein non-homologous ending joining (NHEJ) leads to a mutation during repair of a DSB in the TTR gene.

[0098] Embodiment 87 is the method or composition for use of embodiment 86, wherein NHEJ leads to a deletion or insertion of a nucleotide(s) during repair of a DSB in the TTR gene.

[0099] Embodiment 88 is the method or composition for use of embodiment 87, wherein the deletion or insertion of a nucleotide(s) induces a frame shift or nonsense mutation in the TTR gene.

[00100] Embodiment 89 is the method or composition for use of embodiment 87, wherein a frame shift or nonsense mutation is induced in the TTR gene of at least 50% of liver cells.

[00101] Embodiment 90 is the method or composition for use of embodiment 89, wherein a frame shift or nonsense mutation is induced in the TTR gene of 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100% of liver cells.

[00102] Embodiment 91 is the method or composition for use of any one of embodiments 87-90, wherein a deletion or insertion of a nucleotide(s) occurs in the TTR gene at least 50-fold or more than in off-target sites.

[00103] Embodiment 92 is the method or composition for use of embodiment 91, wherein the deletion or insertion of a nucleotide(s) occurs in the TTR gene 50-fold to 150-fold, 150-fold to 500-fold, 500-fold to 1500-fold, 1500-fold to 5000-fold, 5000-fold to 15000-fold, 15000-fold to 30000-fold, or 30000-fold to 60000-fold more than in off-target sites.

[00104] Embodiment 93 is the method or composition for use of any one of embodiments 87-92, wherein the deletion or insertion of a nucleotide(s) occurs at less than or equal to 3, 2, 1, or 0 off-target site(s) in primary human hepatocytes, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[00105] Embodiment 94 is the method or composition for use of embodiment 93, wherein the deletion or insertion of a nucleotide(s) occurs at a number of off-target sites in primary human hepatocytes that is less than the number of off-target sites at which a deletion or insertion of a nucleotide(s) occurs in Cas9-overexpressing cells, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[00106] Embodiment 95 is the method or composition for use of embodiment 94, wherein the Cas9-overexpressing cells are HEK293 cells stably expressing Cas9.

[00107] Embodiment 96 is the method or composition for use of any one of embodiments 93-95, wherein the number of off-target sites in primary human hepatocytes is determined by analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA and the guide RNA, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[00108] Embodiment 97 is the method or composition for use of any one of embodiments 93-95, wherein the number of off-target sites in primary human hepatocytes is determined by an oligonucleotide insertion assay comprising analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA, the guide RNA, and a donor oligonucleotide, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[00109] Embodiment 98 is the method or composition of any one of embodiments 1-43 or 47-97, wherein the sequence of the guide RNA is:

a) SEQ ID NO: 92 or 104;

b) SEQ ID NO: 87, 89, 96, or 113;

c) SEQ ID NO: 100, 102, 106, 111, or 112; or

d) SEQ ID NO: 88, 90, 91, 93, 94, 95, 97, 101, 103, 108, or 109,

optionally wherein the guide RNA does not produce indels at off-target site(s) that occur in a protein coding region in the genome of primary human hepatocytes.

[00110] Embodiment 99 is the method or composition for use of any one of embodiments 1-5 or 8-98, wherein administering the composition reduces levels of TTR in the subject.

[00111] Embodiment 100 is the method or composition for use of embodiment 99, wherein the levels of TTR are reduced by at least 50%.

[00112] Embodiment 101 is the method or composition for use of embodiment 100, wherein the levels of TTR are reduced by 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100%.

[00113] Embodiment 102 is the method or composition for use of embodiment 100 or 101, wherein the levels of TTR are measured in serum, plasma, blood, cerebral spinal fluid, or sputum.

[00114] Embodiment 103 is the method or composition for use of embodiment 100 or 101, wherein the levels of TTR are measured in liver, choroid plexus, and/or retina.

[00115] Embodiment 104 is the method or composition for use of any one of embodiments 99-103, wherein the levels of TTR are measured via enzyme-linked immunosorbent assay (ELISA).

[00116] Embodiment 105 is the method or composition for use of any one of embodiments 1-5 or 8-104, wherein the subject has ATTR.

[00117] Embodiment 106 is the method or composition for use of any one of embodiments 1-5 or 8-105, wherein the subject is human.

[00118] Embodiment 107 is the method or composition for use of embodiment 105 or 106, wherein the subject has ATTRwt.

[00119] Embodiment 108 is the method or composition for use of embodiment 105 or 106, wherein the subject has hereditary ATTR.

[00120] Embodiment 109 is the method or composition for use of any one of embodiments 1-5, 8-106, or 108, wherein the subject has a family history of ATTR.

[00121] Embodiment 110 is the method or composition for use of any one of embodiments 1-5, 8-106, or 108-109, wherein the subject has familial amyloid polyneuropathy.

[00122] Embodiment 111 is the method or composition for use of any one of embodiments 1-5 or 8-110, wherein the subject has only or predominantly nerve symptoms of ATTR.

[00123] Embodiment 112 is the method or composition for use of any one of embodiments 1-5 or 8-110, wherein the subject has familial amyloid cardiomyopathy.

[00124] Embodiment 113 is the method or composition for use of any one of embodiments 1-5, 8-109, or 112, wherein the subject has only or predominantly cardiac symptoms of ATTR.

[00125] Embodiment 114 is the method or composition for use of any one of embodiments 1-5 or 8-113, wherein the subject expresses TTR having a V30 mutation.

[00126] Embodiment 115 is the method or composition for use of embodiment 114, wherein the V30 mutation is V30A, V30G, V30L, or V30M.

[00127] Embodiment 116 is the method or composition for use of embodiment any one of embodiments 1-5 or 8-113, wherein the subject expresses TTR having a T60 mutation.

[00128] Embodiment 117 is the method or composition for use of embodiment 116, wherein the T60 mutation is T60A.

[00129] Embodiment 118 is the method or composition for use of embodiment any one of embodiments 1-5 or 8-113, wherein the subject expresses TTR having a V122 mutation.

[00130] Embodiment 119 is the method or composition for use of embodiment 118, wherein the V122 mutation is V122A, V122I, or V122(-).

[00131] Embodiment 120 is the method or composition for use of any one of embodiments 1-5 or 8-119, wherein the subject expresses wild-type TTR.

[00132] Embodiment 121 is the method or composition for use of any one of embodiments 1-5, 8-107, or 120, wherein the subject does not express TTR having a V30, T60, or V122 mutation.

[00133] Embodiment 122 is the method or composition for use of any one of embodiments 1-5, 8-107, or 120-121, wherein the subject does not express TTR having a pathological mutation.

[00134] Embodiment 123 is the method or composition for use of embodiment 121, wherein the subject is homozygous for wild-type TTR.

[00135] Embodiment 124 is the method or composition for use of any one of embodiments 1-5 or 8-123, wherein after administration the subject has an improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy.

[00136] Embodiment 125 is the method or composition for use of embodiment 124, wherein the improvement, stabilization, or slowing of change in sensory neuropathy is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

[00137] Embodiment 126 is the method or composition for use of any one of embodiments 1-5 or 8-125, wherein the subject has an improvement, stabilization, or slowing of change in symptoms of congestive heart failure.

[00138] Embodiment 127 is the method or composition for use of embodiment 126, wherein the improvement, stabilization, or slowing of change in congestive heart failure is measured using cardiac biomarker tests, lung function tests, chest x-rays, or electrocardiography.

[00139] Embodiment 128 is the method or composition for use of any one of embodiments 1-5 or 8-127, wherein the composition or pharmaceutical formulation is administered via a viral vector.

[00140] Embodiment 129 is the method or composition for use of any one of embodiments 1-5 or 8-127, wherein the composition or pharmaceutical formulation is administered via lipid nanoparticles.

[00141] Embodiment 130 is the method or composition for use of any one of embodiments 1-5 or 8-129, wherein the subject is tested for specific mutations in the TTR gene before administering the composition or formulation.

[00142] Embodiment 131 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 5.

[00143] Embodiment 132 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 6.

[00144] Embodiment 133 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 7.

[00145] Embodiment 134 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 8.

[00146] Embodiment 135 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 9.

[00147] Embodiment 136 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 10.

[00148] Embodiment 137 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 11.

[00149] Embodiment 138 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 12.

[00150] Embodiment 139 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 13.

[00151] Embodiment 140 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 14.

[00152] Embodiment 141 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 15.

[00153] Embodiment 142 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 16.

[00154] Embodiment 143 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 17.

[00155] Embodiment 144 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 18.

[00156] Embodiment 145 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 19.

[00157] Embodiment 146 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 20.

[00158] Embodiment 147 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 21.

[00159] Embodiment 148 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 22.

[00160] Embodiment 149 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 23.

[00161] Embodiment 150 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 24.

[00162] Embodiment 151 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 25.

[00163] Embodiment 152 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 26.

[00164] Embodiment 153 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 27.

[00165] Embodiment 154 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 28.

[00166] Embodiment 155 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 29.

[00167] Embodiment 156 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 30.

[00168] Embodiment 157 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 31.

[00169] Embodiment 158 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 32.

[00170] Embodiment 159 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 33.

[00171] Embodiment 160 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 34.

[00172] Embodiment 161 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 35.

[00173] Embodiment 162 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 36.

[00174] Embodiment 163 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 37.

[00175] Embodiment 164 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 38.

[00176] Embodiment 165 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 39.

[00177] Embodiment 166 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 40.

[00178] Embodiment 167 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 41.

[00179] Embodiment 168 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 42.

[00180] Embodiment 169 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 43.

[00181] Embodiment 170 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 44.

[00182] Embodiment 171 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 45.

[00183] Embodiment 172 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 46.

[00184] Embodiment 173 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 47.

[00185] Embodiment 174 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 48.

[00186] Embodiment 175 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 49.

[00187] Embodiment 176 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 50.

[00188] Embodiment 177 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 51.

[00189] Embodiment 178 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 52.

[00190] Embodiment 179 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 53.

[00191] Embodiment 180 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 54.

[00192] Embodiment 181 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 55.

[00193] Embodiment 182 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 56.

[00194] Embodiment 183 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 57.

[00195] Embodiment 184 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 58.

[00196] Embodiment 185 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 59.

[00197] Embodiment 186 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 60.

[00198] Embodiment 187 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 61.

[00199] Embodiment 188 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 62.

[00200] Embodiment 189 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 63.

[00201] Embodiment 190 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 64.

[00202] Embodiment 191 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 65.

[00203] Embodiment 192 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 66.

[00204] Embodiment 193 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 67.

[00205] Embodiment 194 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 68.

[00206] Embodiment 195 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 69.

[00207] Embodiment 196 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 70.

[00208] Embodiment 197 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 71.

[00209] Embodiment 198 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 72.

[00210] Embodiment 199 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 73.

[00211] Embodiment 200 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 74.

[00212] Embodiment 201 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 75.

[00213] Embodiment 202 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 76.

[00214] Embodiment 203 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 77.

[00215] Embodiment 204 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 78.

[00216] Embodiment 205 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 79.

[00217] Embodiment 206 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 80.

[00218] Embodiment 207 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 81.

[00219] Embodiment 208 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 82.

[00220] Embodiment 209 is a use of a composition or formulation of any of embodiments 6-208 for the preparation of a medicament for treating a human subject having ATTR.

[00221] Also disclosed is the use of a composition or formulation of any of the foregoing embodiments for the preparation of a medicament for treating a human subject having ATTR. Also disclosed are any of the foregoing compositions or formulations for use in treating ATTR or for use in modifying (e.g., forming an indel in, or forming a frameshift or nonsense mutation in) a *TTR* gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[00222] FIG. 1 shows a schematic of chromosome 18 with the regions of the TTR gene that are targeted by the guide sequences provided in Table 1.

[00223] FIG. 2 shows off-target analysis in HEK293_Cas9 cells of certain dual guide RNAs targeting TTR. The on-target site is designated by a filled square for each dual guide RNA tested, whereas closed circles represent a potential off-target site.

[00224] FIG. 3 shows off-target analysis in HEK_Cas9 cells of certain single guide RNAs targeting TTR. The on-target site is designated by a filled square for each single guide RNA tested, whereas open circles represent a potential off-target site.

[00225] FIG. 4 shows dose response curves of lipid nanoparticle formulated human TTR specific sgRNAs on primary human hepatocytes.

[00226] FIG. 5 shows dose response curves of lipid nanoparticle formulated human TTR specific sgRNAs on primary cyno hepatocytes.

[00227] FIG. 6 shows dose response curves of lipid nanoparticle formulated cyno TTR specific sgRNAs on primary cyno hepatocytes.

[00228] FIG. 7 shows percent editing (% edit) of TTR and reduction of secreted TTR following administration of the guide in HUH7 cells sequences provided on the x-axis. The values are normalized to the amount of alpha-1-antitrypsin (AAT) protein.

[00229] FIG. 8 shows western blot analysis of intracellular TTR following administration of targeted guides (listed in Table 1) in HUH7 cells.

[00230] FIG. 9 shows percentage liver editing of TTR observed following administration of LNP formulations to mice with humanized (G481-G499) or murine (G282) TTR. Note: the first three '0's in each Guide ID is omitted from the Figure, for example "G481" is "G000481" in Tables 2 and 3.

[00231] FIGS. 10A-B show serum TTR levels observed following the dosing regimens indicated on the horizontal axis as μg/ml (FIG. 10A) or percentage of TSS control (FIG. 10B). MPK = mg/kg throughout.

[00232] FIGS. 11A-B show serum TTR levels observed following the dosing regimens indicated on the horizontal axis for 1 mg/kg (FIG. 11A) or 0.5 mg/kg dosages (FIG. 11B). Data for a single 2 mg/kg dose is included as the right column in both panels.

[00233] FIGS. 12A-B show percentage liver editing observed following the dosing regimens indicated on the horizontal axis for 1 mg/kg (FIG. 12A) or 0.5 mg/kg dosages (FIG. 12B). FIG. 12C shows percentage liver editing observed following a single dose at 0.5, 1, or 2 mg/kg.

[00234] FIG. 13 shows percent liver editing observed following administration of LNP formulations to mice humanized with respect to the TTR gene. Note: the first three '0's in each Guide ID is omitted from the Figure, for example "G481" is "G000481" in Tables 2 and 3.

[00235] FIGS. 14A-B show that there is correlation between liver editing (FIG. 14A) and serum human TTR levels (FIG. 14B) following administration of LNP formulations to mice humanized with respect to the TTR gene. Note: the first three '0's in each Guide ID is omitted from the Figure, for example "G481" is "G000481" in Tables 2 and 3.

[00236] FIGS. 15A-B show that there is a dose response with respect to percent editing (FIG. 15A) and serum TTR levels (FIG. 15B) in wild type mice following administration of LNP formulations comprising guide G502, which is cross homologous between mouse and cyno.

[00237] FIG. 16 shows dose response curves of lipid nanoparticle formulated human TTR specific sgRNAs on primary cyno hepatocytes.

[00238] FIG. 17 shows dose response curves of lipid nanoparticle formulated cyno TTR specific sgRNAs on primary human hepatocytes.

[00239] FIG. 18 shows dose response curves of lipid nanoparticle formulated cyno TTR specific sgRNAs on primary cyno hepatocytes.

[00240] FIGS. 19A-D show serum TTR (% TSS; FIGs. 19A and 19C) and editing results following dosing of LNP formulations at the indicated ratios and amounts (FIGs. 19B and 19D).

[00241] FIG. 20 shows off-target analysis of certain single guide RNAs in Primary Human Hepatocytes (PHH) targeting TTR. In the graph, filled squares represent the identification of

the on-target cut site, while open circles represent the identification of potential off-target sites.

[00242] FIGS. 21A-B show percent editing on-target (ONT, FIG. 21A) and at two off-target sites (OT2 and OT4) in primary human hepatocytes following administration of lipid nanoparticle formulated G000480. FIG. 21B is a re-scaled version of the OT2, OT4, and negative control (Neg Cont) data in FIG. 21A.

[00243] FIGS. 22A-B show percent editing on-target (ONT, FIG. 22A) and at an off-target site (OT4) in primary human hepatocytes following administration of lipid nanoparticle formulated G000486. FIG. 22B is a re-scaled version of the OT4 and negative control (Neg Cont) data in FIG. 22A.

[00244] FIGS. 23A-B show percent editing (FIG. 23A) and number of insertion and deletion events at the TTR locus (FIG. 23B). FIG. 23A shows percent editing at the TTR locus in control and treatment (dosed with lipid nanoparticle formulated TTR specific sgRNA) groups. FIG. 23B shows the number of insertion and deletion events at the TTR locus when editing was observed in the treatment group of FIG. 23A.

[00245] FIGS. 24A-B show TTR levels in circulating serum (FIG. 24A) and cerebrospinal fluid (CSF) (FIG. 24B), respectively, in μg/mL for control and treatment (dosed with lipid nanoparticle formulated TTR specific sgRNA) groups. Treatment resulted in >99% knockdown of TTR levels in serum.

[00246] FIGS. 25A-D show immunohistochemistry images with staining for TTR in stomach (FIG. 25A), colon (FIG. 25B), sciatic nerve (FIG. 25C), and dorsal root ganglion (DRG) (FIG. 25D) from control and treatment (dosed with lipid nanoparticle formulated TTR specific sgRNA) mice. At right, bar graphs show reduction in TTR staining 8 weeks after treatment in treated mice as measured by percent occupied area for each tissue type.

[00247] FIGS. 26A-C show liver TTR editing (FIG. 26A) and serum TTR results (in μg/mL (FIG. 26B) and as percentage of TSS-treated control (FIG. 26C)), respectively, from humanized TTR mice dosed with LNP formulations across a range of doses with guides G000480, G000488, G000489 and G000502 and containing Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide.

[00248] FIGS. 27A-C show liver TTR editing (FIG. 27A) and serum TTR results (in μg/mL (FIG. 27B) and as percentage of TSS-treated control (FIG. 27C)), respectively, from humanized TTR mice dosed with LNP formulations across a range of doses with guides G000481, G000482, G000486 and G000499 and containing Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide.

[00249] FIGS. 28A-C show liver TTR editing (FIG. 28A) and serum TTR results (in μg/mL (FIG. 28B) and as percentage of TSS-treated control (FIG. 28C)), respectively, from humanized TTR mice dosed with LNP formulations across a range of doses with guides G000480, G000481, G000486, G000499 and G000502 and containing Cas9 mRNA (SEQ ID NO: 1) in a 1:2 ratio by weight to the guide.

[00250] FIG. 29 shows relative expression of TTR mRNA in primary human hepatocytes (PHH) after treatment with LNPs comprising Cas9 mRNA and a gRNA as indicated, as compared to negative (untreated) controls.

[00251] FIG. 30 shows relative expression of TTR mRNA in primary human hepatocytes (PHH) after treatment with LNPs comprising Cas9 mRNA and a gRNA as indicated, as compared to negative (untreated) controls.

DETAILED DESCRIPTION

[00252] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying drawings. While the invention will be described in conjunction with the illustrated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the invention as defined by the appended claims.

[00253] Before describing the present teachings in detail, it is to be understood that the disclosure is not limited to specific compositions or process steps, as such may vary. It should be noted that, as used in this specification and the appended claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a conjugate" includes a plurality of conjugates and reference to "a cell" includes a plurality of cells and the like.

[00254] Numeric ranges are inclusive of the numbers defining the range. Measured and measureable values are understood to be approximate, taking into account significant digits and the error associated with the measurement. Also, the use of "comprise", "comprises", "comprising", "contain", "contains", "containing", "include", "includes", and "including" are not intended to be limiting. It is to be understood that both the foregoing general description and detailed description are exemplary and explanatory only and are not restrictive of the teachings.

[00255] Unless specifically noted in the above specification, embodiments in the specification that recite "comprising" various components are also contemplated as

"consisting of" or "consisting essentially of" the recited components; embodiments in the specification that recite "consisting of" various components are also contemplated as "comprising" or "consisting essentially of" the recited components; and embodiments in the specification that recite "consisting essentially of" various components are also contemplated as "consisting of" or "comprising" the recited components (this interchangeability does not apply to the use of these terms in the claims). The term "or" is used in an inclusive sense, i.e., equivalent to "and/or," unless the context clearly indicates otherwise.

[00256] The section headings used herein are for organizational purposes only and are not to be construed as limiting the desired subject matter in any way. In the event that any material incorporated by reference contradicts any term defined in this specification or any other express content of this specification, this specification controls. While the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

I. Definitions

[00257] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[00258] "Polynucleotide" and "nucleic acid" are used herein to refer to a multimeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases or base analogs linked together along a backbone, including conventional RNA, DNA, mixed RNA-DNA, and polymers that are analogs thereof. A nucleic acid "backbone" can be made up of a variety of linkages, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds ("peptide nucleic acids" or PNA; PCT No. WO 95/32305), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of a nucleic acid can be ribose, deoxyribose, or similar compounds with substitutions, e.g., 2' methoxy or 2' halide substitutions. Nitrogenous bases can be conventional bases (A, G, C, T, U), analogs thereof (e.g., modified uridines such as 5-methoxyuridine, pseudouridine, or N1-methylpseudouridine, or others); inosine; derivatives of purines or pyrimidines (e.g., N^4-methyl deoxyguanosine, deaza- or aza-purines, deaza- or aza-pyrimidines, pyrimidine bases with substituent groups at the 5 or 6 position (e.g., 5-methylcytosine), purine bases with a substituent at the 2, 6, or 8 positions, 2-amino-6-methylaminopurine, O^6-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-

dimethylhydrazine-pyrimidines, and O^4-alkyl-pyrimidines; US Pat. No. 5,378,825 and PCT No. WO 93/13121). For general discussion see *The Biochemistry of the Nucleic Acids* 5-36, Adams et al., ed., 11th ed., 1992). Nucleic acids can include one or more "abasic" residues where the backbone includes no nitrogenous base for position(s) of the polymer (US Pat. No. 5,585,481). A nucleic acid can comprise only conventional RNA or DNA sugars, bases and linkages, or can include both conventional components and substitutions (e.g., conventional bases with 2' methoxy linkages, or polymers containing both conventional bases and one or more base analogs). Nucleic acid includes "locked nucleic acid" (LNA), an analogue containing one or more LNA nucleotide monomers with a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhance hybridization affinity toward complementary RNA and DNA sequences (Vester and Wengel, 2004, *Biochemistry* 43(42):13233-41). RNA and DNA have different sugar moieties and can differ by the presence of uracil or analogs thereof in RNA and thymine or analogs thereof in DNA.

[00259] "Guide RNA", "gRNA", and "guide" are used herein interchangeably to refer to either a crRNA (also known as CRISPR RNA), or the combination of a crRNA and a trRNA (also known as tracrRNA). The crRNA and trRNA may be associated as a single RNA molecule (single guide RNA, sgRNA) or in two separate RNA molecules (dual guide RNA, dgRNA). "Guide RNA" or "gRNA" refers to each type. The trRNA may be a naturally-occurring sequence, or a trRNA sequence with modifications or variations compared to naturally-occurring sequences.

[00260] As used herein, a "guide sequence" refers to a sequence within a guide RNA that is complementary to a target sequence and functions to direct a guide RNA to a target sequence for binding or modification (e.g., cleavage) by an RNA-guided DNA binding agent. A "guide sequence" may also be referred to as a "targeting sequence," or a "spacer sequence." A guide sequence can be 20 base pairs in length, e.g., in the case of Streptococcus pyogenes (i.e., Spy Cas9) and related Cas9 homologs/orthologs. Shorter or longer sequences can also be used as guides, e.g., 15-, 16-, 17-, 18-, 19-, 21-, 22-, 23-, 24-, or 25-nucleotides in length. For example, in some embodiments, the guide sequence comprises at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82. In some embodiments, the target sequence is in a gene or on a chromosome, for example, and is complementary to the guide sequence. In some embodiments, the degree of complementarity or identity between a guide sequence and its corresponding target sequence may be about 75%, 80%, 85%, 88%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. For example, in some embodiments, the guide sequence comprises a sequence with about 75%,

80%, 85%, 88%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82. In some embodiments, the guide sequence and the target region may be 100% complementary or identical. In other embodiments, the guide sequence and the target region may contain at least one mismatch. For example, the guide sequence and the target sequence may contain 1, 2, 3, or 4 mismatches, where the total length of the target sequence is at least 17, 18, 19, 20 or more base pairs. In some embodiments, the guide sequence and the target region may contain 1-4 mismatches where the guide sequence comprises at least 17, 18, 19, 20 or more nucleotides. In some embodiments, the guide sequence and the target region may contain 1, 2, 3, or 4 mismatches where the guide sequence comprises 20 nucleotides.

[00261] Target sequences for Cas proteins include both the positive and negative strands of genomic DNA (i.e., the sequence given and the sequence's reverse compliment), as a nucleic acid substrate for a Cas protein is a double stranded nucleic acid. Accordingly, where a guide sequence is said to be "complementary to a target sequence", it is to be understood that the guide sequence may direct a guide RNA to bind to the reverse complement of a target sequence. Thus, in some embodiments, where the guide sequence binds the reverse complement of a target sequence, the guide sequence is identical to certain nucleotides of the target sequence (e.g., the target sequence not including the PAM) except for the substitution of U for T in the guide sequence.

[00262] As used herein, an "RNA-guided DNA binding agent" means a polypeptide or complex of polypeptides having RNA and DNA binding activity, or a DNA-binding subunit of such a complex, wherein the DNA binding activity is sequence-specific and depends on the sequence of the RNA. Exemplary RNA-guided DNA binding agents include Cas cleavases/nickases and inactivated forms thereof ("dCas DNA binding agents"). "Cas nuclease", also called "Cas protein", as used herein, encompasses Cas cleavases, Cas nickases, and dCas DNA binding agents. Cas cleavases/nickases and dCas DNA binding agents include a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases. As used herein, a "Class 2 Cas nuclease" is a single-chain polypeptide with RNA-guided DNA binding activity, such as a Cas9 nuclease or a Cpf1 nuclease. Class 2 Cas nucleases include Class 2 Cas cleavases and Class 2 Cas nickases (e.g., H840A, D10A, or N863A variants), which further have RNA-guided DNA cleavases or nickase activity, and Class 2 dCas DNA binding agents, in which cleavase/nickase activity is inactivated. Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, C2c3, HF

Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9 (e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g, K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants) proteins and modifications thereof. Cpf1 protein, Zetsche et al., *Cell*, 163: 1-13 (2015), is homologous to Cas9, and contains a RuvC-like nuclease domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. *See*, *e.g.*, Zetsche, Tables S1 and S3. "Cas9" encompasses Spy Cas9, the variants of Cas9 listed herein, and equivalents thereof. See, *e.g.*, Makarova et al., *Nat Rev Microbiol*, 13(11): 722-36 (2015); Shmakov et al., *Molecular Cell,* 60:385-397 (2015).

[00263] "Modified uridine" is used herein to refer to a nucleoside other than thymidine with the same hydrogen bond acceptors as uridine and one or more structural differences from uridine. In some embodiments, a modified uridine is a substituted uridine, i.e., a uridine in which one or more non-proton substituents (e.g., alkoxy, such as methoxy) takes the place of a proton. In some embodiments, a modified uridine is pseudouridine. In some embodiments, a modified uridine is a substituted pseudouridine, i.e., a pseudouridine in which one or more non-proton substituents (e.g., alkyl, such as methyl) takes the place of a proton. In some embodiments, a modified uridine is any of a substituted uridine, pseudouridine, or a substituted pseudouridine.

[00264] "Uridine position" as used herein refers to a position in a polynucleotide occupied by a uridine or a modified uridine. Thus, for example, a polynucleotide in which "100% of the uridine positions are modified uridines" contains a modified uridine at every position that would be a uridine in a conventional RNA (where all bases are standard A, U, C, or G bases) of the same sequence. Unless otherwise indicated, a U in a polynucleotide sequence of a sequence table or sequence listing in, or accompanying, this disclosure can be a uridine or a modified uridine.

[00265] As used herein, a first sequence is considered to "comprise a sequence with at least X% identity to" a second sequence if an alignment of the first sequence to the second sequence shows that X% or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAGA comprises a sequence with 100% identity to the sequence AAG because an alignment would give 100% identity in that there are matches to all three positions of the second sequence. The differences between RNA and DNA (generally the exchange of uridine for thymidine or vice versa) and the presence of nucleoside analogs such as modified uridines do not contribute to differences in identity or complementarity among polynucleotides as long as the relevant nucleotides (such as thymidine, uridine, or modified uridine) have the same complement (e.g., adenosine for all of

thymidine, uridine, or modified uridine; another example is cytosine and 5-methylcytosine, both of which have guanosine or modified guanosine as a complement). Thus, for example, the sequence 5'-AXG where X is any modified uridine, such as pseudouridine, N1-methyl pseudouridine, or 5-methoxyuridine, is considered 100% identical to AUG in that both are perfectly complementary to the same sequence (5'-CAU). Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the art. One skilled in the art will understand what choice of algorithm and parameter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server is generally appropriate.

[00266] "mRNA" is used herein to refer to a polynucleotide that is not DNA and comprises an open reading frame that can be translated into a polypeptide (i.e., can serve as a substrate for translation by a ribosome and amino-acylated tRNAs). mRNA can comprise a phosphate-sugar backbone including ribose residues or analogs thereof, e.g., 2'-methoxy ribose residues. In some embodiments, the sugars of an mRNA phosphate-sugar backbone consist essentially of ribose residues, 2'-methoxy ribose residues, or a combination thereof. In general, mRNAs do not contain a substantial quantity of thymidine residues (e.g., 0 residues or fewer than 30, 20, 10, 5, 4, 3, or 2 thymidine residues; or less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1% thymidine content). An mRNA can contain modified uridines at some or all of its uridine positions.

[00267] As used herein, the "minimum uridine content" of a given open reading frame (ORF) is the uridine content of an ORF that (a) uses a minimal uridine codon at every position and (b) encodes the same amino acid sequence as the given ORF. The minimal uridine codon(s) for a given amino acid is the codon(s) with the fewest uridines (usually 0 or 1 except for a codon for phenylalanine, where the minimal uridine codon has 2 uridines). Modified uridine residues are considered equivalent to uridines for the purpose of evaluating minimum uridine content.

[00268] As used herein, the "minimum uridine dinucleotide content" of a given open reading frame (ORF) is the lowest possible uridine dinucleotide (UU) content of an ORF that (a) uses a minimal uridine codon (as discussed above) at every position and (b) encodes the same amino acid sequence as the given ORF. The uridine dinucleotide (UU) content can be expressed in absolute terms as the enumeration of UU dinucleotides in an ORF or on a rate basis as the percentage of positions occupied by the uridines of uridine dinucleotides (for

example, AUUAU would have a uridine dinucleotide content of 40% because 2 of 5 positions are occupied by the uridines of a uridine dinucleotide). Modified uridine residues are considered equivalent to uridines for the purpose of evaluating minimum uridine dinucleotide content.

[00269] As used herein, "TTR" refers to transthyretin, which is the gene product of a *TTR* gene.

[00270] As used herein, "amyloid" refers to abnormal aggregates of proteins or peptides that are normally soluble. Amyloids are insoluble, and amyloids can create proteinaceous deposits in organs and tissues. Proteins or peptides in amyloids may be misfolded into a form that allows many copies of the protein to stick together to form fibrils. While some forms of amyloid may have normal functions in the human body, "amyloids" as used herein refers to abnormal or pathologic aggregates of protein. Amyloids may comprise a single protein or peptide, such as TTR, or they may comprise multiple proteins or peptides, such as TTR and additional proteins.

[00271] As used herein, "amyloid fibrils" refers to insoluble fibers of amyloid that are resistant to degradation. Amyloid fibrils can produce symptoms based on the specific protein or peptide and the tissue and cell type in which it has aggregated.

[00272] As used herein, "amyloidosis" refers to a disease characterized by symptoms caused by deposition of amyloid or amyloid fibrils. Amyloidosis can affect numerous organs including the heart, kidney, liver, spleen, nervous system, and digestive track.

[00273] As used herein, "ATTR," "TTR-related amyloidosis," "TTR amyloidosis," "ATTR amyloidosis," or "amyloidosis associated with TTR" refers to amyloidosis associated with deposition of TTR.

[00274] As used herein, "familial amyloid cardiomyopathy" or "FAC" refers to a hereditary transthyretin amyloidosis (ATTR) characterized primarily by restrictive cardiomyopathy. Congestive heart failure is common in FAC. Average age of onset is approximately 60-70 years of age, with an estimated life expectancy of 4-5 years after diagnosis.

[00275] As used herein, "familial amyloid polyneuropathy" or "FAP" refers to a hereditary transthyretin amyloidosis (ATTR) characterized primarily by sensorimotor neuropathy. Autonomic neuropathy is common in FAP. While neuropathy is a primary feature, symptoms of FAP may also include cachexia, renal failure, and cardiac disease. Average age of onset of FAP is approximately 30-50 years of age, with an estimated life expectancy of 5-15 after diagnosis.

[00276] As used herein, "wild-type ATTR" and "ATTRwt" refer to ATTR not associated with a pathological TTR mutation such as T60A, V30M, V30A, V30G, V30L, V122I, V122A, or V122(-). ATTRwt has also been referred to as senile systemic amyloidosis. Onset typically occurs in men aged 60 or higher with the most common symptoms being congestive heart failure and abnormal heart rhythm such as atrial fibrillation. Additional symptoms include consequences of poor heart function such as shortness of breath, fatigue, dizziness, swelling (especially in the legs), nausea, angina, disrupted sleep, and weight loss. A history of carpal tunnel syndrome indicates increased risk for ATTRwt and may in some cases be indicative of early-stage disease. ATTRwt generally leads to decreasing heart function over time but can have a better prognosis than hereditary ATTR because wild-type TTR deposits accumulate more slowly. Existing treatments are similar to other forms of ATTR (other than liver transplantation) and are generally directed to supporting or improving heart function, ranging from diuretics and limited fluid and salt intake to anticoagulants, and in severe cases, heart transplants. Nonetheless, like FAC, ATTRwt can result in death from heart failure, sometimes within 3-5 years of diagnosis.

[00277] Guide sequences useful in the guide RNA compositions and methods described herein are shown in Table 1 and throughout the application.

[00278] As used herein, "hereditary ATTR" refers to ATTR that is associated with a mutation in the sequence of the *TTR* gene. Known mutations in the *TTR* gene associated with ATTR include those resulting in TTR with substitutions of T60A, V30M, V30A, V30G, V30L, V122I, V122A, or V122(-).

[00279] As used herein, "indels" refer to insertion/deletion mutations consisting of a number of nucleotides that are either inserted or deleted at the site of double-stranded breaks (DSBs) in a target nucleic acid.

[00280] As used herein, "knockdown" refers to a decrease in expression of a particular gene product (e.g., protein, mRNA, or both). Knockdown of a protein can be measured either by detecting protein secreted by tissue or population of cells (e.g., in serum or cell media) or by detecting total cellular amount of the protein from a tissue or cell population of interest. Methods for measuring knockdown of mRNA are known, and include sequencing of mRNA isolated from a tissue or cell population of interest. In some embodiments, "knockdown" may refer to some loss of expression of a particular gene product, for example a decrease in the amount of of mRNA transcribed or a decrease in the amount of protein expressed or secreted by a population of cells (including *in vivo* populations such as those found in tissues).

[00281] As used herein, "knockout" refers to a loss of expression of a particular protein in a cell. Knockout can be measured either by detecting the amount of protein secretion from a tissue or population of cells (e.g., in serum or cell media) or by detecting total cellular amount of a protein a tissue or a population of cells. In some embodiments, the methods of the disclosure "knockout" TTR in one or more cells (e.g., in a population of cells including *in vivo* populations such as those found in tissues). In some embodiments, a knockout is not the formation of mutant TTR protein, for example, created by indels, but rather the complete loss of expression of TTR protein in a cell.

[00282] As used herein, "mutant TTR" refers to a gene product of *TTR* (i.e., the TTR protein) having a change in the amino acid sequence of TTR compared to the wildtype amino acid sequence of TTR. The human wild-type TTR sequence is available at NCBI Gene ID: 7276; Ensembl: Ensembl: ENSG00000118271. Mutants forms of TTR associated with ATTR, e.g., in humans, include T60A, V30M, V30A, V30G, V30L, V122I, V122A, or V122(-).

[00283] As used herein, "mutant *TTR*" or "mutant *TTR* allele" refers to a *TTR* sequence having a change in the nucleotide sequence of *TTR* compared to the wildtype sequence (NCBI Gene ID: 7276; Ensembl: ENSG00000118271).

[00284] As used herein, "ribonucleoprotein" (RNP) or "RNP complex" refers to a guide RNA together with an RNA-guided DNA binding agent, such as a Cas nuclease, e.g., a Cas cleavase, Cas nickase, or dCas DNA binding agent (e.g., Cas9). In some embodiments, the guide RNA guides the RNA-guided DNA binding agent such as Cas9 to a target sequence, and the guide RNA hybridizes with and the agent binds to the target sequence; in cases where the agent is a cleavase or nickase, binding can be followed by cleaving or nicking.

[00285] As used herein, a "target sequence" refers to a sequence of nucleic acid in a target gene that has complementarity to the guide sequence of the gRNA. The interaction of the target sequence and the guide sequence directs an RNA-guided DNA binding agent to bind, and potentially nick or cleave (depending on the activity of the agent), within the target sequence.

[00286] As used herein, "treatment" refers to any administration or application of a therapeutic for disease or disorder in a subject, and includes inhibiting the disease, arresting its development, relieving one or more symptoms of the disease, curing the disease, or preventing reoccurrence of one or more symptoms of the disease. For example, treatment of ATTR may comprise alleviating symptoms of ATTR.

[00287] "Modified uridine" is used herein to refer to a nucleoside other than thymidine with the same hydrogen bond acceptors as uridine and one or more structural differences from uridine. In some embodiments, a modified uridine is a substituted uridine, i.e., a uridine in which one or more non-proton substituents (e.g., alkoxy, such as methoxy) takes the place of a proton. In some embodiments, a modified uridine is pseudouridine. In some embodiments, a modified uridine is a substituted pseudouridine, i.e., a pseudouridine in which one or more non-proton substituents (e.g., alkyl, such as methyl) takes the place of a proton, e.g., N1-methyl pseudouridine. In some embodiments, a modified uridine is any of a substituted uridine, pseudouridine, or a substituted pseudouridine.

[00288] As used herein, a first sequence is considered to "comprise a sequence with at least X% identity to" a second sequence if an alignment of the first sequence to the second sequence shows that X% or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAGA comprises a sequence with 100% identity to the sequence AAG because an alignment would give 100% identity in that there are matches to all three positions of the second sequence. The differences between RNA and DNA (generally the exchange of uridine for thymidine or vice versa) and the presence of nucleoside analogs such as modified uridines do not contribute to differences in identity or complementarity among polynucleotides as long as the relevant nucleotides (such as thymidine, uridine, or modified uridine) have the same complement (e.g., adenosine for all of thymidine, uridine, or modified uridine; another example is cytosine and 5-methylcytosine, both of which have guanosine as a complement). Thus, for example, the sequence 5'-AXG where X is any modified uridine, such as pseudouridine, N1-methyl pseudouridine, or 5-methoxyuridine, is considered 100% identical to AUG in that both are perfectly complementary to the same sequence (5'-CAU). Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the art. One skilled in the art will understand what choice of algorithm and parameter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server are generally appropriate.

[00289] The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined.

II. Compositions

A. Compositions Comprising Guide RNA (gRNAs)

[00290] Provided herein are compositions useful for editing the *TTR* gene, e.g., using a guide RNA with an RNA-guided DNA binding agent (e.g., a CRISPR/Cas system). The compositions may be administered to subjects having wild-type or non-wild type *TTR* gene sequences, such as, for example, subjects with ATTR, which may be ATTR wt or a hereditary or familial form of ATTR. Guide sequences targeting the *TTR* gene are shown in Table 1 at SEQ ID Nos: 5-82.

Table 1: *TTR* targeted guide sequences, nomenclature, chromosomal coordinates, and sequence.

SEQ ID No.	Guide ID	Description	Species	Chromosomal Location	Guide Sequences*
5	CR003335	TTR (Exon 1)	Human	chr18:31591917-31591937	CUGCUCCUCCUCUGCCUUGC
6	CR003336	TTR (Exon 1)	Human	chr18:31591922-31591942	CCUCCUCUGCCUUGCUGGAC
7	CR003337	TTR (Exon 1)	Human	chr18:31591925-31591945	CCAGUCCAGCAAGGCAGAGG
8	CR003338	TTR (Exon 1)	Human	chr18:31591928-31591948	AUACCAGUCCAGCAAGGCAG
9	CR003339	TTR (Exon 1)	Human	chr18:31591934-31591954	ACACAAAUACCAGUCCAGCA
10	CR003340	TTR (Exon 1)	Human	chr18:31591937-31591957	UGGACUGGUAUUUGUGUCUG
11	CR003341	TTR (Exon 1)	Human	chr18:31591941-31591961	CUGGUAUUUGUGUCUGAGGC
12	CR003342	TTR (Exon 2)	Human	chr18:31592880-31592900	CUUCUCUACACCCAGGGCAC
13	CR003343	TTR (Exon 2)	Human	chr18:31592902-31592922	CAGAGGACACUUGGAUUCAC
14	CR003344	TTR (Exon 2)	Human	chr18:31592911-31592931	UUUGACCAUCAGAGGACACU
15	CR003345	TTR (Exon 2)	Human	chr18:31592919-31592939	UCUAGAACUUUGACCAUCAG
16	CR003346	TTR (Exon 2)	Human	chr18:31592928-31592948	AAAGUUCUAGAUGCUGUCCG
17	CR003347	TTR (Exon 2)	Human	chr18:31592948-31592968	CAUUGAUGGCAGGACUGCCU
18	CR003348	TTR (Exon 2)	Human	chr18:31592948-31592968	AGGCAGUCCUGCCAUCAAUG
19	CR003349	TTR (Exon 2)	Human	chr18:31592958-31592978	UGCACGGCCACAUUGAUGGC
20	CR003350	TTR (Exon 2)	Human	chr18:31592962-31592982	CACAUGCACGGCCACAUUGA
21	CR003351	TTR (Exon 2)	Human	chr18:31592974-31592994	AGCCUUUCUGAACACAUGCA
22	CR003352	TTR (Exon 2)	Human	chr18:31592986-31593006	GAAAGGCUGCUGAUGACACC

23	CR003353	TTR (Exon 2)	Human	chr18:31592987-31593007	AAAGGCUGCUGAUGACACCU
24	CR003354	TTR (Exon 2)	Human	chr18:31593003-31593023	ACCUGGGAGCCAUUUGCCUC
25	CR003355	TTR (Exon 2)	Human	chr18:31593007-31593027	CCCAGAGGCAAAUGGCUCCC
26	CR003356	TTR (Exon 2)	Human	chr18:31593015-31593035	GCAACUUACCCAGAGGCAAA
27	CR003357	TTR (Exon 2)	Human	chr18:31593022-31593042	UUCUUUGGCAACUUACCCAG
28	CR003358	TTR (Exon 3)	Human	chr18:31595127-31595147	AUGCAGCUCUCCAGACUCAC
29	CR003359	TTR (Exon 3)	Human	chr18:31595126-31595146	AGUGAGUCUGGAGAGCUGCA
30	CR003360	TTR (Exon 3)	Human	chr18:31595127-31595147	GUGAGUCUGGAGAGCUGCAU
31	CR003361	TTR (Exon 3)	Human	chr18:31595140-31595160	GCUGCAUGGGCUCACAACUG
32	CR003362	TTR (Exon 3)	Human	chr18:31595143-31595163	GCAUGGGCUCACAACUGAGG
33	CR003363	TTR (Exon 3)	Human	chr18:31595156-31595176	ACUGAGGAGGAAUUUGUAGA
34	CR003364	TTR (Exon 3)	Human	chr18:31595157-31595177	CUGAGGAGGAAUUUGUAGAA
35	CR003365	TTR (Exon 3)	Human	chr18:31595170-31595190	UGUAGAAGGGAUAUACAAAG
36	CR003366	TTR (Exon 3)	Human	chr18:31595193-31595213	AAAUAGACACCAAAUCUUAC
37	CR003367	TTR (Exon 3)	Human	chr18:31595197-31595217	AGACACCAAAUCUUACUGGA
38	CR003368	TTR (Exon 3)	Human	chr18:31595205-31595225	AAGUGCCUUCCAGUAAGAUU
39	CR003369	TTR (Exon 3)	Human	chr18:31595235-31595255	CUCUGCAUGCUCAUGGAAUG
40	CR003370	TTR (Exon 3)	Human	chr18:31595236-31595256	CCUCUGCAUGCUCAUGGAAU
41	CR003371	TTR (Exon 3)	Human	chr18:31595237-31595257	ACCUCUGCAUGCUCAUGGAA
42	CR003372	TTR (Exon 3)	Human	chr18:31595242-31595262	UACUCACCUCUGCAUGCUCA
43	CR003373	TTR (Exon 4)	Human	chr18:31598570-31598590	GUAUUCACAGCCAACGACUC
44	CR003374	TTR (Exon 4)	Human	chr18:31598583-31598603	GCGGCGGGGGCCGGAGUCGU
45	CR003375	TTR (Exon 4)	Human	chr18:31598592-31598612	AAUGGUGUAGCGGCGGGGGC
46	CR003376	TTR (Exon 4)	Human	chr18:31598596-31598616	CGGCAAUGGUGUAGCGGCGG
47	CR003377	TTR (Exon 4)	Human	chr18:31598597-31598617	GCGGCAAUGGUGUAGCGGCG
48	CR003378	TTR (Exon 4)	Human	chr18:31598598-31598618	GGCGGCAAUGGUGUAGCGGC
49	CR003379	TTR (Exon 4)	Human	chr18:31598599-31598619	GGGCGGCAAUGGUGUAGCGG
50	CR003380	TTR (Exon 4)	Human	chr18:31598602-31598622	GCAGGGCGGCAAUGGUGUAG
51	CR003381	TTR (Exon 4)	Human	chr18:31598610-31598630	GGGGCUCAGCAGGGCGGCAA
52	CR003382	TTR (Exon 4)	Human	chr18:31598616-31598636	GGAGUAGGGGCUCAGCAGGG

53	CR003383	TTR (Exon 4)	Human	chr18:31598619-31598639	AUAGGAGUAGGGGCUCAGCA
54	CR003384	TTR (Exon 4)	Human	chr18:31598620-31598640	AAUAGGAGUAGGGGCUCAGC
55	CR003385	TTR (Exon 4)	Human	chr18:31598626-31598646	CCCCUACUCCUAUUCCACCA
56	CR003386	TTR (Exon 4)	Human	chr18:31598629-31598649	CCGUGGUGGAAUAGGAGUAG
57	CR003387	TTR (Exon 4)	Human	chr18:31598630-31598650	GCCGUGGUGGAAUAGGAGUA
58	CR003388	TTR (Exon 4)	Human	chr18:31598637-31598657	GACGACAGCCGUGGUGGAAU
59	CR003389	TTR (Exon 4)	Human	chr18:31598643-31598663	AUUGGUGACGACAGCCGUGG
60	CR003390	TTR (Exon 4)	Human	chr18:31598646-31598666	GGGAUUGGUGACGACAGCCG
61	CR003391	TTR (Exon 4)	Human	chr18:31598647-31598667	GGCUGUCGUCACCAAUCCCA
62	CR003392	TTR (Exon 4)	Human	chr18:31598661-31598681	AGUCCCUCAUUCCUUGGGAU
63	CR005298	TTR (Exon 1)	Human	chr18:31591883-31591903	UCCACUCAUUCUUGGCAGGA
64	CR005299	TTR (Exon 4)	Human	chr18:31598631-31598651	AGCCGUGGUGGAAUAGGAGU
65	CR005300	TTR (Exon 1)	Human	chr18:31591967-31591987	UCACAGAAACACUCACCGUA
66	CR005301	TTR (Exon 1)	Human	chr18:31591968-31591988	GUCACAGAAACACUCACCGU
67	CR005302	TTR (Exon 2)	Human	chr18:31592874-31592894	ACGUGUCUUCUCUACACCCA
68	CR005303	TTR (Exon 2)	Human	chr18:31592903-31592923	UGAAUCCAAGUGUCCUCUGA
69	CR005304	TTR (Exon 2)	Human	chr18:31592969-31592989	GGCCGUGCAUGUGUUCAGAA
70	CR005305	TTR (Exon 3)	Human	chr18:31595114-31595134	UAUAGGAAAACCAGUGAGUC
71	CR005306	TTR (Exon 3)	Human	chr18:31595204-31595224	AAAUCUUACUGGAAGGCACU
72	CR005307	TTR (Exon 4)	Human	chr18:31598548-31598568	UGUCUGUCUUCUCUCAUAGG
73	CR000689	TTR	Cyno	chr18:50681533-50681553	ACACAAAUACCAGUCCAGCG
74	CR005364	TTR	Cyno	chr18:50680481-50680501	AAAGGCUGCUGAUGAGACCU
75	CR005365	TTR	Cyno	chr18:50680520-50680540	CAUUGACAGCAGGACUGCCU
76	CR005366	TTR	Cyno	chr18:50681539-50681559	AUACCAGUCCAGCGAGGCAG
77	CR005367	TTR	Cyno	chr18:50681542-50681562	CCAGUCCAGCGAGGCAGAGG
78	CR005368	TTR	Cyno	chr18:50681545-50681565	CCUCCUCUGCCUCGCUGGAC
79	CR005369	TTR	Cyno	chr18:50680540-50680560	AAAGUUCUAGAUGCCGUCCG
80	CR005370	TTR	Cyno	chr18:50680594-50680614	ACUUGUCUUCUCUAUACCCA
81	CR005371	TTR	Cyno	chr18:50678216-50678236	AAGUGACUUCCAGUAAGAUU
82	CR005372	TTR	Cyno	chr18:50680482-50680502	AAAAGGCUGCUGAUGAGACC

[00291] Each of the Guide Sequences above may further comprise additional nucleotides to form a crRNA, e.g., with the following exemplary nucleotide sequence following the Guide Sequence at its 3' end: GUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 126). In the case of a sgRNA, the above Guide Sequences may further comprise additional nucleotides to form a sgRNA, e.g., with the following exemplary nucleotide sequence following the 3' end of the Guide Sequence: GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 125) in 5' to 3' orientation.

[00292] In some embodiments, the sgRNA is modified. In some embodiments, the sgRNA comprises the modification pattern shown below in SEQ ID NO: 3, where N is any natural or non-natural nucleotide, and where the totality of the N's comprise a guide sequence as described herein and the modified sgRNA comprises the following sequence: mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmU mAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAm AmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU (SEQ ID NO: 3), where "N" may be any natural or non-natural nucleotide. For example, encompassed herein is SEQ ID NO: 3, where the N's are replaced with any of the guide sequences disclosed herein. The modifications remain as shown in SEQ ID NO: 3 despite the substitution of N's for the nucleotides of a guide. That is, although the nucleotides of the guide replace the "N's", the first three nucleotides are 2'OMe modified and there are phosphorothioate linkages between the first and second nucleotides, the second and third nucleotides and the third and fourth nucleotides.

[00293] In some embodiments, any one of the sequences recited in Table 2 is encompassed.

Table 2: TTR targeted sgRNA sequences

SEQ ID No.	Guide ID	Target and Description	Species	Sequence
87	G000480	TTR sgRNA modified sequence	Human	mA*mA*mA*GGCUGCUGAUGACACCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

88	G000481	TTR sgRNA modified sequence	Human	mU*mC*mU*AGAACUUUGACCAUCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
89	G000482	TTR sgRNA modified sequence	Human	mU*mG*mU*AGAAGGGAUAUACAAAGG UUUUAGAmGmCmUmAmGmAmAmAmUm AmGmCAAGUUAAAAUAAGGCUAGUCCG UUAUCAmAmCmUmUmGmAmAmAmAmA mGmUmGmGmCmAmCmCmGmAmGmUmC mGmGmUmGmCmU*mU*mU*mU
90	G000483	TTR sgRNA modified sequence	Human	mU*mC*mC*ACUCAUUCUUGGCAGGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
91	G000484	TTR sgRNA modified sequence	Human	mA*mG*mA*CACCAAAUCUUACUGGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
92	G000485	TTR sgRNA modified sequence	Human	mC*mC*mU*CCUCUGCCUUGCUGGACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
93	G000486	TTR sgRNA modified sequence	Human	mA*mC*mA*CAAAUACCAGUCCAGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
94	G000487	TTR sgRNA modified sequence	Human	mU*mU*mC*UUUGGCAACUUACCCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
95	G000488	TTR sgRNA modified sequence	Human	mA*mA*mA*GUUCUAGAUGCUGUCCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

96	G000489	TTR sgRNA modified sequence	Human	mU*mU*mU*GACCAUCAGAGGACACUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
97	G000490	TTR sgRNA modified sequence	Human	mA*mA*mA*UAGACACCAAAUCUUACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
98	G000491	TTR sgRNA modified sequence	Human	mA*mU*mA*CCAGUCCAGCAAGGCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
99	G000492	TTR sgRNA modified sequence	Human	mC*mU*mU*CUCUACACCCAGGGCACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
100	G000493	TTR sgRNA modified sequence	Human	mA*mA*mG*UGCCUUCCAGUAAGAUUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
101	G000494	TTR sgRNA modified sequence	Human	mG*mU*mG*AGUCUGGAGAGCUGCAUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
102	G000495	TTR sgRNA modified sequence	Human	mC*mA*mG*AGGACACUUGGAUUCACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
103	G000496	TTR sgRNA modified sequence	Human	mG*mG*mC*CGUGCAUGUGUUCAGAAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

104	G000497	TTR sgRNA modified sequence	Human	mC*mU*mG*CUCCUCCUCUGCCUUGCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
105	G000498	TTR sgRNA modified sequence	Human	mA*mG*mU*GAGUCUGGAGAGCUGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
106	G000499	TTR sgRNA modified sequence	Human	mU*mG*mA*AUCCAAGUGUCCUCUGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
107	G000500	TTR sgRNA modified sequence	Human	mC*mC*mA*GUCCAGCAAGGCAGAGGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
108	G000501	TTR sgRNA modified sequence	Human	mU*mC*mA*CAGAAACACUCACCGUAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
109	G000567	TTR sgRNA modified sequence	Human	mG*mA*mA*AGGCUGCUGAUGACACCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
110	G000568	TTR sgRNA modified sequence	Human	mG*mG*mC*UGUCGUCACCAAUCCCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
111	G000570	TTR sgRNA modified sequence	Human	mC*mA*mU*UGAUGGCAGGACUGCCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

112	G000571	TTR sgRNA modified sequence	Human	mG*mU*mC*ACAGAAACACUCACCGUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
113	G000572	TTR sgRNA modified sequence	Human	mC*mC*mC*CUACUCCUAUUCCACCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
114	G000502	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mC*mA*CAAAUACCAGUCCAGCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
115	G000503	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*AGGCUGCUGAUGAGACCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
116	G000504	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*GGCUGCUGAUGAGACCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
117	G000505	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mA*mU*UGACAGCAGGACUGCCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
118	G000506	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mU*mA*CCAGUCCAGCGAGGCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
119	G000507	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mC*mA*GUCCAGCGAGGCAGAGGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

120	G000508	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mC*mU*CCUCUGCCUCGCUGGACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
121	G000509	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*GUUCUAGAUGCCGUCCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
122	G000510	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mC*mU*UGUCUUCUCUAUACCCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
123	G000511	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mG*UGACUUCCAGUAAGAUUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
124	G000282	TTR	Mouse	mU*mU*mA*CAGCCACGUCUACAGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

* = PS linkage; 'm' = 2'-O-Me nucleotide

[00294] An alignment mapping of the Guide IDs with the corresponding sgRNA IDs as well as homology to the cyno genome and cyno matched guide IDs are provided in Table 3.

Table 3: TTR targeted guide sequence ID mapping and Cyno Homology

Description	Human Dual Guide ID	Human Single Guide ID	Number Mismatches to Cyno Genome	Cyno Matched dgRNA ID	Cyno Matched sgRNA ID
TTR	CR003335	G000497	1		
TTR	CR003336	G000485	1	CR005368	G000508
TTR	CR003337	G000500	1	CR005367	G000507
TTR	CR003338	G000491	1	CR005366	G000506
TTR	CR003339	G000486	1	CR000689	G000502
TTR	CR003340		0		
TTR	CR003341		0		
TTR	CR003342	G000492	no PAM in cyno		

TTR	CR003343	G000495	no PAM in cyno		
TTR	CR003344	G000489	0		
TTR	CR003345	G000481	0		
TTR	CR003346	G000488	1	CR005369	G000509
TTR	CR003347	G000570	2	CR005365	G000505
TTR	CR003348		2		
TTR	CR003349		>3		
TTR	CR003350		no PAM in cyno		
TTR	CR003351		no PAM in cyno		
TTR	CR003352	G000567	2	CR005372	G000503
TTR	CR003353	G000480	1	CR005364	G000504
TTR	CR003354		1		
TTR	CR003355		1		
TTR	CR003356		3		
TTR	CR003357	G000487	>3		
TTR	CR003358		0		
TTR	CR003359	G000498	0		
TTR	CR003360	G000494	0		
TTR	CR003361		0		
TTR	CR003362		0		
TTR	CR003363		0		
TTR	CR003364		0		
TTR	CR003365	G000482	0		
TTR	CR003366	G000490	0		
TTR	CR003367	G000484	no PAM in cyno		
TTR	CR003368	G000493	1	CR005371	G000511
TTR	CR003369		0		
TTR	CR003370		0		
TTR	CR003371		0		
TTR	CR003372		0		
TTR	CR003373		1		
TTR	CR003374		2		
TTR	CR003375		2		
TTR	CR003376		2		
TTR	CR003377		2		
TTR	CR003378		2		
TTR	CR003379		2		
TTR	CR003380		1		
TTR	CR003381		1		
TTR	CR003382		0		
TTR	CR003383		0		
TTR	CR003384		0		
TTR	CR003385	G000572	0		
TTR	CR003386		0		
TTR	CR003387		0		
TTR	CR003388		0		

TTR	CR003389	G000569	0		
TTR	CR003390		0		
TTR	CR003391	G000568	0		
TTR	CR003392		0		
TTR	CR005298	G000483	1		
TTR	CR005299		0		
TTR	CR005300	G000501	no PAM in cyno		
TTR	CR005301	G000571	0		
TTR	CR005302		2	CR005370	G000510
TTR	CR005303	G000499	0		
TTR	CR005304	G000496	>3		
TTR	CR005305		0		
TTR	CR005306		1		
TTR	CR005307		0		

[00295] In some embodiments, the invention provides a composition comprising one or more guide RNA (gRNA) comprising guide sequences that direct an RNA-guided DNA binding agent, which can be a nuclease (e.g., a Cas nuclease such as Cas9), to a target DNA sequence in *TTR*. The gRNA may comprise a crRNA comprising a guide sequence shown in Table 1. The gRNA may comprise a crRNA comprising 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the gRNA comprises a crRNA comprising a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to at least 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the gRNA comprises a crRNA comprising a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a guide sequence shown in Table 1. The gRNA may further comprise a trRNA. In each composition and method embodiment described herein, the crRNA and trRNA may be associated as a single RNA (sgRNA), or may be on separate RNAs (dgRNA). In the context of sgRNAs, the crRNA and trRNA components may be covalently linked, e.g., via a phosphodiester bond or other covalent bond.

[00296] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise two RNA molecules as a "dual guide RNA" or "dgRNA". The dgRNA comprises a first RNA molecule comprising a crRNA comprising, e.g., a guide sequence shown in Table 1, and a second RNA molecule comprising a trRNA. The first and second RNA molecules may not be covalently linked, but may form a RNA duplex via the base pairing between portions of the crRNA and the trRNA.

[00297] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise a single RNA molecule as a "single guide RNA" or "sgRNA". The sgRNA may comprise a crRNA (or a portion thereof) comprising a guide sequence shown in Table 1 covalently linked to a trRNA. The sgRNA may comprise 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the crRNA and the trRNA are covalently linked via a linker. In some embodiments, the sgRNA forms a stem-loop structure via the base pairing between portions of the crRNA and the trRNA. In some embodiments, the crRNA and the trRNA are covalently linked via one or more bonds that are not a phosphodiester bond.

[00298] In some embodiments, the trRNA may comprise all or a portion of a trRNA sequence derived from a naturally-occurring CRISPR/Cas system. In some embodiments, the trRNA comprises a truncated or modified wild type trRNA. The length of the trRNA depends on the CRISPR/Cas system used. In some embodiments, the trRNA comprises or consists of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or more than 100 nucleotides. In some embodiments, the trRNA may comprise certain secondary structures, such as, for example, one or more hairpin or stem-loop structures, or one or more bulge structures.

[00299] In some embodiments, the invention provides a composition comprising one or more guide RNAs comprising a guide sequence of any one of SEQ ID NOs: 5-82.

[00300] In one aspect, the invention provides a composition comprising a gRNA that comprises a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 5-82.

[00301] In other embodiments, the composition comprises at least one, e.g., at least two gRNA's comprising guide sequences selected from any two or more of the guide sequences of SEQ ID NOs: 5-82. In some embodiments, the composition comprises at least two gRNA's that each comprise a guide sequence at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 5-82.

[00302] In some embodiments, the gRNA is a sgRNA comprising any one of the sequences shown in Table 2 (SEQ ID Nos. 87-124). In some embodiments, the gRNA is a sgRNA comprising any one of the sequences shown in Table 2 (SEQ ID Nos. 87-124, but without the modifications as shown (i.e., unmodified SEQ ID Nos. 87-124). In some embodiments, the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID Nos. 87-124. In some embodiments, the sgRNA comprises a sequence that is at least 99%, 98%, 97%,

96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID Nos. 87-124, but without the modifications as shown (i.e., unmodified SEQ ID Nos. 87-124). In some embodiments, the sgRNA comprises any one of the guide sequences shown in Table 1 in place of the guide sequences shown in the sgRNA sequences of Table 2 at SEQ ID Nos: 87-124, with or without the modifications.

[00303] The guide RNA compositions of the present invention are designed to recognize (e.g., hybridize to) a target sequence in the *TTR* gene. For example, the *TTR* target sequence may be recognized and cleaved by a provided Cas cleavase comprising a guide RNA. In some embodiments, an RNA-guided DNA binding agent, such as a Cas cleavase, may be directed by a guide RNA to a target sequence of the *TTR* gene, where the guide sequence of the guide RNA hybridizes with the target sequence and the RNA-guided DNA binding agent, such as a Cas cleavase, cleaves the target sequence.

[00304] In some embodiments, the selection of the one or more guide RNAs is determined based on target sequences within the *TTR* gene.

[00305] Without being bound by any particular theory, mutations (e.g., frameshift mutations resulting from indels occurring as a result of a nuclease-mediated DSB) in certain regions of the gene may be less tolerable than mutations in other regions of the gene, thus the location of a DSB is an important factor in the amount or type of protein knockdown that may result. In some embodiments, a gRNA complementary or having complementarity to a target sequence within *TTR* is used to direct the RNA-guided DNA binding agent to a particular location in the *TTR* gene. In some embodiments, gRNAs are designed to have guide sequences that are complementary or have complementarity to target sequences in exon 1, exon 2, exon 3, or exon 4 of *TTR*.

[00306] In some embodiments, the guide sequence is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a target sequence present in the human *TTR* gene. In some embodiments, the target sequence may be complementary to the guide sequence of the guide RNA. In some embodiments, the degree of complementarity or identity between a guide sequence of a guide RNA and its corresponding target sequence may be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the target sequence and the guide sequence of the gRNA may be 100% complementary or identical. In other embodiments, the target sequence and the guide sequence of the gRNA may contain at least one mismatch. For example, the target sequence and the guide sequence of the gRNA may contain 1, 2, 3, or 4 mismatches, where the total length of the guide sequence is 20. In some

embodiments, the target sequence and the guide sequence of the gRNA may contain 1-4 mismatches where the guide sequence is 20 nucleotides.

[00307] In some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered.

[00308] In some embodiments, the RNA-guided DNA-binding agent is a Class 2 Cas nuclease. In some embodiments, the RNA-guided DNA-binding agent has cleavase activity, which can also be referred to as double-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nuclease, such as a Class 2 Cas nuclease (which may be, e.g., a Cas nuclease of Type II, V, or VI). Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, and C2c3 proteins and modifications thereof. Examples of Cas9 nucleases include those of the type II CRISPR systems of *S. pyogenes, S. aureus*, and other prokaryotes (see, e.g., the list in the next paragraph), and modified (e.g., engineered or mutant) versions thereof. See, e.g., US2016/0312198 A1; US 2016/0312199 A1. Other examples of Cas nucleases include a Csm or Cmr complex of a type III CRISPR system or the Cas10, Csm1, or Cmr2 subunit thereof; and a Cascade complex of a type I CRISPR system, or the Cas3 subunit thereof. In some embodiments, the Cas nuclease may be from a Type-IIA, Type-IIB, or Type-IIC system. For discussion of various CRISPR systems and Cas nucleases see, e.g., Makarova et al., NAT. REV. MICROBIOL. 9:467-477 (2011); Makarova et al., NAT. REV. MICROBIOL, 13: 722-36 (2015); Shmakov et al., MOLECULAR CELL, 60:385-397 (2015).

[00309] Non-limiting exemplary species that the Cas nuclease can be derived from include *Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Staphylococcus aureus, Listeria innocua, Lactobacillus gasseri, Francisella novicida, Wolinella succinogenes, Sutterella wadsworthensis, Gammaproteobacterium, Neisseria meningitidis, Campylobacter jejuni, Pasteurella multocida, Fibrobacter succinogene, Rhodospirillum rubrum, Nocardiopsis dassonvillei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Lactobacillus buchneri, Treponema denticola, Microscilla marina, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas sp.,*

Crocosphaera watsonii, Cyanothece sp., Microcystis aeruginosa, Synechococcus sp., Acetohalobium arabaticum, Ammonifex degensii, Caldicelulosiruptor becscii, Candidatus Desulforudis, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter sp., Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Arthrospira maxima, Arthrospira platensis, Arthrospira sp., Lyngbya sp., Microcoleus chthonoplastes, Oscillatoria sp., Petrotoga mobilis, Thermosipho africanus, Streptococcus pasteurianus, Neisseria cinerea, Campylobacter lari, Parvibaculum lavamentivorans, Corynebacterium diphtheria, Acidaminococcus sp., Lachnospiraceae bacterium ND2006, and *Acaryochloris marina*.

[00310] In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Neisseria meningitidis*. In some embodiments, the Cas nuclease is the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Acidaminococcus sp.* In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Lachnospiraceae* bacterium ND2006. In further embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella tularensis, Lachnospiraceae bacterium, Butyrivibrio proteoclasticus, Peregrinibacteria bacterium, Parcubacteria bacterium, Smithella, Acidaminococcus, Candidatus Methanoplasma termitum, Eubacterium eligens, Moraxella bovoculi, Leptospira inadai, Porphyromonas crevioricanis, Prevotella disiens,* or *Porphyromonas macacae*. In certain embodiments, the Cas nuclease is a Cpf1 nuclease from an *Acidaminococcus* or *Lachnospiraceae*.

[00311] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 nuclease comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 nuclease is a wild type Cas9. In some embodiments, the Cas9 is capable of inducing a double strand break in target DNA. In certain embodiments, the Cas nuclease may cleave dsDNA, it may cleave one strand of dsDNA, or it may not have DNA cleavase or nickase activity. An exemplary Cas9 amino acid sequence is provided as SEQ ID NO: 203. An exemplary Cas9 mRNA ORF sequence, which

includes start and stop codons, is provided as SEQ ID NO: 204. An exemplary Cas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 210.

[00312] In some embodiments, chimeric Cas nucleases are used, where one domain or region of the protein is replaced by a portion of a different protein. In some embodiments, a Cas nuclease domain may be replaced with a domain from a different nuclease such as Fok1. In some embodiments, a Cas nuclease may be a modified nuclease.

[00313] In other embodiments, the Cas nuclease may be from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a component of the Cascade complex of a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a Cas3 protein. In some embodiments, the Cas nuclease may be from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease may have an RNA cleavage activity.

[00314] In some embodiments, the RNA-guided DNA-binding agent has single-strand nickase activity, i.e., can cut one DNA strand to produce a single-strand break, also known as a "nick." In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. A nickase is an enzyme that creates a nick in dsDNA, i.e., cuts one strand but not the other of the DNA double helix. In some embodiments, a Cas nickase is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which an endonucleolytic active site is inactivated, e.g., by one or more alterations (e.g., point mutations) in a catalytic domain. See, e.g., US Pat. No. 8,889,356 for discussion of Cas nickases and exemplary catalytic domain alterations. In some embodiments, a Cas nickase such as a Cas9 nickase has an inactivated RuvC or HNH domain. An exemplary Cas9 nickase amino acid sequence is provided as SEQ ID NO: 206. An exemplary Cas9 nickase mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 207. An exemplary Cas9 nickase mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 211.

[00315] In some embodiments, the RNA-guided DNA-binding agent is modified to contain only one functional nuclease domain. For example, the agent protein may be modified such that one of the nuclease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, a nickase is used having a RuvC domain with reduced activity. In some embodiments, a nickase is used having an inactive RuvC domain. In some embodiments, a nickase is used having an HNH domain with reduced activity. In some embodiments, a nickase is used having an inactive HNH domain.

[00316] In some embodiments, a conserved amino acid within a Cas protein nuclease domain is substituted to reduce or alter nuclease activity. In some embodiments, a Cas nuclease may comprise an amino acid substitution in the RuvC or RuvC-like nuclease

domain. Exemplary amino acid substitutions in the RuvC or RuvC-like nuclease domain include D10A (based on the S. *pyogenes* Cas9 protein). *See, e.g.*, Zetsche et al. (2015) *Cell* Oct 22:163(3): 759-771. In some embodiments, the Cas nuclease may comprise an amino acid substitution in the HNH or HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the S. *pyogenes* Cas9 protein). *See, e.g.*, Zetsche et al. (2015). Further exemplary amino acid substitutions include D917A, E1006A, and D1255A (based on the *Francisella novicida* U112 Cpf1 (FnCpf1) sequence (UniProtKB - A0Q7Q2 (CPF1_FRATN)).

[00317] In some embodiments, an mRNA encoding a nickase is provided in combination with a pair of guide RNAs that are complementary to the sense and antisense strands of the target sequence, respectively. In this embodiment, the guide RNAs direct the nickase to a target sequence and introduce a DSB by generating a nick on opposite strands of the target sequence (i.e., double nicking). In some embodiments, use of double nicking may improve specificity and reduce off-target effects. In some embodiments, a nickase is used together with two separate guide RNAs targeting opposite strands of DNA to produce a double nick in the target DNA. In some embodiments, a nickase is used together with two separate guide RNAs that are selected to be in close proximity to produce a double nick in the target DNA.

[00318] In some embodiments, the RNA-guided DNA-binding agent lacks cleavase and nickase activity. In some embodiments, the RNA-guided DNA-binding agent comprises a dCas DNA-binding polypeptide. A dCas polypeptide has DNA-binding activity while essentially lacking catalytic (cleavase/nickase) activity. In some embodiments, the dCas polypeptide is a dCas9 polypeptide. In some embodiments, the RNA-guided DNA-binding agent lacking cleavase and nickase activity or the dCas DNA-binding polypeptide is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which its endonucleolytic active sites are inactivated, e.g., by one or more alterations (e.g., point mutations) in its catalytic domains. See, e.g., US 2014/0186958 A1; US 2015/0166980 A1. An exemplary dCas9 amino acid sequence is provided as SEQ ID NO: 208. An exemplary dCas9 mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 209. An exemplary dCas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 212.

[00319] In some embodiments, the RNA-guided DNA-binding agent comprises one or more heterologous functional domains (e.g., is or comprises a fusion polypeptide).

[00320] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent into the nucleus of a cell. For example, the heterologous functional domain may be a nuclear localization signal (NLS). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-10 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with one NLS. Where one NLS is used, the NLS may be linked at the N-terminus or the C-terminus of the RNA-guided DNA-binding agent sequence. It may also be inserted within the RNA-guided DNA binding agent sequence. In other embodiments, the RNA-guided DNA-binding agent may be fused with more than one NLS. In some embodiments, the RNA-guided DNA-binding agent may be fused with 2, 3, 4, or 5 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (*e.g.*, two SV40 NLSs) or different. In some embodiments, the RNA-guided DNA-binding agent is fused to two SV40 NLS sequences linked at the carboxy terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs, one linked at the N-terminus and one at the C-terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with 3 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with no NLS. In some embodiments, the NLS may be a monopartite sequence, such as, *e.g.*, the SV40 NLS, PKKKRKV (SEQ ID NO: 274) or PKKKRRV (SEQ ID NO: 275). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 276). In a specific embodiment, a single PKKKRKV (SEQ ID NO: 274) NLS may be linked at the C-terminus of the RNA-guided DNA-binding agent. One or more linkers are optionally included at the fusion site.

[00321] In some embodiments, the heterologous functional domain may be capable of modifying the intracellular half-life of the RNA-guided DNA binding agent. In some embodiments, the half-life of the RNA-guided DNA binding agent may be increased. In some embodiments, the half-life of the RNA-guided DNA-binding agent may be reduced. In some embodiments, the heterologous functional domain may be capable of increasing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may be capable of reducing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may act as a signal peptide for protein degradation. In some embodiments, the protein degradation may be mediated by proteolytic enzymes, such as, for example, proteasomes, lysosomal proteases, or calpain

proteases. In some embodiments, the heterologous functional domain may comprise a PEST sequence. In some embodiments, the RNA-guided DNA-binding agent may be modified by addition of ubiquitin or a polyubiquitin chain. In some embodiments, the ubiquitin may be a ubiquitin-like protein (UBL). Non-limiting examples of ubiquitin-like proteins include small ubiquitin-like modifier (SUMO), ubiquitin cross-reactive protein (UCRP, also known as interferon-stimulated gene-15 (ISG15)), ubiquitin-related modifier-1 (URM1), neuronal-precursor-cell-expressed developmentally downregulated protein-8 (NEDD8, also called Rub1 in *S. cerevisiae*), human leukocyte antigen F-associated (FAT10), autophagy-8 (ATG8) and -12 (ATG12), Fau ubiquitin-like protein (FUB1), membrane-anchored UBL (MUB), ubiquitin fold-modifier-1 (UFM1), and ubiquitin-like protein-5 (UBL5).

[00322] In some embodiments, the heterologous functional domain may be a marker domain. Non-limiting examples of marker domains include fluorescent proteins, purification tags, epitope tags, and reporter gene sequences. In some embodiments, the marker domain may be a fluorescent protein. Non-limiting examples of suitable fluorescent proteins include green fluorescent proteins (*e.g.*, GFP, GFP-2, tagGFP, turboGFP, sfGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreen1), yellow fluorescent proteins (*e.g.*, YFP, EYFP, Citrine, Venus, YPet, PhiYFP, ZsYellow1), blue fluorescent proteins (*e.g.*, EBFP, EBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire,), cyan fluorescent proteins (*e.g.*, ECFP, Cerulean, CyPet, AmCyan1, Midoriishi-Cyan), red fluorescent proteins (*e.g.*, mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRed1, AsRed2, eqFP611, mRasberry, mStrawberry, Jred), and orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato) or any other suitable fluorescent protein. In other embodiments, the marker domain may be a purification tag and/or an epitope tag. Non-limiting exemplary tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein (MBP), thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1, AU5, E, ECS, E2, FLAG, HA, nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, 6xHis, 8xHis, biotin carboxyl carrier protein (BCCP), poly-His, and calmodulin. Non-limiting exemplary reporter genes include glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), beta-galactosidase, beta-glucuronidase, luciferase, or fluorescent proteins.

[00323] In additional embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to a specific organelle, cell type, tissue, or organ. In some

embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to mitochondria.

[00324] In further embodiments, the heterologous functional domain may be an effector domain. When the RNA-guided DNA-binding agent is directed to its target sequence, *e.g.*, when a Cas nuclease is directed to a target sequence by a gRNA, the effector domain may modify or affect the target sequence. In some embodiments, the effector domain may be chosen from a nucleic acid binding domain, a nuclease domain (e.g., a non-Cas nuclease domain), an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. In some embodiments, the heterologous functional domain is a nuclease, such as a FokI nuclease. See, e.g., US Pat. No. 9,023,649. In some embodiments, the heterologous functional domain is a transcriptional activator or repressor. See, e.g., Qi et al., "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression," *Cell* 152:1173-83 (2013); Perez-Pinera et al., "RNA-guided gene activation by CRISPR-Cas9-based transcription factors," *Nat. Methods* 10:973-6 (2013); Mali et al., "CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," *Nat. Biotechnol.* 31:833-8 (2013); Gilbert et al., "CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes," *Cell* 154:442-51 (2013). As such, the RNA-guided DNA-binding agent essentially becomes a transcription factor that can be directed to bind a desired target sequence using a guide RNA.

B. Modified gRNAs and mRNAs

[00325] In some embodiments, the gRNA is chemically modified. A gRNA comprising one or more modified nucleosides or nucleotides is called a "modified" gRNA or "chemically modified" gRNA, to describe the presence of one or more non-naturally and/or naturally occurring components or configurations that are used instead of or in addition to the canonical A, G, C, and U residues. In some embodiments, a modified gRNA is synthesized with a non-canonical nucleoside or nucleotide, is here called "modified." Modified nucleosides and nucleotides can include one or more of: (i) alteration, *e.g.*, replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage (an exemplary backbone modification); (ii) alteration, *e.g.*, replacement, of a constituent of the ribose sugar, *e.g.*, of the 2' hydroxyl on the ribose sugar (an exemplary sugar modification); (iii) wholesale replacement of the phosphate moiety with "dephospho" linkers (an exemplary backbone

modification); (iv) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); (v) replacement or modification of the ribose-phosphate backbone (an exemplary backbone modification); (vi) modification of the 3' end or 5' end of the oligonucleotide, *e.g.*, removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, cap or linker (such 3' or 5' cap modifications may comprise a sugar and/or backbone modification); and (vii) modification or replacement of the sugar (an exemplary sugar modification).

[00326] As noted above, in some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered. In some embodiments, the ORF encoding an RNA-guided DNA nuclease is a "modified RNA-guided DNA binding agent ORF" or simply a "modified ORF," which is used as shorthand to indicate that the ORF is modified in one or more of the following ways: (1) the modified ORF has a uridine content ranging from its minimum uridine content to 150% of the minimum uridine content; (2) the modified ORF has a uridine dinucleotide content ranging from its minimum uridine dinucleotide content to 150% of the minimum uridine dinucleotide content; (3) the modified ORF has at least 90% identity to any one of SEQ ID NOs: 201, 204, 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266; (4) the modified ORF consists of a set of codons of which at least 75% of the codons are codons listed in the Table 3A of Minimal Uridine Codons; or (5) the modified ORF comprises at least one modified uridine. In some embodiments, the modified ORF is modified in at least two, three, or four of the foregoing ways. In some embodiments, the modified ORF comprises at least one modified uridine and is modified in at least one, two, three, or all of (1)-(4) above.

Table 3A of Minimal Uridine Codons

	Amino Acid	Minimal uridine codon
A	Alanine	GCA or GCC or GCG
G	Glycine	GGA or GGC or GGG
V	Valine	GUC or GUA or GUG
D	Aspartic acid	GAC
E	Glutamic acid	GAA or GAG
I	Isoleucine	AUC or AUA
T	Threonine	ACA or ACC or ACG
N	Asparagine	AAC
K	Lysine	AAG or AAA

S	Serine	AGC
R	Arginine	AGA or AGG
L	Leucine	CUG or CUA or CUC
P	Proline	CCG or CCA or CCC
H	Histidine	CAC
Q	Glutamine	CAG or CAA
F	Phenylalanine	UUC
Y	Tyrosine	UAC
C	Cysteine	UGC
W	Tryptophan	UGG
M	Methionine	AUG

[00327] In any of the foregoing embodiments, the modified ORF may consist of a set of codons of which at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the codons are codons listed in Table 3A showing Minimal Uridine Codons.

[00328] In any of the foregoing embodiments, the modified ORF may comprise a sequence with at least 90%, 95%, 98%, 99%, or 100% identity to any one of SEQ ID NO: 201, 204, 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[00329] In any of the foregoing embodiments, the modified ORF may have a uridine content ranging from its minimum uridine content to 150%, 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%, 102%, or 101% of the minimum uridine content.

[00330] In any of the foregoing embodiments, the modified ORF may have a uridine dinucleotide content ranging from its minimum uridine dinucleotide content to 150%, 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%, 102%, or 101% of the minimum uridine dinucleotide content.

[00331] In any of the foregoing embodiments, the modified ORF may comprise a modified uridine at least at one, a plurality of, or all uridine positions. In some embodiments, the modified uridine is a uridine modified at the 5 position, e.g., with a halogen, methyl, or ethyl. In some embodiments, the modified uridine is a pseudouridine modified at the 1 position, e.g., with a halogen, methyl, or ethyl. The modified uridine can be, for example, pseudouridine, N1-methyl-pseudouridine, 5-methoxyuridine, 5-iodouridine, or a combination thereof. In some embodiments, the modified uridine is 5-methoxyuridine. In some embodiments, the modified uridine is 5-iodouridine. In some embodiments, the modified uridine is pseudouridine. In some embodiments, the modified uridine is N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination

of pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a combination of N1-methyl pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a combination of 5-iodouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and 5-iodouridine. In some embodiments, the modified uridine is a combination of 5-iodouridine and 5-methoxyuridine.

[00332] In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the uridine positions in an mRNA according to the disclosure are modified uridines. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are modified uridines, e.g., 5-methoxyuridine, 5-iodouridine, N1-methyl pseudouridine, pseudouridine, or a combination thereof. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are 5-methoxyuridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are pseudouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are N1-methyl pseudouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are 5-iodouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are 5-methoxyuridine, and the remainder are N1-methyl pseudouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are 5-iodouridine, and the remainder are N1-methyl pseudouridine.

[00333] In some embodiments, the mRNA comprises at least one UTR from an expressed mammalian mRNA, such as a constitutively expressed mRNA. An mRNA is considered constitutively expressed in a mammal if it is continually transcribed in at least one tissue of a healthy adult mammal. In some embodiments, the mRNA comprises a 5' UTR, 3' UTR, or 5' and 3' UTRs from an expressed mammalian RNA, such as a constitutively expressed mammalian mRNA. Actin mRNA is an example of a constitutively expressed mRNA.

[00334] In some embodiments, the mRNA comprises at least one UTR from Hydroxysteroid 17-Beta Dehydrogenase 4 (HSD17B4 or HSD), e.g., a 5' UTR from HSD. In some embodiments, the mRNA comprises at least one UTR from a globin mRNA, for example, human alpha globin (HBA) mRNA, human beta globin (HBB) mRNA, or Xenopus laevis beta globin (XBG) mRNA. In some embodiments, the mRNA comprises a 5' UTR, 3' UTR, or 5' and 3' UTRs from a globin mRNA, such as HBA, HBB, or XBG. In some embodiments, the mRNA comprises a 5' UTR from bovine growth hormone, cytomegalovirus (CMV), mouse Hba-a1, HSD, an albumin gene, HBA, HBB, or XBG. In some embodiments, the mRNA comprises a 3' UTR from bovine growth hormone, cytomegalovirus, mouse Hba-a1, HSD, an albumin gene, HBA, HBB, or XBG. In some embodiments, the mRNA comprises 5' and 3' UTRs from bovine growth hormone, cytomegalovirus, mouse Hba-a1, HSD, an albumin gene, HBA, HBB, XBG, heat shock protein 90 (Hsp90), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), beta-actin, alpha-tubulin, tumor protein (p53), or epidermal growth factor receptor (EGFR).

[00335] In some embodiments, the mRNA comprises 5' and 3' UTRs that are from the same source, e.g., a constitutively expressed mRNA such as actin, albumin, or a globin such as HBA, HBB, or XBG.

[00336] In some embodiments, the mRNA does not comprise a 5' UTR, e.g., there are no additional nucleotides between the 5' cap and the start codon. In some embodiments, the mRNA comprises a Kozak sequence (described below) between the 5' cap and the start codon, but does not have any additional 5' UTR. In some embodiments, the mRNA does not comprise a 3' UTR, e.g., there are no additional nucleotides between the stop codon and the poly-A tail.

[00337] In some embodiments, the mRNA comprises a Kozak sequence. The Kozak sequence can affect translation initiation and the overall yield of a polypeptide translated from an mRNA. A Kozak sequence includes a methionine codon that can function as the start codon. A minimal Kozak sequence is NNNRUGN wherein at least one of the following is true: the first N is A or G and the second N is G. In the context of a nucleotide sequence, R means a purine (A or G). In some embodiments, the Kozak sequence is RNNRUGN, NNNRUGG, RNNRUGG, RNNAUGN, NNNAUGG, or RNNAUGG. In some embodiments, the Kozak sequence is rccRUGg with zero mismatches or with up to one or two mismatches to positions in lowercase. In some embodiments, the Kozak sequence is rccAUGg with zero mismatches or with up to one or two mismatches to positions in lowercase. In some embodiments, the Kozak sequence is gccRccAUGG (SEQ ID NO: 277)

with zero mismatches or with up to one, two, or three mismatches to positions in lowercase. In some embodiments, the Kozak sequence is gccAccAUG with zero mismatches or with up to one, two, three, or four mismatches to positions in lowercase. In some embodiments, the Kozak sequence is GCCACCAUG. In some embodiments, the Kozak sequence is gccgccRccAUGG (SEQ ID NO: 278) with zero mismatches or with up to one, two, three, or four mismatches to positions in lowercase.

[00338] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 1, optionally wherein the ORF of SEQ ID NO: 1 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[00339] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 244, optionally wherein the ORF of SEQ ID NO: 244 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[00340] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 256, optionally wherein the ORF of SEQ ID NO: 256 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[00341] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 257, optionally wherein the ORF of SEQ ID NO: 257 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[00342] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 257, optionally wherein the ORF of SEQ ID NO: 258 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[00343] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 259, optionally wherein the ORF of SEQ ID NO: 259 (i.e., SEQ ID NO: 204) is substituted with

an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[00344] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 260, optionally wherein the ORF of SEQ ID NO: 260 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

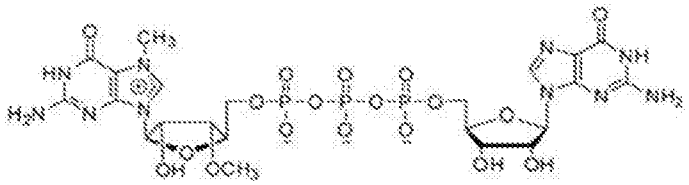
[00345] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 261, optionally wherein the ORF of SEQ ID NO: 261 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[00346] In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, or 256-261 is 95%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, or 256-261 is 98%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, or 256-261 is 99%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, or 256-261 is 100%.

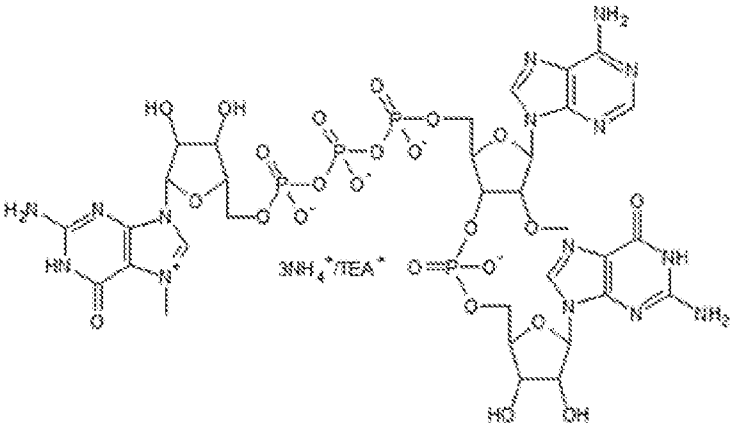
[00347] In some embodiments, an mRNA disclosed herein comprises a 5' cap, such as a Cap0, Cap1, or Cap2. A 5' cap is generally a 7-methylguanine ribonucleotide (which may be further modified, as discussed below e.g. with respect to ARCA) linked through a 5'-triphosphate to the 5' position of the first nucleotide of the 5'-to-3' chain of the mRNA, i.e., the first cap-proximal nucleotide. In Cap0, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-hydroxyl. In Cap1, the riboses of the first and second transcribed nucleotides of the mRNA comprise a 2'-methoxy and a 2'-hydroxyl, respectively. In Cap2, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-methoxy. See, e.g., Katibah et al. (2014) *Proc Natl Acad Sci USA* 111(33):12025-30; Abbas et al. (2017) *Proc Natl Acad Sci USA* 114(11):E2106-E2115. Most endogenous higher eukaryotic mRNAs, including mammalian mRNAs such as human mRNAs, comprise Cap1 or Cap2. Cap0 and other cap structures differing from Cap1 and Cap2 may be immunogenic in mammals, such as humans, due to recognition as "non-self" by components of the innate immune system such as IFIT-1 and IFIT-5, which can result in elevated cytokine levels including type I interferon. Components of the innate immune

system such as IFIT-1 and IFIT-5 may also compete with eIF4E for binding of an mRNA with a cap other than Cap1 or Cap2, potentially inhibiting translation of the mRNA.

[00348] A cap can be included co-transcriptionally. For example, ARCA (anti-reverse cap analog; Thermo Fisher Scientific Cat. No. AM8045) is a cap analog comprising a 7-methylguanine 3'-methoxy-5'-triphosphate linked to the 5' position of a guanine ribonucleotide which can be incorporated in vitro into a transcript at initiation. ARCA results in a Cap0 cap in which the 2' position of the first cap-proximal nucleotide is hydroxyl. See, e.g., Stepinski et al., (2001) "Synthesis and properties of mRNAs containing the novel 'anti-reverse' cap analogs 7-methyl(3'-O-methyl)GpppG and 7-methyl(3'deoxy)GpppG," *RNA* 7: 1486–1495. The ARCA structure is shown below.



[00349] CleanCap™ AG (m7G(5')ppp(5')(2'OMeA)pG; TriLink Biotechnologies Cat. No. N-7113) or CleanCap™ GG (m7G(5')ppp(5')(2'OMeG)pG; TriLink Biotechnologies Cat. No. N-7133) can be used to provide a Cap1 structure co-transcriptionally. 3'-O-methylated versions of CleanCap™ AG and CleanCap™ GG are also available from TriLink Biotechnologies as Cat. Nos. N-7413 and N-7433, respectively. The CleanCap™ AG structure is shown below.



[0001] Alternatively, a cap can be added to an RNA post-transcriptionally. For example, Vaccinia capping enzyme is commercially available (New England Biolabs Cat. No. M2080S) and has RNA triphosphatase and guanylyltransferase activities, provided by its D1 subunit, and guanine methyltransferase, provided by its D12 subunit. As such, it can add a 7-methylguanine to an RNA, so as to give Cap0, in the presence of S-adenosyl methionine and

GTP. See, e.g., Guo, P. and Moss, B. (1990) *Proc. Natl. Acad. Sci. USA* 87, 4023-4027; Mao, X. and Shuman, S. (1994) *J. Biol. Chem.* 269, 24472-24479. For additional discussion of caps and capping approaches, see, e.g., WO2017/053297 and Ishikawa et al., *Nucl. Acids. Symp. Ser.* (2009) No. 53, 129-130.

[0002] In some embodiments, the mRNA further comprises a poly-adenylated (poly-A) tail. In some embodiments, the poly-A tail comprises at least 20, 30, 40, 50, 60, 70, 80, 90, or 100 adenines, optionally up to 300 adenines. In some embodiments, the poly-A tail comprises 95, 96, 97, 98, 99, or 100 adenine nucleotides. In some instances, the poly-A tail is "interrupted" with one or more non-adenine nucleotide "anchors" at one or more locations within the poly-A tail. The poly-A tails may comprise at least 8 consecutive adenine nucleotides, but also comprise one or more non-adenine nucleotide. As used herein, "non-adenine nucleotides" refer to any natural or non-natural nucleotides that do not comprise adenine. Guanine, thymine, and cytosine nucleotides are exemplary non-adenine nucleotides. Thus, the poly-A tails on the mRNA described herein may comprise consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA binding agent or a sequence of interest. In some instances, the poly-A tails on mRNA comprise non-consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA binding agent or a sequence of interest, wherein non-adenine nucleotides interrupt the adenine nucleotides at regular or irregularly spaced intervals.

[0003] In some embodiments, the one or more non-adenine nucleotides are positioned to interrupt the consecutive adenine nucleotides so that a poly(A) binding protein can bind to a stretch of consecutive adenine nucleotides. In some embodiments, one or more non-adenine nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-100 consecutive adenine nucleotides. In some embodiments, the non-adenine nucleotide is after one, two, three, four, five, six, or seven adenine nucleotides and is followed by at least 8 consecutive adenine nucleotides.

[0004] The poly-A tail may comprise one sequence of consecutive adenine nucleotides followed by one or more non-adenine nucleotides, optionally followed by additional adenine nucleotides.

[0005] In some embodiments, the poly-A tail comprises or contains one non-adenine nucleotide or one consecutive stretch of 2-10 non-adenine nucleotides. In some embodiments, the non-adenine nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine

nucleotides. In some instances, the one or more non-adenine nucleotides are located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotides are located after at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 consecutive adenine nucleotides.

[0006] In some embodiments, the non-adenine nucleotide is guanine, cytosine, or thymine. In some instances, the non-adenine nucleotide is a guanine nucleotide. In some embodiments, the non-adenine nucleotide is a cytosine nucleotide. In some embodiments, the non-adenine nucleotide is a thymine nucleotide. In some instances, where more than one non-adenine nucleotide is present, the non-adenine nucleotide may be selected from: a) guanine and thymine nucleotides; b) guanine and cytosine nucleotides; c) thymine and cytosine nucleotides; or d) guanine, thymine and cytosine nucleotides. An exemplary poly-A tail comprising non-adenine nucleotides is provided as SEQ ID NO: 4.

[0007] In some embodiments, the mRNA further comprises a poly-adenylated (poly-A) tail. In some instances, the poly-A tail is "interrupted" with one or more non-adenine nucleotide "anchors" at one or more locations within the poly-A tail. The poly-A tails may comprise at least 8 consecutive adenine nucleotides, but also comprise one or more non-adenine nucleotide. As used herein, "non-adenine nucleotides" refer to any natural or non-natural nucleotides that do not comprise adenine. Guanine, thymine, and cytosine nucleotides are exemplary non-adenine nucleotides. Thus, the poly-A tails on the mRNA described herein may comprise consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA-binding agent or a sequence of interest. In some instances, the poly-A tails on mRNA comprise non-consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA-binding agent or a sequence of interest, wherein non-adenine nucleotides interrupt the adenine nucleotides at regular or irregularly spaced intervals.

[0008] In some embodiments, the one or more non-adenine nucleotides are positioned to interrupt the consecutive adenine nucleotides so that a poly(A) binding protein can bind to a stretch of consecutive adenine nucleotides. In some embodiments, one or more non-adenine nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-100 consecutive adenine nucleotides. In some embodiments, the non-adenine nucleotide is after one, two, three, four, five, six, or seven adenine nucleotides and is followed by at least 8 consecutive adenine nucleotides.

[0009] The poly-A tail of the present invention may comprise one sequence of consecutive adenine nucleotides followed by one or more non-adenine nucleotides, optionally followed by additional adenine nucleotides.

[0010] In some embodiments, the poly-A tail comprises or contains one non-adenine nucleotide or one consecutive stretch of 2-10 non-adenine nucleotides. In some embodiments, the non-adenine nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine nucleotides. In some instances, the one or more non-adenine nucleotides are located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotides are located after at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 consecutive adenine nucleotides.

[0011] In some embodiments, the non-adenine nucleotide is guanine, cytosine, or thymine. In some instances, the non-adenine nucleotide is a guanine nucleotide. In some embodiments, the non-adenine nucleotide is a cytosine nucleotide. In some embodiments, the non-adenine nucleotide is a thymine nucleotide. In some instances, where more than one non-adenine nucleotide is present, the non-adenine nucleotide may be selected from: a) guanine and thymine nucleotides; b) guanine and cytosine nucleotides; c) thymine and cytosine nucleotides; or d) guanine, thymine and cytosine nucleotides. An exemplary poly-A tail comprising non-adenine nucleotides is provided as SEQ ID NO: 4:
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCGAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAACCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
A.

[0012] Chemical modifications such as those listed above can be combined to provide modified gRNAs and/or mRNAs comprising nucleosides and nucleotides (collectively "residues") that can have two, three, four, or more modifications. For example, a modified residue can have a modified sugar and a modified nucleobase. In some embodiments, every base of a gRNA is modified, *e.g.*, all bases have a modified phosphate group, such as a phosphorothioate group. In certain embodiments, all, or substantially all, of the phosphate groups of an gRNA molecule are replaced with phosphorothioate groups. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 5' end of the RNA. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 3' end of the RNA.

[0013] In some embodiments, the gRNA comprises one, two, three or more modified residues. In some embodiments, at least 5% (*e.g.*, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the positions in a modified gRNA are modified nucleosides or nucleotides.

[0014] Unmodified nucleic acids can be prone to degradation by, *e.g.*, intracellular nucleases or those found in serum. For example, nucleases can hydrolyze nucleic acid phosphodiester bonds. Accordingly, in one aspect the gRNAs described herein can contain one or more modified nucleosides or nucleotides, *e.g.*, to introduce stability toward intracellular or serum-based nucleases. In some embodiments, the modified gRNA molecules described herein can exhibit a reduced innate immune response when introduced into a population of cells, both *in vivo* and *ex vivo*. The term "innate immune response" includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, which involves the induction of cytokine expression and release, particularly the interferons, and cell death.

[0015] In some embodiments of a backbone modification, the phosphate group of a modified residue can be modified by replacing one or more of the oxygens with a different substituent. Further, the modified residue, *e.g.*, modified residue present in a modified nucleic acid, can include the wholesale replacement of an unmodified phosphate moiety with a modified phosphate group as described herein. In some embodiments, the backbone modification of the phosphate backbone can include alterations that result in either an uncharged linker or a charged linker with unsymmetrical charge distribution.

[0016] Examples of modified phosphate groups include, phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. The phosphorous atom in an unmodified phosphate group is achiral. However, replacement of one of the non-bridging oxygens with one of the above atoms or groups of atoms can render the phosphorous atom chiral. The stereogenic phosphorous atom can possess either the "R" configuration (herein Rp) or the "S" configuration (herein Sp). The backbone can also be modified by replacement of a bridging oxygen, (*i.e.*, the oxygen that links the phosphate to the nucleoside), with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates)

and carbon (bridged methylenephosphonates). The replacement can occur at either linking oxygen or at both of the linking oxygens.

[0017] The phosphate group can be replaced by non-phosphorus containing connectors in certain backbone modifications. In some embodiments, the charged phosphate group can be replaced by a neutral moiety. Examples of moieties which can replace the phosphate group can include, without limitation, *e.g.*, methyl phosphonate, hydroxylamino, siloxane, carbonate, carboxymethyl, carbamate, amide, thioether, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, oxime, methyleneimino, methylenemethylimino, methylenehydrazo, methylenedimethylhydrazo and methyleneoxymethylimino.

[0018] Scaffolds that can mimic nucleic acids can also be constructed wherein the phosphate linker and ribose sugar are replaced by nuclease resistant nucleoside or nucleotide surrogates. Such modifications may comprise backbone and sugar modifications. In some embodiments, the nucleobases can be tethered by a surrogate backbone. Examples can include, without limitation, the morpholino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates.

[0019] The modified nucleosides and modified nucleotides can include one or more modifications to the sugar group, *i.e.* at sugar modification. For example, the 2' hydroxyl group (OH) can be modified, *e.g.* replaced with a number of different "oxy" or "deoxy" substituents. In some embodiments, modifications to the 2' hydroxyl group can enhance the stability of the nucleic acid since the hydroxyl can no longer be deprotonated to form a 2'-alkoxide ion.

[0020] Examples of 2' hydroxyl group modifications can include alkoxy or aryloxy (OR, wherein "R" can be, *e.g.*, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or a sugar); polyethyleneglycols (PEG), $O(CH_2CH_2O)_nCH_2CH_2OR$ wherein R can be, *e.g.*, H or optionally substituted alkyl, and n can be an integer from 0 to 20 (*e.g.*, from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and from 4 to 20). In some embodiments, the 2' hydroxyl group modification can be 2'-O-Me. In some embodiments, the 2' hydroxyl group modification can be a 2'-fluoro modification, which replaces the 2' hydroxyl group with a fluoride. In some embodiments, the 2' hydroxyl group modification can include "locked" nucleic acids (LNA) in which the 2' hydroxyl can be connected, *e.g.*, by a C_{1-6} alkylene or C_{1-6} heteroalkylene bridge, to the 4' carbon of the same ribose sugar, where exemplary bridges can include

methylene, propylene, ether, or amino bridges; O-amino (wherein amino can be, *e.g.*, NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino) and aminoalkoxy, $O(CH_2)_n$-amino, (wherein amino can be, *e.g.*, NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino). In some embodiments, the 2' hydroxyl group modification can included "unlocked" nucleic acids (UNA) in which the ribose ring lacks the C2'-C3' bond. In some embodiments, the 2' hydroxyl group modification can include the methoxyethyl group (MOE), ($OCH_2CH_2OCH_3$, *e.g.*, a PEG derivative).

[0021] "Deoxy" 2' modifications can include hydrogen (*i.e.* deoxyribose sugars, *e.g.*, at the overhang portions of partially dsRNA); halo (*e.g.*, bromo, chloro, fluoro, or iodo); amino (wherein amino can be, *e.g.*, NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, diheteroarylamino, or amino acid); $NH(CH_2CH_2NH)_nCH2CH_2$- amino (wherein amino can be, *e.g.*, as described herein), -NHC(O)R (wherein R can be, *e.g.*, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with *e.g.*, an amino as described herein.

[0022] The sugar modification can comprise a sugar group which may also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified nucleic acid can include nucleotides containing *e.g.*, arabinose, as the sugar. The modified nucleic acids can also include abasic sugars. These abasic sugars can also be further modified at one or more of the constituent sugar atoms. The modified nucleic acids can also include one or more sugars that are in the L form, *e.g.* L- nucleosides.

[0023] The modified nucleosides and modified nucleotides described herein, which can be incorporated into a modified nucleic acid, can include a modified base, also called a nucleobase. Examples of nucleobases include, but are not limited to, adenine (A), guanine (G), cytosine (C), and uracil (U). These nucleobases can be modified or wholly replaced to provide modified residues that can be incorporated into modified nucleic acids. The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine analog, or pyrimidine analog. In some embodiments, the nucleobase can include, for example, naturally-occurring and synthetic derivatives of a base.

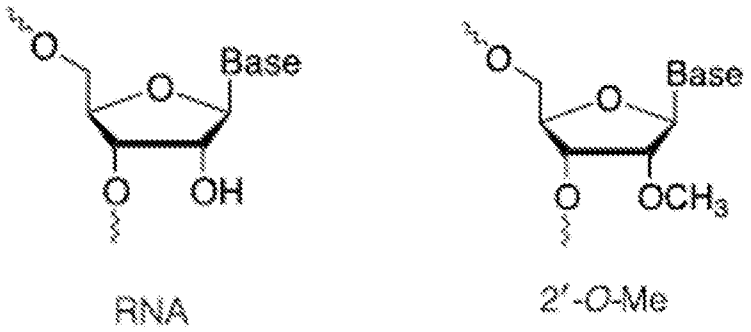
[0024] In embodiments employing a dual guide RNA, each of the crRNA and the tracr RNA can contain modifications. Such modifications may be at one or both ends of the crRNA and/or tracr RNA. In embodiments comprising an sgRNA, one or more residues at one or both ends of the sgRNA may be chemically modified, or the entire sgRNA may be chemically modified. Certain embodiments comprise a 5' end modification. Certain embodiments comprise a 3' end modification. In certain embodiments, one or more or all of the nucleotides in single stranded overhang of a guide RNA molecule are deoxynucleotides.

[0025] In some embodiments, the guide RNAs disclosed herein comprise one of the modification patterns disclosed in US 62/431,756, filed December 8, 2016, titled "Chemically Modified Guide RNAs," the contents of which are hereby incorporated by reference in their entirety.

[0026] In some embodiments, the invention comprises a gRNA comprising one or more modifications. In some embodiments, the modification comprises a 2'-O-methyl (2'-O-Me) modified nucleotide. In some embodiments, the modification comprises a phosphorothioate (PS) bond between nucleotides.

[0027] The terms "mA," "mC," "mU," or "mG" may be used to denote a nucleotide that has been modified with 2'-O-Me.

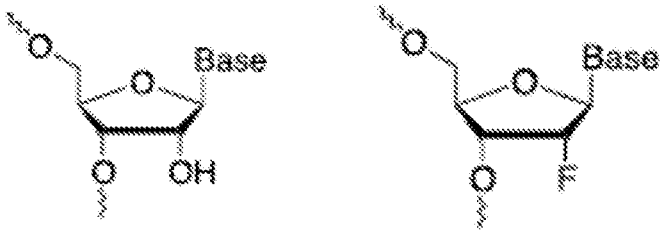
[0028] Modification of 2'-*O*-methyl can be depicted as follows:



[0029] Another chemical modification that has been shown to influence nucleotide sugar rings is halogen substitution. For example, 2'-fluoro (2'-F) substitution on nucleotide sugar rings can increase oligonucleotide binding affinity and nuclease stability.

[0030] In this application, the terms "fA," "fC," "fU," or "fG" may be used to denote a nucleotide that has been substituted with 2'-F.

[0031] Substitution of 2'-F can be depicted as follows:



RNA — Natural composition of RNA

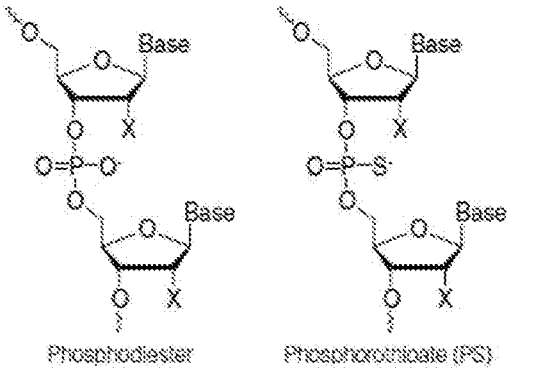
2'F-RNA — 2'F substitution

[0032] Phosphorothioate (PS) linkage or bond refers to a bond where a sulfur is substituted for one nonbridging phosphate oxygen in a phosphodiester linkage, for example in the bonds between nucleotides bases. When phosphorothioates are used to generate oligonucleotides, the modified oligonucleotides may also be referred to as S-oligos.

[0033] A "*" may be used to depict a PS modification. In this application, the terms A*, C*, U*, or G* may be used to denote a nucleotide that is linked to the next (e.g., 3') nucleotide with a PS bond.

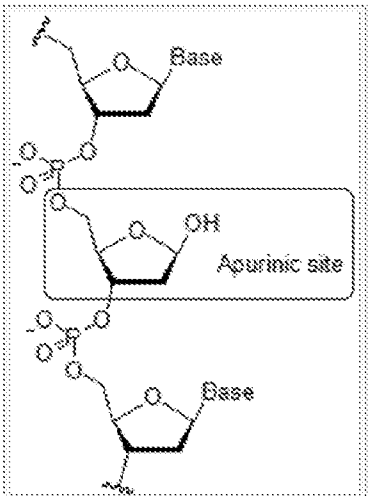
[0034] In this application, the terms "mA*," "mC*," "mU*," or "mG*" may be used to denote a nucleotide that has been substituted with 2'-O-Me and that is linked to the next (e.g., 3') nucleotide with a PS bond.

[0035] The diagram below shows the substitution of S- into a nonbridging phosphate oxygen, generating a PS bond in lieu of a phosphodiester bond:

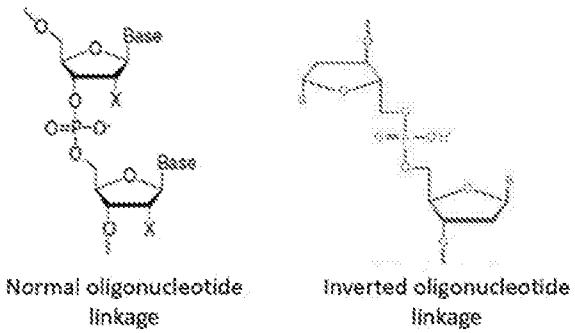


Natural phosphodiester linkage of RNA — Modified phosphorothioate (PS) bond

[0036] Abasic nucleotides refer to those which lack nitrogenous bases. The figure below depicts an oligonucleotide with an abasic (also known as apurinic) site that lacks a base:



[0037] Inverted bases refer to those with linkages that are inverted from the normal 5' to 3' linkage (i.e., either a 5' to 5' linkage or a 3' to 3' linkage). For example:



[0038] An abasic nucleotide can be attached with an inverted linkage. For example, an abasic nucleotide may be attached to the terminal 5' nucleotide via a 5' to 5' linkage, or an abasic nucleotide may be attached to the terminal 3' nucleotide via a 3' to 3' linkage. An inverted abasic nucleotide at either the terminal 5' or 3' nucleotide may also be called an inverted abasic end cap.

[0039] In some embodiments, one or more of the first three, four, or five nucleotides at the 5' terminus, and one or more of the last three, four, or five nucleotides at the 3' terminus are modified. In some embodiments, the modification is a 2'-O-Me, 2'-F, inverted abasic nucleotide, PS bond, or other nucleotide modification well known in the art to increase stability and/or performance.

[0040] In some embodiments, the first four nucleotides at the 5' terminus, and the last four nucleotides at the 3' terminus are linked with phosphorothioate (PS) bonds.

[0041] In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise a 2'-O-methyl (2'-O-Me) modified nucleotide. In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise a 2'-fluoro (2'-F) modified nucleotide. In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise an inverted abasic nucleotide.

[0042] In some embodiments, the guide RNA comprises a modified sgRNA. In some embodiments, the sgRNA comprises the modification pattern shown in SEQ ID No: 3, where N is any natural or non-natural nucleotide, and where the totality of the N's comprise a guide sequence that directs a nuclease to a target sequence.

[0043] In some embodiments, the guide RNA comprises a sgRNA shown in any one of SEQ ID No: 87-124. In some embodiments, the guide RNA comprises a sgRNA comprising any one of the guide sequences of SEQ ID No: 5-82 and the nucleotides of SEQ ID No: 125, wherein the nucleotides of SEQ ID No: 125 are on the 3' end of the guide sequence, and wherein the guide sequence may be modified as shown in SEQ ID No: 3.

C. Ribonucleoprotein complex

[0044] In some embodiments, a composition is encompassed comprising one or more gRNAs comprising one or more guide sequences from Table 1 or one or more sgRNAs from Table 2 and an RNA-guided DNA binding agent, e.g., a nuclease, such as a Cas nuclease, such as Cas9. In some embodiments, the encoded RNA-guided DNA-binding agent has cleavase activity, which can also be referred to as double-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nuclease. Examples of Cas9 nucleases include those of the type II CRISPR systems of *S. pyogenes, S. aureus*, and other prokaryotes (see, e.g., the list in the next paragraph), and modified (e.g., engineered or mutant) versions thereof. See, e.g., US2016/0312198 A1; US 2016/0312199 A1. Other examples of Cas nucleases include a Csm or Cmr complex of a type III CRISPR system or the Cas10, Csm1, or Cmr2 subunit thereof; and a Cascade complex of a type I CRISPR system, or the Cas3 subunit thereof. In some embodiments, the Cas nuclease may be from a Type-IIA, Type-IIB, or Type-IIC system. For discussion of various CRISPR systems and Cas nucleases see, e.g., Makarova et al., NAT. REV. MICROBIOL. 9:467-477 (2011);

Makarova et al., NAT. REV. MICROBIOL, 13: 722-36 (2015); Shmakov et al., MOLECULAR CELL, 60:385-397 (2015).

[0045] Non-limiting exemplary species that the Cas nuclease can be derived from include *Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Staphylococcus aureus, Listeria innocua, Lactobacillus gasseri, Francisella novicida, Wolinella succinogenes, Sutterella wadsworthensis, Gammaproteobacterium, Neisseria meningitidis, Campylobacter jejuni, Pasteurella multocida, Fibrobacter succinogene, Rhodospirillum rubrum, Nocardiopsis dassonvillei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Lactobacillus buchneri, Treponema denticola, Microscilla marina, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas sp., Crocosphaera watsonii, Cyanothece sp., Microcystis aeruginosa, Synechococcus sp., Acetohalobium arabaticum, Ammonifex degensii, Caldicelulosiruptor becscii, Candidatus Desulforudis, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter sp., Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Arthrospira maxima, Arthrospira platensis, Arthrospira sp., Lyngbya sp., Microcoleus chthonoplastes, Oscillatoria sp., Petrotoga mobilis, Thermosipho africanus, Streptococcus pasteurianus, Neisseria cinerea, Campylobacter lari, Parvibaculum lavamentivorans, Corynebacterium diphtheria, Acidaminococcus sp., Lachnospiraceae* bacterium ND2006, and *Acaryochloris marina*.

[0046] In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Neisseria meningitidis*. In some embodiments, the Cas nuclease is the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Acidaminococcus sp.* In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Lachnospiraceae* bacterium ND2006. In further embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella tularensis, Lachnospiraceae bacterium, Butyrivibrio*

proteoclasticus, Peregrinibacteria bacterium, Parcubacteria bacterium, Smithella, Acidaminococcus, Candidatus Methanoplasma termitum, Eubacterium eligens, Moraxella bovoculi, Leptospira inadai, Porphyromonas crevioricanis, Prevotella disiens, or *Porphyromonas macacae*. In certain embodiments, the Cas nuclease is a Cpf1 nuclease from an *Acidaminococcus* or *Lachnospiraceae*.

[0047] In some embodiments, the gRNA together with an RNA-guided DNA binding agent is called a ribonucleoprotein complex (RNP). In some embodiments, the RNA-guided DNA binding agent is a Cas nuclease. In some embodiments, the gRNA together with a Cas nuclease is called a Cas RNP. In some embodiments, the RNP comprises Type-I, Type-II, or Type-III components. In some embodiments, the Cas nuclease is the Cas9 protein from the Type-II CRISPR/Cas system. In some embodiment, the gRNA together with Cas9 is called a Cas9 RNP.

[0048] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 protein comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 protein is a wild type Cas9. In each of the composition, use, and method embodiments, the Cas induces a double strand break in target DNA.

[0049] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 nuclease comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 nuclease is a wild type Cas9. In some embodiments, the Cas9 is capable of inducing a double strand break in target DNA. In certain embodiments, the Cas nuclease may cleave dsDNA, it may cleave one strand of dsDNA, or it may not have DNA cleavase or nickase activity. An exemplary Cas9 amino acid sequence is provided as SEQ ID NO: 203. An exemplary Cas9 mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 204. An exemplary Cas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 210.

[0050] In some embodiments, chimeric Cas nucleases are used, where one domain or region of the protein is replaced by a portion of a different protein. In some embodiments, a Cas nuclease domain may be replaced with a domain from a different nuclease such as Fok1. In some embodiments, a Cas nuclease may be a modified nuclease.

[0051] In other embodiments, the Cas nuclease may be from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a component of the Cascade

complex of a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a Cas3 protein. In some embodiments, the Cas nuclease may be from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease may have an RNA cleavage activity.

[0052] In some embodiments, the RNA-guided DNA-binding agent has single-strand nickase activity, i.e., can cut one DNA strand to produce a single-strand break, also known as a "nick." In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. A nickase is an enzyme that creates a nick in dsDNA, i.e., cuts one strand but not the other of the DNA double helix. In some embodiments, a Cas nickase is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which an endonucleolytic active site is inactivated, e.g., by one or more alterations (e.g., point mutations) in a catalytic domain. See, e.g., US Pat. No. 8,889,356 for discussion of Cas nickases and exemplary catalytic domain alterations. In some embodiments, a Cas nickase such as a Cas9 nickase has an inactivated RuvC or HNH domain. An exemplary Cas9 nickase amino acid sequence is provided as SEQ ID NO: 206. An exemplary Cas9 nickase mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 207. An exemplary Cas9 nickase mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 211.

[0053] In some embodiments, the RNA-guided DNA-binding agent is modified to contain only one functional nuclease domain. For example, the agent protein may be modified such that one of the nuclease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, a nickase is used having a RuvC domain with reduced activity. In some embodiments, a nickase is used having an inactive RuvC domain. In some embodiments, a nickase is used having an HNH domain with reduced activity. In some embodiments, a nickase is used having an inactive HNH domain.

[0054] In some embodiments, a conserved amino acid within a Cas protein nuclease domain is substituted to reduce or alter nuclease activity. In some embodiments, a Cas nuclease may comprise an amino acid substitution in the RuvC or RuvC-like nuclease domain. Exemplary amino acid substitutions in the RuvC or RuvC-like nuclease domain include D10A (based on the S. *pyogenes* Cas9 protein). *See, e.g.*, Zetsche et al. (2015) *Cell* Oct 22:163(3): 759-771. In some embodiments, the Cas nuclease may comprise an amino acid substitution in the HNH or HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the S. *pyogenes* Cas9 protein). *See, e.g.*, Zetsche et al. (2015). Further exemplary amino acid substitutions include D917A, E1006A, and D1255A (based on

the *Francisella novicida* U112 Cpf1 (FnCpf1) sequence (UniProtKB - A0Q7Q2 (CPF1_FRATN)).

[0055] In some embodiments, an mRNA encoding a nickase is provided in combination with a pair of guide RNAs that are complementary to the sense and antisense strands of the target sequence, respectively. In this embodiment, the guide RNAs direct the nickase to a target sequence and introduce a DSB by generating a nick on opposite strands of the target sequence (i.e., double nicking). In some embodiments, use of double nicking may improve specificity and reduce off-target effects. In some embodiments, a nickase is used together with two separate guide RNAs targeting opposite strands of DNA to produce a double nick in the target DNA. In some embodiments, a nickase is used together with two separate guide RNAs that are selected to be in close proximity to produce a double nick in the target DNA.

[0056] In some embodiments, the RNA-guided DNA-binding agent lacks cleavase and nickase activity. In some embodiments, the RNA-guided DNA-binding agent comprises a dCas DNA-binding polypeptide. A dCas polypeptide has DNA-binding activity while essentially lacking catalytic (cleavase/nickase) activity. In some embodiments, the dCas polypeptide is a dCas9 polypeptide. In some embodiments, the RNA-guided DNA-binding agent lacking cleavase and nickase activity or the dCas DNA-binding polypeptide is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which its endonucleolytic active sites are inactivated, e.g., by one or more alterations (e.g., point mutations) in its catalytic domains. See, e.g., US 2014/0186958 A1; US 2015/0166980 A1. An exemplary dCas9 amino acid sequence is provided as SEQ ID NO: 208. An exemplary Cas9 mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 209. An exemplary Cas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 212.

[0057] In some embodiments, the RNA-guided DNA-binding agent comprises one or more heterologous functional domains (e.g., is or comprises a fusion polypeptide).

[0058] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent into the nucleus of a cell. For example, the heterologous functional domain may be a nuclear localization signal (NLS). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-10 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with one NLS. Where one NLS is used, the NLS may be linked at the N-terminus or the C-terminus of the RNA-guided DNA-binding agent sequence. In some embodiments, the RNA-guided DNA-binding agent

may be fused C-terminally to at least one NLS. An NLS may also be inserted within the RNA-guided DNA binding agent sequence. In other embodiments, the RNA-guided DNA-binding agent may be fused with more than one NLS. In some embodiments, the RNA-guided DNA-binding agent may be fused with 2, 3, 4, or 5 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (*e.g.*, two SV40 NLSs) or different. In some embodiments, the RNA-guided DNA-binding agent is fused to two SV40 NLS sequences linked at the carboxy terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs, one linked at the N-terminus and one at the C-terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with 3 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with no NLS. In some embodiments, the NLS may be a monopartite sequence, such as, *e.g.*, the SV40 NLS, PKKKRKV (SEQ ID NO: 274) or PKKKRRV (SEQ ID NO: 275). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 276). In a specific embodiment, a single PKKKRKV (SEQ ID NO: 274) NLS may be linked at the C-terminus of the RNA-guided DNA-binding agent. One or more linkers are optionally included at the fusion site. In some embodiments, one or more NLS(s) according to any of the foregoing embodiments are present in the RNA-guided DNA-binding agent in combination with one or more additional heterologous functional domains, such as any of the heterologous functional domains described below.

[0059] In some embodiments, the heterologous functional domain may be capable of modifying the intracellular half-life of the RNA-guided DNA binding agent. In some embodiments, the half-life of the RNA-guided DNA binding agent may be increased. In some embodiments, the half-life of the RNA-guided DNA-binding agent may be reduced. In some embodiments, the heterologous functional domain may be capable of increasing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may be capable of reducing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may act as a signal peptide for protein degradation. In some embodiments, the protein degradation may be mediated by proteolytic enzymes, such as, for example, proteasomes, lysosomal proteases, or calpain proteases. In some embodiments, the heterologous functional domain may comprise a PEST sequence. In some embodiments, the RNA-guided DNA-binding agent may be modified by addition of ubiquitin or a polyubiquitin chain. In some embodiments, the ubiquitin may be a ubiquitin-like protein (UBL). Non-limiting examples of ubiquitin-like proteins include small

ubiquitin-like modifier (SUMO), ubiquitin cross-reactive protein (UCRP, also known as interferon-stimulated gene-15 (ISG15)), ubiquitin-related modifier-1 (URM1), neuronal-precursor-cell-expressed developmentally downregulated protein-8 (NEDD8, also called Rub1 in *S. cerevisiae*), human leukocyte antigen F-associated (FAT10), autophagy-8 (ATG8) and -12 (ATG12), Fau ubiquitin-like protein (FUB1), membrane-anchored UBL (MUB), ubiquitin fold-modifier-1 (UFM1), and ubiquitin-like protein-5 (UBL5).

[0060] In some embodiments, the heterologous functional domain may be a marker domain. Non-limiting examples of marker domains include fluorescent proteins, purification tags, epitope tags, and reporter gene sequences. In some embodiments, the marker domain may be a fluorescent protein. Non-limiting examples of suitable fluorescent proteins include green fluorescent proteins (*e.g.*, GFP, GFP-2, tagGFP, turboGFP, sfGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreen1), yellow fluorescent proteins (*e.g.*, YFP, EYFP, Citrine, Venus, YPet, PhiYFP, ZsYellow1), blue fluorescent proteins (*e.g.*, EBFP, EBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire,), cyan fluorescent proteins (*e.g.*, ECFP, Cerulean, CyPet, AmCyan1, Midoriishi-Cyan), red fluorescent proteins (*e.g.*, mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRed1, AsRed2, eqFP611, mRasberry, mStrawberry, Jred), and orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato) or any other suitable fluorescent protein. In other embodiments, the marker domain may be a purification tag and/or an epitope tag. Non-limiting exemplary tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein (MBP), thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1, AU5, E, ECS, E2, FLAG, HA, nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, 6xHis, 8xHis, biotin carboxyl carrier protein (BCCP), poly-His, and calmodulin. Non-limiting exemplary reporter genes include glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), beta-galactosidase, beta-glucuronidase, luciferase, or fluorescent proteins.

[0061] In additional embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to a specific organelle, cell type, tissue, or organ. In some embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to mitochondria.

[0062] In further embodiments, the heterologous functional domain may be an effector domain. When the RNA-guided DNA-binding agent is directed to its target sequence, *e.g.*,

when a Cas nuclease is directed to a target sequence by a gRNA, the effector domain may modify or affect the target sequence. In some embodiments, the effector domain may be chosen from a nucleic acid binding domain, a nuclease domain (e.g., a non-Cas nuclease domain), an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. In some embodiments, the heterologous functional domain is a nuclease, such as a FokI nuclease. See, e.g., US Pat. No. 9,023,649. In some embodiments, the heterologous functional domain is a transcriptional activator or repressor. See, e.g., Qi et al., "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression," *Cell* 152:1173-83 (2013); Perez-Pinera et al., "RNA-guided gene activation by CRISPR-Cas9-based transcription factors," *Nat. Methods* 10:973-6 (2013); Mali et al., "CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," *Nat. Biotechnol.* 31:833-8 (2013); Gilbert et al., "CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes," *Cell* 154:442-51 (2013). As such, the RNA-guided DNA-binding agent essentially becomes a transcription factor that can be directed to bind a desired target sequence using a guide RNA.

D. Determination of efficacy of gRNAs

[0063] In some embodiments, the efficacy of a gRNA is determined when delivered or expressed together with other components forming an RNP. In some embodiments, the gRNA is expressed together with an RNA-guided DNA nuclease, such as a Cas protein. In some embodiments, the gRNA is delivered to or expressed in a cell line that already stably expresses an RNA-guided DNA nuclease, such as a Cas protein. In some embodiments the gRNA is delivered to a cell as part of a RNP. In some embodiments, the gRNA is delivered to a cell along with a mRNA encoding an RNA-guided DNA nuclease, such as a Cas nuclease.

[0064] As described herein, use of an RNA-guided DNA nuclease and a guide RNA disclosed herein can lead to double-stranded breaks in the DNA which can produce errors in the form of insertion/deletion (indel) mutations upon repair by cellular machinery. Many mutations due to indels alter the reading frame or introduce premature stop codons and, therefore, produce a non-functional protein.

[0065] In some embodiments, the efficacy of particular gRNAs is determined based on *in vitro* models. In some embodiments, the *in vitro* model is HEK293 cells stably expressing Cas9 (HEK293_Cas9). In some embodiments, the *in vitro* model is HUH7 human hepatocarcinoma cells. In some embodiments, the *in vitro* model is HepG2 cells. In some

embodiments, the *in vitro* model is primary human hepatocytes. In some embodiments, the *in vitro* model is primary cynomolgus hepatocytes. With respect to using primary human hepatocytes, commercially available primary human hepatocytes can be used to provide greater consistency between experiments. In some embodiments, the number of off-target sites at which a deletion or insertion occurs in an in vitro model (e.g., in primary human hepatocytes) is determined, e.g., by analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA and the guide RNA. In some embodiments, such a determination comprises analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA, the guide RNA, and a donor oligonucleotide. Exemplary procedures for such determinations are provided in the working examples below.

[0066] In some embodiments, the efficacy of particular gRNAs is determined across multiple *in vitro* cell models for a gRNA selection process. In some embodiments, a cell line comparison of data with selected gRNAs is performed. In some embodiments, cross screening in multiple cell models is performed.

[0067] In some embodiments, the efficacy of particular gRNAs is determined based on *in vivo* models. In some embodiments, the *in vivo* model is a rodent model. In some embodiments, the rodent model is a mouse which expresses a human *TTR* gene, which may be a mutant human *TTR* gene. In some embodiments, the *in vivo* model is a non-human primate, for example cynomolgus monkey.

[0068] In some embodiments, the efficacy of a guide RNA is measured by percent editing of *TTR*. In some embodiments, the percent editing of *TTR* is compared to the percent editing necessary to acheive knockdown of TTR protein, e.g., in the cell culture media in the case of an *in vitro* model or in serum or tissue in the case of an *in vivo* model.

[0069] In some embodiements, the efficacy of a guide RNA is measured by the number and/or frequency of indels at off-target sequences within the genome of the target cell type. In some embodiments, efficacious guide RNAs are provided which produce indels at off target sites at very low frequencies (e.g., <5%) in a cell population and/or relative to the frequency of indel creation at the target site. Thus, the disclosure provides for guide RNAs which do not exhibit off-target indel formation in the target cell type (e.g., a hepatocyte), or which produce a frequency of off-target indel formation of <5% in a cell population and/or relative to the frequency of indel creation at the target site. In some embodiments, the disclosure provides guide RNAs which do not exhibit any off target indel formation in the target cell type (e.g., hepatocyte). In some embodiments, guide RNAs are provided which produce indels at less than 5 off-target sites, e.g., as evaluated by one or more methods

described herein. In some embodiments, guide RNAs are provided which produce indels at less than or equal to 4, 3, 2, or 1 off-target site(s) e.g., as evaluated by one or more methods described herein. In some embodiments, the off-target site(s) does not occur in a protein coding region in the target cell (e.g., hepatocyte) genome.

[0070] In some embodiments, detecting gene editing events, such as the formation of insertion/deletion ("indel") mutations and homology directed repair (HDR) events in target DNA utilize linear amplification with a tagged primer and isolating the tagged amplification products (herein after referred to as "LAM-PCR," or "Linear Amplification (LA)" method).

[0071] In some embodiments, the method comprises isolating cellular DNA from a cell that has been induced to have a double strand break (DSB) and optionally that has been provided with an HDR template to repair the DSB; performing at least one cycle of linear amplification of the DNA with a tagged primer; isolating the linear amplification products that comprise tag, thereby discarding any amplification product that was amplified with a non-tagged primer; optionally further amplifying the isolated products; and analyzing the linear amplification products, or the further amplified products, to determine the presence or absence of an editing event such as, for example, a double strand break, an insertion, deletion, or HDR template sequence in the target DNA. In some instances, the editing event can be quantified. Quantification and the like as used herein (including in the context of HDR and non-HDR editing events such as indels) includes detecting the frequency and/or type(s) of editing events in a population.

[0072] In some embodiments, only one cycle of linear amplification is conducted.

[0073] In some instances, the tagged primer comprises a molecular barcode. In some embodiments, the tagged primer comprises a molecular barcode, and only one cycle of linear amplification is conducted.

[0074] In some embodiments, the analyzing step comprises sequencing the linear amplified products or the further amplified products. Sequencing may comprise any method known to those of skill in the art, including, next generation sequencing, and cloning the linear amplification products or further amplified products into a plasmid and sequencing the plasmid or a portion of the plasmid. In other aspects, the analyzing step comprises performing digital PCR (dPCR) or droplet digital PCR (ddPCR) on the linear amplified products or the further amplified products. In other instances, the analyzing step comprises contacting the linear amplified products or the further amplified products with a nucleic acid probe designed to identify DNA comprising HDR template sequence and detecting the probes that have bound to the linear amplified product(s) or further amplified product(s). In some

embodiments, the method further comprises determining the location of the HDR template in the target DNA.

[0075] In certain embodiments, the method further comprises determining the sequence of an insertion site in the target DNA, wherein the insertion site is the location where the HDR template incorporates into the target DNA, and wherein the insertion site may include some target DNA sequence and some HDR template sequence.

[0076] In some embodiments, the linear amplification of the target DNA with a tagged primer is performed for 1-50 cycles, 1-60 cycles, 1-70 cycles, 1-80 cycles, 1-90 cycles, or 1-100 cycles.

[0077] In some embodiments, the linear amplification of the target DNA with a tagged primer comprises a denaturation step to separate DNA duplexes, an annealing step to allow primer binding, and an elongation step. In some embodiments, the linear amplification is isothermal (does not require a change in temperature). In some embodiments, the isothermal linear amplification is a loop-mediated isothermal amplification (LAMP), a strand displacement amplification (SDA), a helicase-dependent amplification, or a nicking enzyme amplification reaction.

[0078] In some embodiments, the tagged primer anneals to the target DNA at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, at least 210, at least 220, at least 230, at least 240, at least 250, at least 260, at least 270, at least 280, at least 290, at least 300, at least 1,000, at least 5,000, or at least 10,000 nucleotides away from of the expected editing event location, e.g., the insertion, deletion, or template insertion site.

[0079] In some embodiments, the tagged primer comprises a molecular barcode. In some embodiments, the molecular barcode comprises a sequence that is not complementary to the target DNA. In some embodiments, the molecular barcode comprises 6, 8, 10, or 12 nucleotides.

[0080] In some embodiments, the tag on the primer is biotin, streptavidin, digoxigenin, a DNA sequence, or fluorescein isothiocyanate (FITC).

[0081] In some embodiments, the linear amplification product(s) are isolated using a capture reagent specific for the tag on the primer. In some embodiments, the capture reagent is on a bead, solid support, matrix, or column. In some embodiments, the isolation step comprises contacting the linear amplification product(s) with a capture reagent specific for

the tag on the primer. In some embodiments, the capture reagent is biotin, streptavidin, digoxigenin, a DNA sequence, or fluorescein isothiocyanate (FITC).

[0082] In some embodiments, the tag is biotin and capture reagent is streptavidin. In some embodiments, the tag is streptavidin and the capture reagent is biotin. In some embodiments, the tag is on the 5' terminus of the primer, the 3' terminus of the primer, or internal to the primer. In some embodiments, the tag and/or the capture reagent is removed after the isolation step. In some embodiments, the tag and/or the capture reagent is not removed, and the further amplifying and analyzing steps are performed in the presence of tag and/or capture.

[0083] In some embodiments, the further amplification is non-linear. In some embodiments, the further amplification is digital PCR, qPCR, or RT-PCR. In some embodiments, the sequencing is next generation sequencing (NGS).

[0084] In some embodiments, the target DNA is genomic or mitochondrial. In some embodiments, the target DNA is genomic DNA of a prokaryotic or eukaryotic cell. In some embodiments, the target DNA is mammalian. The target DNA may be from a non-dividing cell or a dividing cell. In some embodiments, the target DNA may be from a primary cell. In some embodiments, the target DNA is from a replicating cell.

[0085] In some instances, the cellular DNA is sheared prior to linear amplification. In some embodiments, the sheared DNA has an average size between 0.5 kb and 20 kb. In some instances, the cellular DNA is sheared to an average size of 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 3.75, 4.0, 4.25, 4.5, 4.75, 5.0, 5.25, 5.5, 5.75, 6.0, 6.25, 6.5, 6.75, 7.0, 7.25, 7.5, 7.75, 8.0, 8.25, 8.5, 8.75, 9.0, 9.25, 9.5, 9.75, 10.0, 10.25, 10.5, 10.75, 11.0, 11.25, 11.5, 11.75, 12.0, 12.25, 12.5, 12.75, 13.0, 13.25, 13.5, 13.75, 14.0, 14.25, 14.5, 14.75, 15.0, 15.25, 15.5, 15.75, 16.0, 16.25, 16.5, 16.75, 17.0, 17.25, 17.5, 17.75, 18.0, 18.25, 18.5, 18.75, 19.0, 19.25, 19.5, 19.75, or 20.0 kb. In some instances, the cellular DNA is sheared to an average size of about 1.5 kb.

[0086] In some embodiments, the efficacy of a guide RNA is measured by secretion of TTR. In some embodiments, secretion of TTR is measured using an enzyme-linked immunosorbent assay (ELISA) assay with cell culture media or serum. In some embodiments, secretion of TTR is measured in the same *in vitro* or *in vivo* systems or models used to measure editing. In some embodiments, secretion of TTR is measured in primary human hepatocytes. In some embodiments, secretion of TTR is measured in HUH7 cells. In some embodiments, secretion of TTR is measured in HepG2 cells.

[0087] ELISA assays are generally known to the skilled artisan and can be designed to determine serum TTR levels. In one exemplary embodiment, blood is collected and the serum is isolated. The total TTR serum levels may be determined using a Mouse Prealbumin (Transthyretin) ELISA Kit (Aviva Systems Biology, Cat. OKIA00111) or similar kit for measuring human TTR. If no kit is available, an ELISA can be developed using plates that are pre-coated with with capture antibody specific for the TTR one is measuring. The plate is next incubated at room temperature for a period of time before washing. Enzyme-anti-TTR antibody conjugate is added and inncubated. Unbound antibody conjugate is removed and the plate washed before the addition of the chromogenic substrate solution that reactes with the enzyme. The plate is read on an appropriate plate reader at an absorbance specific for the enzyme and substrate used.

[0088] In some embodiments, the amount of TTR in cells (including those from tissue) measures efficacy of a gRNA. In some embodiments, the amount of TTR in cells is measured using western blot. In some embodiments, the cell used is HUH7 cells. In some embodiments, the cell used is a primary human hepatocyte. In some embodiments, the cell used is a primar cell obtained from an animal. In some embodiments, the amount of TTR is compared to the amount of glyceraldehyde 3-phosphate dehydrogenase GAPDH (a housekeeping gene) to control for changes in cell number.

III. LNP formulations and Treatment of ATTR

[0089] In some embodiments, a method of inducing a double-stranded break (DSB) within the *TTR* gene is provided comprising administering a composition comprising a guide RNA comprising any one or more guide sequences of SEQ ID Nos: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID Nos: 5-82 are administered to induce a DSB in the *TTR* gene. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0090] In some embodiments, a method of modifying the *TTR* gene is provided comprising administering a composition comprising a guide RNA comprising any one or more of the guide sequences of SEQ ID Nos: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID Nos: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124, are administered to modify the *TTR* gene. The guide RNAs may be administered

together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0091] In some embodiments, a method of treating ATTR is provided comprising administering a composition comprising a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124 are administered to treat ATTR. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0092] In some embodiments, a method of reducing TTR serum concentration is provided comprising administering a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-82 or any one or more of the sgRNAs of SEQ ID Nos: 87-124 are administered to reduce or prevent the accumulation of TTR in amyloids or amyloid fibrils. The gRNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0093] In some embodiments, a method of reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject is provided comprising administering a composition comprising a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, a method of reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject is provided comprising administering a composition comprising any one or more of the sgRNAs of SEQ ID Nos: 87-113. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-82 or any one or more of the sgRNAs of SEQ ID Nos: 87-124 are administered to reduce or prevent the accumulation of TTR in amyloids or amyloid fibrils. The gRNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0094] In some embodiments, the gRNAs comprising the guide sequences of Table 1 or one or more sgRNAs from Table 2 together with an RNA-guided DNA nuclease such as a Cas nuclease induce DSBs, and non-homologous ending joining (NHEJ) during repair leads

to a mutation in the *TTR* gene. In some embodiments, NHEJ leads to a deletion or insertion of a nucleotide(s), which induces a frame shift or nonsense mutation in the *TTR* gene.

[0095] In some embodiments, administering the guide RNAs of the invention (e.g., in a composition provided herein) reduces levels (e.g., serum levels) of TTR in the subject, and therefore prevents accumulation and aggregation of TTR in amyloids or amyloid fibrils.

[0096] In some embodiments, reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject comprises reducing or preventing TTR deposition in one or more tissues of the subject, such as stomach, colon, or nervous tissue. In some embodiments, the nervous tissue comprises sciatic nerve or dorsal root ganglion. In some embodiments, TTR deposition is reduced in two, three, or four of the stomach, colon, dorsal root ganglion, and sciatic nerve. The level of deposition in a given tissue can be determined using a biopsy sample, e.g., using immunostaining. In some embodiments, reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject and/or reducing or preventing TTR deposition is inferred based on reducing serum TTR levels for a period of time. As discussed in the examples, it has been found that reducing serum TTR levels in accordance with methods and uses provided herein can result in clearance of deposited TTR from tissues such as those discussed above and in the examples, e.g., as measured 8 weeks after administration of the composition.

[0097] In some embodiments, the subject is mammalian. In some embodiments, the subject is human. In some embodiments, the subject is cow, pig, monkey, sheep, dog, cat, fish, or poultry.

[0098] In some embodiments, the use of a guide RNAs comprising any one or more of the guide sequences in Table 1 or one or more sgRNAs from Table 2 (e.g., in a composition provided herein) is provided for the preparation of a medicament for treating a human subject having ATTR.

[0099] In some embodiments, the guide RNAs, compositions, and formulations are administered intravenously. In some embodiments, the guide RNAs, compositions, and formulations are administered into the hepatic circulation.

[00100] In some embodiments, a single administration of a composition comprising a guide RNA provided herein is sufficient to knock down expression of the mutant protein. In some embodiments, a single administration of a composition comprising a guide RNA provided herein is sufficient to knock out expression of the mutant protein in a population of cells. In other embodiments, more than one administration of a composition comprising a guide RNA provided herein may be beneficial to maximize editing via cumulative effects.

For example, a composition provided herein can be administered 2, 3, 4, 5, or more times, such as 2 times. Administrations can be separated by a period of time ranging from, e.g., 1 day to 2 years, such as 1 to 7 days, 7 to 14 days, 14 days to 30 days, 30 days to 60 days, 60 days to 120 days, 120 days to 183 days, 183 days to 274 days, 274 days to 366 days, or 366 days to 2 years.

[00101] In some embodiments, a composition is administered in an effective amount in the range of 0.01 to 10 mg/kg (mpk), e.g., 0.01 to 0.1 mpk, 0.1 to 0.3 mpk, 0.3 to 0.5 mpk, 0.5 to 1 mpk, 1 to 2 mpk, 2 to 3 mpk, 3 to 5 mpk, 5 to 10 mpk, or 0.1, 0.2, 0.3, 0.5, 1, 2, 3, 5, or 10 mpk.

[00102] In some embodiments, the efficacy of treatment with the compositions of the invention is seen at 1 year, 2 years, 3 years, 4 years, 5 years, or 10 years after delivery. In some embodiments, efficacy of treatment with the compositions of the invention is assessed by measuring serum levels of TTR before and after treatment. In some embodiments, efficacy of treatment with the compositions assessed via a reduction of serum levels of TTR is seen at 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, or at 11 months.

[00103] In some embodiments, treatment slows or halts disease progression.

[00104] In some embodiments, treatment slows or halts progression of FAP. In some embodiments, treatment results in improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy or autonomic neuropathy.

[00105] In some embodiments, treatment results in improvement, stabilization, or slowing of change in symptoms of FAC. In some embodiments, treatment results in improvement, stabilization, or slowing of change symptoms of restrictive cardiomyopathy or congestive heart failure.

[00106] In some embodiments, efficacy of treatment is measured by increased survival time of the subject.

[00107] In some embodiments, efficacy of treatment is measured by improvement or slowing of progression in symptoms of sensorimotor or autonomic neuropathy. In some embodiments, efficacy of treatment is measured by an increase or a a slowing of decrease in ability to move an area of the body or to feel in any area of the body. In some embodiments, efficacy of treatment is measured by improvement or a slowing of decrease in the ability to swallow; breath; use arms, hands, legs, or feet; or walk. In some embodiments, efficacy of treatment is measured by improvement or a slowing of progression of neuralgia. In some embodiments, the neuralgia is characterized by pain, burning, tingling, or abnormal feeling.

In some embodiments, efficacy of treatment is measured by improvement or a slowing of increase in postural hypotension, dizziness, gastrointestinal dysmotility, bladder dysfunction, or sexual dysfunction. In some embodiments, efficacy of treatment is measured by improvement or a slowing of progression of weakness. In some embodiments, efficacy of treatment is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

[00108] In some embodiments, efficacy of treatment is measured by improvement or slowing of progression of symptoms of congestive heart failure or CHF. In some embodiments, efficacy of treatment is measured by an decrease or a slowing of increase in shortness of breath, trouble breathing, fatigue, or swelling in the ankles, feet, legs, abdomen, or veins in the the neck. In some embodiments, efficacy of treatment is measured by improvement or a slowing of progression of fluid buildup in the body, which may be assessed by measures such as weight gain, frequent urination, or nighttime cough. In some embodiments, efficacy of treatment is measured using cardiac biomarker tests (such as B-type natriuretic peptide [BNP] or N-terminal pro b-type natriuretic peptide [NT-proBNP]), lung function tests, chest x-rays, or electrocardiography.

A. Combination Therapy

[00109] In some embodiments, the invention comprises combination therapies comprising any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein) together with an additional therapy suitable for alleviating symptoms of ATTR.

[00110] In some embodiments, the additional therapy for ATTR is a treatment for sensorimotor or autonomic neuropathy. In some embodiments, the treatment for sensorimotor or autonomic neuropathy is a nonsteroidal anti-inflammatory drug, antidepressant, anticonvulsant medication, antiarrythmic medication, or narcotic agent. In some embodiments, the antidepressant is a tricylic agent or a serotonin-norepinephrine reuptake inhibitor. In some embodiments, the antidepressant is amitriptyline, duloxetine, or venlafaxine. In some embodiments, the anticonvulsant agent is gabapentin, pregabalin, topiramate, or carbamazepine. In some embodiments, the additional therapy for sensorimotor neuropathy is transcutaneous electrical nerve stimulation.

[00111] In some embodiments, the additional therapy for ATTR is a treatment for restrictive cardiomyopathy or congestive heart failure (CHF). In some embodiments, the treatment for CHF is a ACE inhibitor, aldosterone antagonist, angiotensin receptor blocker,

beta blocker, digoxin, diuretic, or isosorbide dinitrate/hydralazine hydrochloride. In some embodiments, the ACE inhibitor is enalapril, captopril, ramipril, perindopril, imidapril, or quinapril. In some embodiments, the aldosterone antagonist is eplerenone or spironolactone. In some embodiments, the angiotensin receptor blocker is azilsartan, cadesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, or valsartan. In some embodiments, the beta blocker is acebutolol, atenolol, bisoprolol, metoprolol, nadolol, nebivolol, or propranolol. In some embodiments, the diuretic is chlorothiazide, chlorthalidone, hydrochlorothiazide, indapamide, metolazone, bumetanide, furosemide, torsemide, amiloride, or triameterene.

[00112] In some embodiments, the combination therapy comprises any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein) together with a siRNA that targets TTR or mutant TTR. In some embodiments, the siRNA is any siRNA capable of further reducing or eliminating the expression of wild type or mutant TTR. In some embodiments, the siRNA is the drug Patisiran (ALN-TTR02) or ALN-TTRsc02. In some embodiments, the siRNA is administered after any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein). In some embodiments, the siRNA is administered on a regular basis following treatment with any of the gRNA compositions provided herein.

[00113] In some embodiments, the combination therapy comprises any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein) together with antisense nucleotide that targets TTR or mutant TTR. In some embodiments, the antisense nucleotide is any antisense nucleotide capable of further reducing or eliminating the expression of wild type or mutant TTR. In some embodiments, the antisense nucleotide is the drug Inotersen (IONS-TTR_{Rx}). In some embodiments, the antisense nucleotide is administered after any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein). In some embodiments, the antisense nucleotide is administered on a regular basis following treatment with any of the gRNA compositions provided herein.

[00114] In some embodiments, the combination therapy comprises any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein) together with a small molecule stabilizer that promotes kinetic stabilization of the correctly folded tetrameric form of TTR. In some embodiments, the small molecule stabilizer is the drug tafamidis

(Vyndaqel®) or diflunisal. In some embodiments, the small molecule stabilizer is administered after any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein). In some embodiments, the small molecule stabilizer is administered on a regular basis following treatment with any of the gRNA compositions provided herein.

B. Delivery of gRNA Compositions

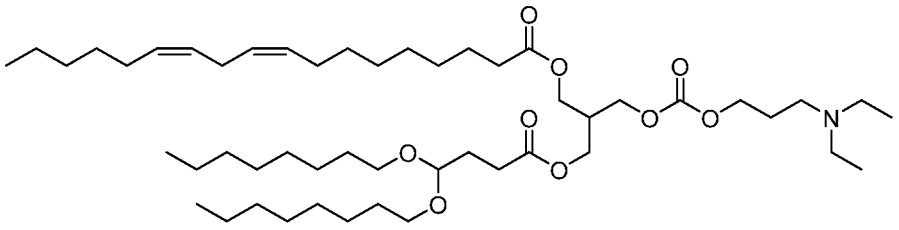
[00115] In some embodiments, the guide RNA compositions described herein, alone or encoded on one or more vectors, are formulated in or administered via a lipid nanoparticle; see e.g., PCT/US2017/024973, filed March 30, 2017 entitled "LIPID NANOPARTICLE FORMULATIONS FOR CRISPR/CAS COMPONENTS," the contents of which are hereby incorporated by reference in their entirety. Any lipid nanoparticle (LNP) known to those of skill in the art to be capable of delivering nucleotides to subjects may be utilized with the guide RNAs described herein, as well as either mRNA encoding an RNA-guided DNA nuclease such as Cas or Cas9, or an RNA-guided DNA nuclease such as Cas or Cas9 protein itself.

[00116] Disclosed herein are various embodiments of LNP formulations for RNAs, including CRISPR/Cas cargoes. Such LNP formulations may include (i) a CCD lipid, such as an amine lipid, (ii) a neutral lipid, (iii) a helper lipid, and (iv) a stealth lipid, such as a PEG lipid. Some embodiments of the LNP formulations include an "amine lipid", along with a helper lipid, a neutral lipid, and a stealth lipid such as a PEG lipid. By "lipid nanoparticle" is meant a particle that comprises a plurality of (i.e. more than one) lipid molecules physically associated with each other by intermolecular forces.

[00117] CCD Lipids

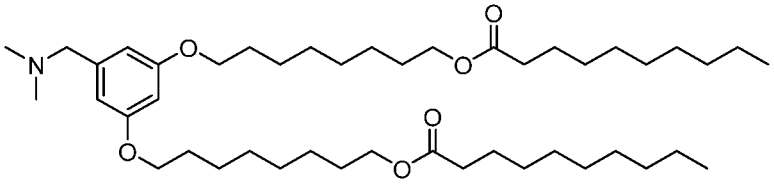
[00118] Lipid compositions for delivery of CRISPR/Cas mRNA and guide RNA components to a liver cell comprise a CCD Lipid.

[00119] In some embodiments, the CCD lipid is Lipid A, which is (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate. Lipid A can be depicted as:

[00120] 

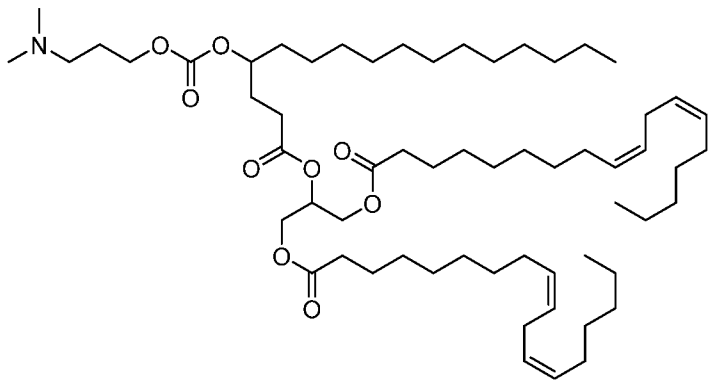
[00121] Lipid A may be synthesized according to WO2015/095340 (e.g., pp. 84-86).

[00122] In some embodiments, the CCD lipid is Lipid B, which is ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate), also called ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl) bis(decanoate). Lipid B can be depicted as:

[00123] 

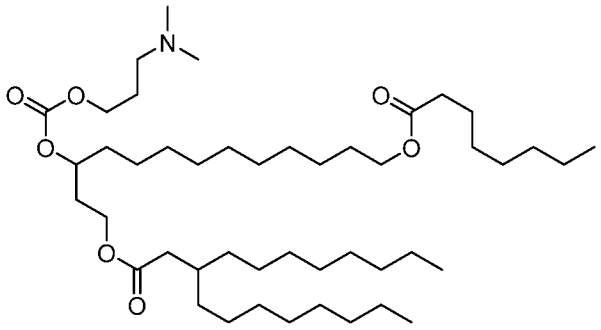
[00124] Lipid B may be synthesized according to WO2014/136086 (e.g., pp. 107-09).

[00125] In some embodiments, the CCD lipid is Lipid C, which is 2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1,3-diyl (9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate). Lipid C can be depicted as:



[00126] In some embodiments, the CCD lipid is Lipid D, which is 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl 3-octylundecanoate.

[00127] Lipid D can be depicted as:



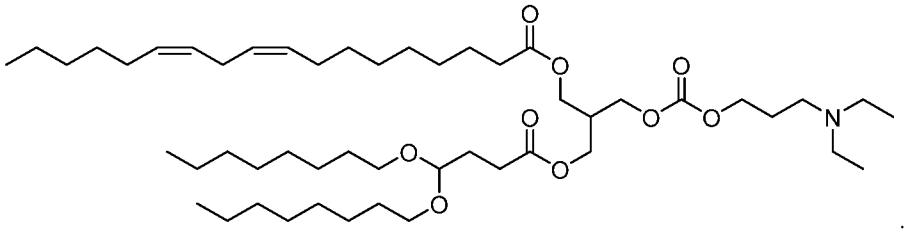
[00128] Lipid C and Lipid D may be synthesized according to WO2015/095340.

[00129] The CCD lipid can also be an equivalent to Lipid A, Lipid B, Lipid C, or Lipid D. In certain embodiments, the CCD lipid is an equivalent to Lipid A, an equivalent to Lipid B, an equivalent to Lipid C, or an equivalent to Lipid D.

[00130] Amine Lipids

[00131] In some embodiments, the LNP compositions for the delivery of biologically active agents comprise an "amine lipid", which is defined as Lipid A, Lipid B, Lipid C, Lipid D or equivalents of Lipid A (including acetal analogs of Lipid A), equivalents of Lipid B, equivalents of Lipid C, and equivalents of Lipid D.

[00132] In some embodiments, the amine lipid is Lipid A, which is (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate. Lipid A can be depicted as:



[00133] Lipid A may be synthesized according to WO2015/095340 (e.g., pp. 84-86). In certain embodiments, the amine lipid is an equivalent to Lipid A.

[00134] In certain embodiments, an amine lipid is an analog of Lipid A. In certain embodiments, a Lipid A analog is an acetal analog of Lipid A. In particular LNP compositions, the acetal analog is a C4-C12 acetal analog. In some embodiments, the acetal analog is a C5-C12 acetal analog. In additional embodiments, the acetal analog is a C5-C10

acetal analog. In further embodiments, the acetal analog is chosen from a C4, C5, C6, C7, C9, C10, C11, and C12 acetal analog.

[00135] Amine lipids suitable for use in the LNPs described herein are biodegradable in vivo. The amine lipids have low toxicity (e.g., are tolerated in animal models without adverse effect in amounts of greater than or equal to 10 mg/kg). In certain embodiments, LNPs comprising an amine lipid include those where at least 75% of the amine lipid is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days. In certain embodiments, LNPs comprising an amine lipid include those where at least 50% of the mRNA or gRNA is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days. In certain embodiments, LNPs comprising an amine lipid include those where at least 50% of the LNP is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days, for example by measuring a lipid (e.g. an amine lipid), RNA (e.g. mRNA), or other component. In certain embodiments, lipid-encapsulated versus free lipid, RNA, or nucleic acid component of the LNP is measured.

[00136] Lipid clearance may be measured as described in literature. See Maier, M.A., et al. Biodegradable Lipids Enabling Rapidly Eliminated Lipid Nanoparticles for Systemic Delivery of RNAi Therapeutics. Mol. Ther. 2013, 21(8), 1570-78 ("Maier"). For example, in Maier, LNP-siRNA systems containing luciferases-targeting siRNA were administered to six- to eight-week old male C57Bl/6 mice at 0.3 mg/kg by intravenous bolus injection via the lateral tail vein. Blood, liver, and spleen samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, and 168 hours post-dose. Mice were perfused with saline before tissue collection and blood samples were processed to obtain plasma. All samples were processed and analyzed by LC-MS. Further, Maier describes a procedure for assessing toxicity after administration of LNP-siRNA formulations. For example, a luciferase-targeting siRNA was administered at 0, 1, 3, 5, and 10 mg/kg (5 animals/group) via single intravenous bolus injection at a dose volume of 5 mL/kg to male Sprague-Dawley rats. After 24 hours, about 1 mL of blood was obtained from the jugular vein of conscious animals and the serum was isolated. At 72 hours post-dose, all animals were euthanized for necropsy. Assessment of clinical signs, body weight, serum chemistry, organ weights and histopathology was performed. Although Maier describes methods for assessing siRNA-LNP formulations, these methods may be applied to assess clearance, pharmacokinetics, and toxicity of administration of LNP compositions of the present disclosure.

[00137] The amine lipids lead to an increased clearance rate. In some embodiments, the clearance rate is a lipid clearance rate, for example the rate at which an amine lipid is cleared from the blood, serum, or plasma. In some embodiments, the clearance rate is an RNA clearance rate, for example the rate at which an mRNA or a gRNA is cleared from the blood, serum, or plasma. In some embodiments, the clearance rate is the rate at which LNP is cleared from the blood, serum, or plasma. In some embodiments, the clearance rate is the rate at which LNP is cleared from a tissue, such as liver tissue or spleen tissue. In certain embodiments, a high rate of clearance rate leads to a safety profile with no substantial adverse effects. The amine lipids reduce LNP accumulation in circulation and in tissues. In some embodiments, a reduction in LNP accumulation in circulation and in tissues leads to a safety profile with no substantial adverse effects.

[00138] The amine lipids of the present disclosure may be ionizable depending upon the pH of the medium they are in. For example, in a slightly acidic medium, the amine lipids may be protonated and thus bear a positive charge. Conversely, in a slightly basic medium, such as, for example, blood where pH is approximately 7.35, the amine lipids may not be protonated and thus bear no charge. In some embodiments, the amine lipids of the present disclosure may be protonated at a pH of at least about 9. In some embodiments, the amine lipids of the present disclosure may be protonated at a pH of at least about 9. In some embodiments, the amine lipids of the present disclosure may be protonated at a pH of at least about 10.

[00139] The ability of an amine lipid to bear a charge is related to its intrinsic pKa. For example, the amine lipids of the present disclosure may each, independently, have a pKa in the range of from about 5.8 to about 6.2. For example, the amine lipids of the present disclosure may each, independently, have a pKa in the range of from about 5.8 to about 6.5. This may be advantageous as it has been found that cationic lipids with a pKa ranging from about 5.1 to about 7.4 are effective for delivery of cargo in vivo, e.g. to the liver. Further, it has been found that cationic lipids with a pKa ranging from about 5.3 to about 6.4 are effective for delivery in vivo, e.g. to tumors. See, e.g., WO2014/136086.

[00140] Additional Lipids

[00141] "Neutral lipids" suitable for use in a lipid composition of the disclosure include, for example, a variety of neutral, uncharged or zwitterionic lipids. Examples of neutral phospholipids suitable for use in the present disclosure include, but are not limited to, 5-heptadecylbenzene-1,3-diol (resorcinol), dipalmitoylphosphatidylcholine (DPPC),

distearoylphosphatidylcholine (DSPC), pohsphocholine (DOPC), dimyristoylphosphatidylcholine (DMPC), phosphatidylcholine (PLPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DAPC), phosphatidylethanolamine (PE), egg phosphatidylcholine (EPC), dilauryloylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), 1-myristoyl-2-palmitoyl phosphatidylcholine (MPPC), 1-palmitoyl-2-myristoyl phosphatidylcholine (PMPC), 1-palmitoyl-2-stearoyl phosphatidylcholine (PSPC), 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DBPC), 1-stearoyl-2-palmitoyl phosphatidylcholine (SPPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine (DEPC), palmitoyloleoyl phosphatidylcholine (POPC), lysophosphatidyl choline, dioleoyl phosphatidylethanolamine (DOPE), dilinoleoylphosphatidylcholine distearoylphosphatidylethanolamine (DSPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyl phosphatidylethanolamine (DPPE), palmitoyloleoyl phosphatidylethanolamine (POPE), lysophosphatidylethanolamine and combinations thereof. In one embodiment, the neutral phospholipid may be selected from the group consisting of distearoylphosphatidylcholine (DSPC) and dimyristoyl phosphatidyl ethanolamine (DMPE). In another embodiment, the neutral phospholipid may be distearoylphosphatidylcholine (DSPC).

[00142] "Helper lipids" include steroids, sterols, and alkyl resorcinols. Helper lipids suitable for use in the present disclosure include, but are not limited to, cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate. In one embodiment, the helper lipid may be cholesterol. In one embodiment, the helper lipid may be cholesterol hemisuccinate.

[00143] "Stealth lipids" are lipids that alter the length of time the nanoparticles can exist in vivo (e.g., in the blood). Stealth lipids may assist in the formulation process by, for example, reducing particle aggregation and controlling particle size. Stealth lipids used herein may modulate pharmacokinetic properties of the LNP. Stealth lipids suitable for use in a lipid composition of the disclosure include, but are not limited to, stealth lipids having a hydrophilic head group linked to a lipid moiety. Stealth lipids suitable for use in a lipid composition of the present disclosure and information about the biochemistry of such lipids can be found in Romberg et al., Pharmaceutical Research, Vol. 25, No. 1, 2008, pg. 55-71 and Hoekstra et al., Biochimica et Biophysica Acta 1660 (2004) 41-52. Additional suitable PEG lipids are disclosed, e.g., in WO 2006/007712.

[00144] In one embodiment, the hydrophilic head group of stealth lipid comprises a polymer moiety selected from polymers based on PEG. Stealth lipids may comprise a lipid moiety. In some embodiments, the stealth lipid is a PEG lipid.

[00145] In one embodiment, a stealth lipid comprises a polymer moiety selected from polymers based on PEG (sometimes referred to as poly(ethylene oxide)), poly(oxazoline), poly(vinyl alcohol), poly(glycerol), poly(N-vinylpyrrolidone), polyaminoacids and poly[N-(2-hydroxypropyl)methacrylamide].

[00146] In one embodiment, the PEG lipid comprises a polymer moiety based on PEG (sometimes referred to as poly(ethylene oxide)).

[00147] The PEG lipid further comprises a lipid moiety. In some embodiments, the lipid moiety may be derived from diacylglycerol or diacylglycamide, including those comprising a dialkylglycerol or dialkylglycamide group having alkyl chain length independently comprising from about C4 to about C40 saturated or unsaturated carbon atoms, wherein the chain may comprise one or more functional groups such as, for example, an amide or ester. In some embodiments, the alkyl chail length comprises about C10 to C20. The dialkylglycerol or dialkylglycamide group can further comprise one or more substituted alkyl groups. The chain lengths may be symmetrical or assymetric.

[00148] Unless otherwise indicated, the term "PEG" as used herein means any polyethylene glycol or other polyalkylene ether polymer. In one embodiment, PEG is an optionally substituted linear or branched polymer of ethylene glycol or ethylene oxide. In one embodiment, PEG is unsubstituted. In one embodiment, the PEG is substituted, e.g., by one or more alkyl, alkoxy, acyl, hydroxy, or aryl groups. In one embodiment, the term includes PEG copolymers such as PEG-polyurethane or PEG-polypropylene (see, e.g., J. Milton Harris, Poly(ethylene glycol) chemistry: biotechnical and biomedical applications (1992)); in another embodiment, the term does not include PEG copolymers. In one embodiment, the PEG has a molecular weight of from about 130 to about 50,000, in a sub-embodiment, about 150 to about 30,000, in a sub-embodiment, about 150 to about 20,000, in a sub-embodiment about 150 to about 15,000, in a sub-embodiment, about 150 to about 10,000, in a sub-embodiment, about 150 to about 6,000, in a sub-embodiment, about 150 to about 5,000, in a sub-embodiment, about 150 to about 4,000, in a sub-embodiment, about 150 to about 3,000, in a sub-embodiment, about 300 to about 3,000, in a sub-embodiment, about 1,000 to about 3,000, and in a sub-embodiment, about 1,500 to about 2,500.

[00149] In certain embodiments, the PEG (e.g., conjugated to a lipid moiety or lipid, such as a stealth lipid), is a "PEG-2K," also termed "PEG 2000," which has an average molecular weight of about 2,000 daltons. PEG-2K is represented herein by the following formula (I), wherein n is 45, meaning that the number averaged degree of polymerization comprises about 45 subunits. However, other PEG embodiments known in the art may be used, including, e.g., those where the number-averaged degree of polymerization comprises about 23 subunits (n=23), and/or 68 subunits (n=68). In some embodiments, n may range from about 30 to about 60. In some embodiments, n may range from about 35 to about 55. In some embodiments, n may range from about 40 to about 50. In some embodiments, n may range from about 42 to about 48. In some embodiments, n may be 45. In some embodiments, R may be selected from H, substituted alkyl, and unsubstituted alkyl. In some embodiments, R may be unsubstituted alkyl. In some embodiments, R may be methyl.

[00150] In any of the embodiments described herein, the PEG lipid may be selected from PEG-dilauroylglycerol, PEG-dimyristoylglycerol (PEG-DMG) (catalog # GM-020 from NOF, Tokyo, Japan), PEG-dipalmitoylglycerol, PEG-distearoylglycerol (PEG-DSPE) (catalog # DSPE-020CN, NOF, Tokyo, Japan), PEG-dilaurylglycamide, PEG-dimyristylglycamide, PEG-dipalmitoylglycamide, and PEG-distearoylglycamide, PEG-cholesterol (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl]carbamoyl-[omega]-methyl-poly(ethylene glycol), PEG-DMB (3,4-ditetradecoxylbenzyl-[omega]-methyl-poly(ethylene glycol)ether), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DMG) (cat. #880150P from Avanti Polar Lipids, Alabaster, Alabama, USA), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSPE) (cat. #880120C from Avanti Polar Lipids, Alabaster, Alabama, USA), 1,2-distearoyl-sn-glycerol, methoxypolyethylene glycol (PEG2k-DSG; GS-020, NOF Tokyo, Japan), poly(ethylene glycol)-2000-dimethacrylate (PEG2k-DMA), and 1,2-distearyloxypropyl-3-amine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSA). In one embodiment, the PEG lipid may be PEG2k-DMG. In some embodiments, the PEG lipid may be PEG2k-DSG. In one embodiment, the PEG lipid may be PEG2k-DSPE. In one embodiment, the PEG lipid may be PEG2k-DMA. In one embodiment, the PEG lipid may be PEG2k-C-DMA. In one embodiment, the PEG lipid may be compound S027, disclosed in WO2016/010840 (paragraphs [00240] to [00244]). In one embodiment, the PEG lipid may be PEG2k-DSA. In one embodiment, the PEG lipid may be PEG2k-C11. In some embodiments, the PEG lipid may be PEG2k-C14. In some

embodiments, the PEG lipid may be PEG2k-C16. In some embodiments, the PEG lipid may be PEG2k-C18.

[00151] LNP Formulations

[00152] The LNP may contain (i) an amine lipid for encapsulation and for endosomal escape, (ii) a neutral lipid for stabilization, (iii) a helper lipid, also for stabilization, and (iv) a stealth lipid, such as a PEG lipid.

[00153] In some embodiments, an LNP composition may comprise an RNA component that includes one or more of an RNA-guided DNA-binding agent, a Cas nuclease mRNA, a Class 2 Cas nuclease mRNA, a Cas9 mRNA, and a gRNA. In some embodiments, an LNP composition may include a Class 2 Cas nuclease and a gRNA as the RNA component. In certain embodiments, an LNP composition may comprise the RNA component, an amine lipid, a helper lipid, a neutral lipid, and a stealth lipid. In certain LNP compositions, the helper lipid is cholesterol. In other compositions, the neutral lipid is DSPC. In additional embodiments, the stealth lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the LNP composition comprises Lipid A or an equivalent of Lipid A; a helper lipid; a neutral lipid; a stealth lipid; and a guide RNA. In certain compositions, the amine lipid is Lipid A. In certain compositions, the amine lipid is Lipid A or an acetal analog thereof; the helper lipid is cholesterol; the neutral lipid is DSPC; and the stealth lipid is PEG2k-DMG.

[00154] In certain embodiments, lipid compositions are described according to the respective molar ratios of the component lipids in the formulation. Embodiments of the present disclosure provide lipid compositions described according to the respective molar ratios of the component lipids in the formulation. In one embodiment, the mol-% of the amine lipid may be from about 30 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 40 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 45 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 50 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 55 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 50 mol-% to about 55 mol-%. In one embodiment, the mol-% of the amine lipid may be about 50 mol-%. In one embodiment, the mol-% of the amine lipid may be about 55 mol-%. In some embodiments, the amine lipid mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target mol-%. In some embodiments, the amine lipid mol-% of the LNP batch will be ±4 mol-%, ±3 mol-%, ±2 mol-%, ±1.5 mol-%, ±1 mol-%, ±0.5 mol-%,

or ±0.25 mol-% of the target mol-%. All mol-% numbers are given as a fraction of the lipid component of the LNP compositions. In certain embodiments, LNP inter-lot variability of the amine lipid mol-% will be less than 15%, less than 10% or less than 5%.

[00155] In one embodiment, the mol-% of the neutral lipid may be from about 5 mol-% to about 15 mol-%. In one embodiment, the mol-% of the neutral lipid may be from about 7 mol-% to about 12 mol-%. In one embodiment, the mol-% of the neutral lipid may be about 9 mol-%. In some embodiments, the neutral lipid mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target neutral lipid mol-%. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[00156] In one embodiment, the mol-% of the helper lipid may be from about 20 mol-% to about 60 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 25 mol-% to about 55 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 25 mol-% to about 50 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 25 mol-% to about 40 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 30 mol-% to about 50 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 30 mol-% to about 40 mol-%. In one embodiment, the mol-% of the helper lipid is adjusted based on amine lipid, neutral lipid, and PEG lipid concentrations to bring the lipid component to 100 mol-%. In some embodiments, the helper mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target mol-%. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[00157] In one embodiment, the mol-% of the PEG lipid may be from about 1 mol-% to about 10 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2 mol-% to about 10 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2 mol-% to about 8 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2 mol-% to about 4 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2.5 mol-% to about 4 mol-%. In one embodiment, the mol-% of the PEG lipid may be about 3 mol-%. In one embodiment, the mol-% of the PEG lipid may be about 2.5 mol-%. In some embodiments, the PEG lipid mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target PEG lipid mol-%. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[00158] In certain embodiments, the cargo includes an mRNA encoding an RNA-guided DNA-binding agent (e.g. a Cas nuclease, a Class 2 Cas nuclease, or Cas9), and a gRNA or a

nucleic acid encoding a gRNA, or a combination of mRNA and gRNA. In one embodiment, an LNP composition may comprise a Lipid A or its equivalents. In some aspects, the amine lipid is Lipid A. In some aspects, the amine lipid is a Lipid A equivalent, e.g. an analog of Lipid A. In certain aspects, the amine lipid is an acetal analog of Lipid A. In various embodiments, an LNP composition comprises an amine lipid, a neutral lipid, a helper lipid, and a PEG lipid. In certain embodiments, the helper lipid is cholesterol. In certain embodiments, the neutral lipid is DSPC. In specific embodiments, PEG lipid is PEG2k-DMG. In some embodiments, an LNP composition may comprise a Lipid A, a helper lipid, a neutral lipid, and a PEG lipid. In some embodiments, an LNP composition comprises an amine lipid, DSPC, cholesterol, and a PEG lipid. In some embodiments, the LNP composition comprises a PEG lipid comprising DMG. In certain embodiments, the amine lipid is selected from Lipid A, and an equivalent of Lipid A, including an acetal analog of Lipid A. In additional embodiments, an LNP composition comprises Lipid A, cholesterol, DSPC, and PEG2k-DMG.

[00159] Embodiments of the present disclosure also provide lipid compositions described according to the molar ratio between the positively charged amine groups of the amine lipid (N) and the negatively charged phosphate groups (P) of the nucleic acid to be encapsulated. This may be mathematically represented by the equation N/P. In some embodiments, an LNP composition may comprise a lipid component that comprises an amine lipid, a helper lipid, a neutral lipid, and a helper lipid; and a nucleic acid component, wherein the N/P ratio is about 3 to 10. In some embodiments, an LNP composition may comprise a lipid component that comprises an amine lipid, a helper lipid, a neutral lipid, and a helper lipid; and an RNA component, wherein the N/P ratio is about 3 to 10. In one embodiment, the N/P ratio may about 5-7. In one embodiment, the N/P ratio may about 4.5-8. In one embodiment, the N/P ratio may about 6. In one embodiment, the N/P ratio may be 6 ±1. In one embodiment, the N/P ratio may about 6 ± 0.5. In some embodiments, the N/P ratio will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target N/P ratio. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[00160] In some embodiments, the RNA component may comprise an mRNA, such as an mRNA disclosed herein, e.g., encoding a Cas nuclease. In one embodiment, RNA component may comprise a Cas9 mRNA. In some compositions comprising an mRNA encoding a Cas nuclease, the LNP further comprises a gRNA nucleic acid, such as a gRNA. In some embodiments, the RNA component comprises a Cas nuclease mRNA and a gRNA.

In some embodiments, the RNA component comprises a Class 2 Cas nuclease mRNA and a gRNA.

[00161] In certain embodiments, an LNP composition may comprise an mRNA disclosed herein, e.g., encoding a Cas nuclease, such as a Class 2 Cas nuclease, an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid. In certain LNP compositions comprising an mRNA encoding a Cas nuclease such as a Class 2 Cas nuclease, the helper lipid is cholesterol. In other compositions comprising an mRNA encoding a Cas nuclease such as a Class 2 Cas nuclease, the neutral lipid is DSPC. In additional embodiments comprising an mRNA encoding a Cas nuclease such as a Class 2 Cas nuclease, the PEG lipid is PEG2k-DMG or PEG2k-C11. In specific compositions comprising an mRNA encoding a Cas nuclease such as a Class 2 Cas nuclease, the amine lipid is selected from Lipid A and its equivalents, such as an acetal analog of Lipid A.

[00162] In some embodiments, an LNP composition may comprise a gRNA. In certain embodiments, an LNP composition may comprise an amine lipid, a gRNA, a helper lipid, a neutral lipid, and a PEG lipid. In certain LNP compositions comprising a gRNA, the helper lipid is cholesterol. In some compositions comprising a gRNA, the neutral lipid is DSPC. In additional embodiments comprising a gRNA, the PEG lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the amine lipid is selected from Lipid A and its equivalents, such as an acetal analog of Lipid A.

[00163] In one embodiment, an LNP composition may comprise an sgRNA. In one embodiment, an LNP composition may comprise a Cas9 sgRNA. In one embodiment, an LNP composition may comprise a Cpf1 sgRNA. In some compositions comprising an sgRNA, the LNP includes an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid. In certain compositions comprising an sgRNA, the helper lipid is cholesterol. In other compositions comprising an sgRNA, the neutral lipid is DSPC. In additional embodiments comprising an sgRNA, the PEG lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the amine lipid is selected from Lipid A and its equivalents, such as acetal analogs of Lipid A.

[00164] In certain embodiments, an LNP composition comprises an mRNA encoding a Cas nuclease and a gRNA, which may be an sgRNA. In one embodiment, an LNP composition may comprise an amine lipid, an mRNA encoding a Cas nuclease, a gRNA, a helper lipid, a neutral lipid, and a PEG lipid. In certain compositions comprising an mRNA encoding a Cas nuclease and a gRNA, the helper lipid is cholesterol. In some compositions

comprising an mRNA encoding a Cas nuclease and a gRNA, the neutral lipid is DSPC. In additional embodiments comprising an mRNA encoding a Cas nuclease and a gRNA, the PEG lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the amine lipid is selected from Lipid A and its equivalents, such as acetal analogs of Lipid A.

[00165] In certain embodiments, the LNP compositions include a Cas nuclease mRNA, such as a Class 2 Cas mRNA and at least one gRNA. In certain embodiments, the LNP composition includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease mRNA from about 25:1 to about 1:25. In certain embodiments, the LNP formulation includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease mRNA from about 10:1 to about 1:10. In certain embodiments, the LNP formulation includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease mRNA from about 8:1 to about 1:8. As measured herein, the ratios are by weight. In some embodiments, the LNP formulation includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas mRNA from about 5:1 to about 1:5. In some embodiments, ratio range is about 3:1 to 1:3, about 2:1 to 1:2, about 5:1 to 1:2, about 5:1 to 1:1, about 3:1 to 1:2, about 3:1 to 1:1, about 3:1, about 2:1 to 1:1. In some embodiments, the gRNA to mRNA ratio is about 3:1 or about 2:1 In some embodiments the ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease is about 1:1. The ratio may be about 25:1, 10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10, or 1:25.

[00166] The LNP compositions disclosed herein may include a template nucleic acid. The template nucleic acid may be co-formulated with an mRNA encoding a Cas nuclease, such as a Class 2 Cas nuclease mRNA. In some embodiments, the template nucleic acid may be co-formulated with a guide RNA. In some embodiments, the template nucleic acid may be co-formulated with both an mRNA encoding a Cas nuclease and a guide RNA. In some embodiments, the template nucleic acid may be formulated separately from an mRNA encoding a Cas nuclease or a guide RNA. The template nucleic acid may be delivered with, or separately from the LNP compositions. In some embodiments, the template nucleic acid may be single- or double-stranded, depending on the desired repair mechanism. The template may have regions of homology to the target DNA, or to sequences adjacent to the target DNA.

[00167] In some embodiments, LNPs are formed by mixing an aqueous RNA solution with an organic solvent-based lipid solution, e.g., 100% ethanol. Suitable solutions or solvents include or may contain: water, PBS, Tris buffer, NaCl, citrate buffer, ethanol, chloroform, diethylether, cyclohexane, tetrahydrofuran, methanol, isopropanol. A

pharmaceutically acceptable buffer, e.g., for in vivo administration of LNPs, may be used. In certain embodiments, a buffer is used to maintain the pH of the composition comprising LNPs at or above pH 6.5. In certain embodiments, a buffer is used to maintain the pH of the composition comprising LNPs at or above pH 7.0. In certain embodiments, the composition has a pH ranging from about 7.2 to about 7.7. In additional embodiments, the composition has a pH ranging from about 7.3 to about 7.7 or ranging from about 7.4 to about 7.6. In further embodiments, the composition has a pH of about 7.2, 7.3, 7.4, 7.5, 7.6, or 7.7. The pH of a composition may be measured with a micro pH probe. In certain embodiments, a cryoprotectant is included in the composition. Non-limiting examples of cryoprotectants include sucrose, trehalose, glycerol, DMSO, and ethylene glycol. Exemplary compositions may include up to 10% cryoprotectant, such as, for example, sucrose. In certain embodiments, the LNP composition may include about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10% cryoprotectant. In certain embodiments, the LNP composition may include about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10% sucrose. In some embodiments, the LNP composition may include a buffer. In some embodiments, the buffer may comprise a phosphate buffer (PBS), a Tris buffer, a citrate buffer, and mixtures thereof. In certain exemplary embodiments, the buffer comprises NaCl. In certain emboidments, NaCl is omitted. Exemplary amounts of NaCl may range from about 20 mM to about 45 mM. Exemplary amounts of NaCl may range from about 40 mM to about 50 mM. In some embodiments, the amount of NaCl is about 45 mM. In some embodiments, the buffer is a Tris buffer. Exemplary amounts of Tris may range from about 20 mM to about 60 mM. Exemplary amounts of Tris may range from about 40 mM to about 60 mM. In some embodiments, the amount of Tris is about 50 mM. In some embodiments, the buffer comprises NaCl and Tris. Certain exemplary embodiments of the LNP compositions contain 5% sucrose and 45 mM NaCl in Tris buffer. In other exemplary embodiments, compositions contain sucrose in an amount of about 5% w/v, about 45 mM NaCl, and about 50 mM Tris at pH 7.5. The salt, buffer, and cryoprotectant amounts may be varied such that the osmolality of the overall formulation is maintained. For example, the final osmolality may be maintained at less than 450 mOsm/L. In further embodiments, the osmolality is between 350 and 250 mOsm/L. Certain embodiments have a final osmolality of 300 +/- 20 mOsm/L.

[00168] In some embodiments, microfluidic mixing, T-mixing, or cross-mixing is used. In certain aspects, flow rates, junction size, junction geometry, junction shape, tube diameter, solutions, and/or RNA and lipid concentrations may be varied. LNPs or LNP compositions

may be concentrated or purified, e.g., via dialysis, tangential flow filtration, or chromatography. The LNPs may be stored as a suspension, an emulsion, or a lyophilized powder, for example. In some embodiments, an LNP composition is stored at 2-8° C, in certain aspects, the LNP compositions are stored at room temperature. In additional embodiments, an LNP composition is stored frozen, for example at -20° C or -80° C. In other embodiments, an LNP composition is stored at a temperature ranging from about 0° C to about -80° C. Frozen LNP compositions may be thawed before use, for example on ice, at 4° C, at room temperature, or at 25° C. Frozen LNP compositions may be maintained at various temperatures, for example on ice, at 4° C, at room temperature, at 25° C, or at 37° C.

[00169] In some embodiments, an LNP composition has greater than about 80% encapsulation. In some embodiments, an LNP composition has a particle size less than about 120 nm. In some embodiments, an LNP composition has a pdi less than about 0.2. In some embodiments, at least two of these features are present. In some embodiments, each of these three features is present. Analytical methods for determining these parameters are discussed below in the general reagents and methods section.

[00170] In some embodiments, microfluidic mixing, T-mixing, or cross-mixing is used. In certain aspects, flow rates, junction size, junction geometry, junction shape, tube diameter, solutions, and/or RNA and lipid concentrations may be varied. LNPs or LNP compositions may be concentrated or purified, e.g., via dialysis or chromatography. The LNPs may be stored as a suspension, an emulsion, or a lyophilized powder, for example. In some embodiments, the LNP compositions are stored at 2-8° C, in certain aspects, the LNP compositions are stored at room temperature. In additional embodiments, the LNP composition is stored frozen, for example at -20° C or -80° C. In other embodiments, the LNP compositionis stored at a temperature ranging from 0° C to -80° C. Frozen LNP compositions may be thawed before use, for example on ice, at room temperature, or at 25° C.

[00171] Dynamic Light Scattering ("DLS") can be used to characterize the polydispersity index ("pdi") and size of the LNPs of the present disclosure. DLS measures the scattering of light that results from subjecting a sample to a light source. PDI, as determined from DLS measurements, represents the distribution of particle size (around the mean particle size) in a population, with a perfectly uniform population having a PDI of zero. In some embodiments, the pdi may range from 0.005 to 0.75. In some embodiments, the pdi may range from 0.01 to 0.5. In some embodiments, the pdi may range from 0.02 to 0.4. In some embodiments, the pdi may range from 0.03 to 0.35. In some embodiments, the pdi may range from 0.1 to 0.35.

[00172] In some embodiments, LNPs disclosed herein have a size of 1 to 250 nm. In some embodiments, the LNPs have a size of 10 to 200 nm. In further embodiments, the LNPs have a size of 20 to 150 nm. In some embodiments, the LNPs have a size of 50 to 150 nm. In some embodiments, the LNPs have a size of 50 to 100 nm. In some embodiments, the LNPs have a size of 50 to 120 nm. In some embodiments, the LNPs have a size of 75 to 150 nm. In some embodiments, the LNPs have a size of 30 to 200 nm. Unless indicated otherwise, all sizes referred to herein are the average sizes (diameters) of the fully formed nanoparticles, as measured by dynamic light scattering on a Malvern Zetasizer. The nanoparticle sample is diluted in phosphate buffered saline (PBS) so that the count rate is approximately 200-400 kcts. The data is presented as a weighted-average of the intensity measure. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 50% to 100%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 50% to 70%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 70% to 90%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 90% to 100%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 75% to 95%.

[00173] In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in preparing a medicament for treating ATTR. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in preparing a medicament for reducing or preventing accumulation and aggregation of TTR in amyloids or amyloid fibrils in subjects having ATTR. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in preparing a medicament for reducing serum TTR concentration. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in treating ATTR in a subject, such as a mammal, e.g., a primate such as a human. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in reducing or preventing accumulation and aggregation of TTR in amyloids or amyloid fibrils in subjects having ATTR, such as a mammal, e.g., a primate such as a human. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in reducing serum TTR concentration in a subject, such as a mammal, e.g., a primate such as a human.

[00174] Electroporation is also a well-known means for delivery of cargo, and any electroporation methodology may be used for delivery of any one of the gRNAs disclosed herein. In some embodiments, electroporation may be used to deliver any one of the gRNAs disclosed herein and an RNA-guided DNA nuclease such as Cas9 or an mRNA encoding an RNA-guided DNA nuclease such as Cas9.

[00175] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein to an ex vivo cell, wherein the gRNA is associated with an LNP or not associated with an LNP. In some embodiments, the gRNA/LNP or gRNA is also associated with an RNA-guided DNA nuclease such as Cas9 or an mRNA encoding an RNA-guided DNA nuclease such as Cas9.

[00176] In certain embodiments, the invention comprises DNA or RNA vectors encoding any of the guide RNAs comprising any one or more of the guide sequences described herein. In some embodiments, in addition to guide RNA sequences, the vectors further comprise nucleic acids that do not encode guide RNAs. Nucleic acids that do not encode guide RNA include, but are not limited to, promoters, enhancers, regulatory sequences, and nucleic acids encoding an RNA-guided DNA nuclease, which can be a nuclease such as Cas9. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, or a crRNA and trRNA. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a sgRNA and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas nuclease, such as Cas9 or Cpf1. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas protein, such as, Cas9. In one embodiment, the Cas9 is from *Streptococcus pyogenes* (i.e., Spy Cas9). In some embodiments, the nucleotide sequence encoding the crRNA, trRNA, or crRNA and trRNA (which may be a sgRNA) comprises or consists of a guide sequence flanked by all or a portion of a repeat sequence from a naturally-occurring CRISPR/Cas system. The nucleic acid comprising or consisting of the crRNA, trRNA, or crRNA and trRNA may further comprise a vector sequence wherein the vector sequence comprises or consists of nucleic acids that are not naturally found together with the crRNA, trRNA, or crRNA and trRNA.

[00177] In some embodiments, the crRNA and the trRNA are encoded by non-contiguous nucleic acids within one vector. In other embodiments, the crRNA and the trRNA may be encoded by a contiguous nucleic acid. In some embodiments, the crRNA and the trRNA are encoded by opposite strands of a single nucleic acid. In other embodiments, the crRNA and the trRNA are encoded by the same strand of a single nucleic acid.

[00178] In some embodiments, the vector may be circular. In other embodiments, the vector may be linear. In some embodiments, the vector may be enclosed in a lipid nanoparticle, liposome, non-lipid nanoparticle, or viral capsid. Non-limiting exemplary vectors include plasmids, phagemids, cosmids, artificial chromosomes, minichromosomes, transposons, viral vectors, and expression vectors.

[00179] In some embodiments, the vector may be a viral vector. In some embodiments, the viral vector may be genetically modified from its wild type counterpart. For example, the viral vector may comprise an insertion, deletion, or substitution of one or more nucleotides to facilitate cloning or such that one or more properties of the vector is changed. Such properties may include packaging capacity, transduction efficiency, immunogenicity, genome integration, replication, transcription, and translation. In some embodiments, a portion of the viral genome may be deleted such that the virus is capable of packaging exogenous sequences having a larger size. In some embodiments, the viral vector may have an enhanced transduction efficiency. In some embodiments, the immune response induced by the virus in a host may be reduced. In some embodiments, viral genes (such as, e.g., integrase) that promote integration of the viral sequence into a host genome may be mutated such that the virus becomes non-integrating. In some embodiments, the viral vector may be replication defective. In some embodiments, the viral vector may comprise exogenous transcriptional or translational control sequences to drive expression of coding sequences on the vector. In some embodiments, the virus may be helper-dependent. For example, the virus may need one or more helper virus to supply viral components (such as, e.g., viral proteins) required to amplify and package the vectors into viral particles. In such a case, one or more helper components, including one or more vectors encoding the viral components, may be introduced into a host cell along with the vector system described herein. In other embodiments, the virus may be helper-free. For example, the virus may be capable of amplifying and packaging the vectors without any helper virus. In some embodiments, the vector system described herein may also encode the viral components required for virus amplification and packaging.

[00180] Non-limiting exemplary viral vectors include adeno-associated virus (AAV) vector, lentivirus vectors, adenovirus vectors, helper dependent adenoviral vectors (HDAd), herpes simplex virus (HSV-1) vectors, bacteriophage T4, baculovirus vectors, and retrovirus vectors. In some embodiments, the viral vector may be an AAV vector. In some embodiments, the viral vector is AAV2, AAV3, AAV3B, AAV5, AAV6, AAV6.2, AAV7, AAVrh.64R1, AAVhu.37, AAVrh.8, AAVrh.32.33, AAV8, AAV9, AAVrh10, or AAVLK03. In other embodiments, the viral vector may a lentivirus vector.

[00181] In some embodiments, the lentivirus may be non-integrating. In some embodiments, the viral vector may be an adenovirus vector. In some embodiments, the adenovirus may be a high-cloning capacity or "gutless" adenovirus, where all coding viral regions apart from the 5' and 3' inverted terminal repeats (ITRs) and the packaging signal ('I') are deleted from the virus to increase its packaging capacity. In yet other embodiments, the viral vector may be an HSV-1

vector. In some embodiments, the HSV-1-based vector is helper dependent, and in other embodiments it is helper independent. For example, an amplicon vector that retains only the packaging sequence requires a helper virus with structural components for packaging, while a 30kb-deleted HSV-1 vector that removes non-essential viral functions does not require helper virus. In additional embodiments, the viral vector may be bacteriophage T4. In some embodiments, the bacteriophage T4 may be able to package any linear or circular DNA or RNA molecules when the head of the virus is emptied. In further embodiments, the viral vector may be a baculovirus vector. In yet further embodiments, the viral vector may be a retrovirus vector. In embodiments using AAV or lentiviral vectors, which have smaller cloning capacity, it may be necessary to use more than one vector to deliver all the components of a vector system as disclosed herein. For example, one AAV vector may contain sequences encoding an RNA-guided DNA nuclease such as a Cas nuclease, while a second AAV vector may contain one or more guide sequences.

[00182] In some embodiments, the vector may be capable of driving expression of one or more coding sequences in a cell. In some embodiments, the cell may be a prokaryotic cell, such as, e.g., a bacterial cell. In some embodiments, the cell may be a eukaryotic cell, such as, e.g., a yeast, plant, insect, or mammalian cell. In some embodiments, the eukaryotic cell may be a mammalian cell. In some embodiments, the eukaryotic cell may be a rodent cell. In some embodiments, the eukaryotic cell may be a human cell. Suitable promoters to drive expression in different types of cells are known in the art. In some embodiments, the promoter may be wild type. In other embodiments, the promoter may be modified for more efficient or efficacious expression. In yet other embodiments, the promoter may be truncated yet retain its function. For example, the promoter may have a normal size or a reduced size that is suitable for proper packaging of the vector into a virus.

[00183] In some embodiments, the vector may comprise a nucleotide sequence encoding an RNA-guided DNA nuclease such as a nuclease described herein. In some embodiments, the nuclease encoded by the vector may be a Cas protein. In some embodiments, the vector system may comprise one copy of the nucleotide sequence encoding the nuclease. In other embodiments, the vector system may comprise more than one copy of the nucleotide sequence encoding the nuclease. In some embodiments, the nucleotide sequence encoding the nuclease may be operably linked to at least one transcriptional or translational control sequence. In some embodiments, the nucleotide sequence encoding the nuclease may be operably linked to at least one promoter.

[00184] In some embodiments, the promoter may be constitutive, inducible, or tissue-specific. In some embodiments, the promoter may be a constitutive promoter. Non-limiting

exemplary constitutive promoters include cytomegalovirus immediate early promoter (CMV), simian virus (SV40) promoter, adenovirus major late (MLP) promoter, Rous sarcoma virus (RSV) promoter, mouse mammary tumor virus (MMTV) promoter, phosphoglycerate kinase (PGK) promoter, elongation factor-alpha (EF1a) promoter, ubiquitin promoters, actin promoters, tubulin promoters, immunoglobulin promoters, a functional fragment thereof, or a combination of any of the foregoing. In some embodiments, the promoter may be a CMV promoter. In some embodiments, the promoter may be a truncated CMV promoter. In other embodiments, the promoter may be an EF1a promoter. In some embodiments, the promoter may be an inducible promoter. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech).

[00185] In some embodiments, the promoter may be a tissue-specific promoter, e.g., a promoter specific for expression in the liver.

[00186] The vector may further comprise a nucleotide sequence encoding the guide RNA described herein. In some embodiments, the vector comprises one copy of the guide RNA. In other embodiments, the vector comprises more than one copy of the guide RNA. In embodiments with more than one guide RNA, the guide RNAs may be non-identical such that they target different target sequences, or may be identical in that they target the same target sequence. In some embodiments where the vectors comprise more than one guide RNA, each guide RNA may have other different properties, such as activity or stability within a complex with an RNA-guided DNA nuclease, such as a Cas RNP complex. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to at least one transcriptional or translational control sequence, such as a promoter, a 3' UTR, or a 5' UTR. In one embodiment, the promoter may be a tRNA promoter, *e.g.*, $tRNA^{Lys3}$, or a tRNA chimera. *See* Mefferd et al., *RNA*. 2015 21:1683-9; Scherer et al., *Nucleic Acids Res*. 2007 35: 2620–2628. In some embodiments, the promoter may be recognized by RNA polymerase III (Pol III). Non-limiting examples of Pol III promoters include U6 and H1 promoters. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human U6 promoter. In other embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human H1 promoter. In embodiments with more than one guide RNA, the promoters used to drive expression may be the same or different. In some embodiments, the nucleotide encoding the crRNA of the guide RNA and the nucleotide encoding the trRNA of the guide RNA may be provided on the same vector. In some embodiments, the nucleotide encoding the crRNA

and the nucleotide encoding the trRNA may be driven by the same promoter. In some embodiments, the crRNA and trRNA may be transcribed into a single transcript. For example, the crRNA and trRNA may be processed from the single transcript to form a double-molecule guide RNA. Alternatively, the crRNA and trRNA may be transcribed into a single-molecule guide RNA (sgRNA). In other embodiments, the crRNA and the trRNA may be driven by their corresponding promoters on the same vector. In yet other embodiments, the crRNA and the trRNA may be encoded by different vectors.

[00187] In some embodiments, the nucleotide sequence encoding the guide RNA may be located on the same vector comprising the nucleotide sequence encoding an RNA-guided DNA nuclease such as a Cas nuclease. In some embodiments, expression of the guide RNA and of the RNA-guided DNA nuclease such as a Cas protein may be driven by their own corresponding promoters. In some embodiments, expression of the guide RNA may be driven by the same promoter that drives expression of the RNA-guided DNA nuclease such as a Cas protein. In some embodiments, the guide RNA and the RNA-guided DNA nuclease such as a Cas protein transcript may be contained within a single transcript. For example, the guide RNA may be within an untranslated region (UTR) of the RNA-guided DNA nuclease such as a Cas protein transcript. In some embodiments, the guide RNA may be within the 5' UTR of the transcript. In other embodiments, the guide RNA may be within the 3' UTR of the transcript. In some embodiments, the intracellular half-life of the transcript may be reduced by containing the guide RNA within its 3' UTR and thereby shortening the length of its 3' UTR. In additional embodiments, the guide RNA may be within an intron of the transcript. In some embodiments, suitable splice sites may be added at the intron within which the guide RNA is located such that the guide RNA is properly spliced out of the transcript. In some embodiments, expression of the RNA-guided DNA nuclease such as a Cas protein and the guide RNA from the same vector in close temporal proximity may facilitate more efficient formation of the CRISPR RNP complex.

[00188] In some embodiments, the compositions comprise a vector system. In some embodiments, the vector system may comprise one single vector. In other embodiments, the vector system may comprise two vectors. In additional embodiments, the vector system may comprise three vectors. When different guide RNAs are used for multiplexing, or when multiple copies of the guide RNA are used, the vector system may comprise more than three vectors.

[00189] In some embodiments, the vector system may comprise inducible promoters to start expression only after it is delivered to a target cell. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or

alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech).

[00190] In additional embodiments, the vector system may comprise tissue-specific promoters to start expression only after it is delivered into a specific tissue.

[00191] The vector may be delivered by liposome, a nanoparticle, an exosome, or a microvesicle. The vector may also be delivered by a lipid nanoparticle (LNP); see e.g., U.S.S.N. 62/433,228, filed December 12, 2016 and entitled "LIPID NANOPARTICLE FORMULATIONS FOR CRISPR/CAS COMPONENTS," the contents of which are hereby incorporated by reference in their entirety. Any of the LNPs and LNP formulations described herein are suitable for delivery of the guides alone or together a cas nuclease or an mRNA encoding a cas nuclease. In some embodiments, an LNP composition is encompassed comprising: an RNA component and a lipid component, wherein the lipid component comprises an amine lipid, a neutral lipid, a helper lipid, and a stealth lipid; and wherein the N/P ratio is about 1-10.

[00192] In some instances, the the lipid component comprises Lipid A or its acetal analog, cholesterol, DSPC, and PEG-DMG; and wherein the N/P ratio is about 1-10. In some embodiments, the lipid component comprises: about 40-60 mol-% amine lipid; about 5-15 mol-% neutral lipid; and about 1.5-10 mol-% PEG lipid, wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-10. In some embodiments, the lipid component comprises about 50-60 mol-% amine lipid; about 8-10 mol-% neutral lipid; and about 2.5-4 mol-% PEG lipid, wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-8. In some instances, the lipid component comprises: about 50-60 mol-% amine lipid; about 5-15 mol-% DSPC; and about 2.5-4 mol-% PEG lipid, wherein the remainder of the lipid component is cholesterol, and wherein the N/P ratio of the LNP composition is about 3-8. In some instances, the lipid component comprises: 48-53 mol-% Lipid A; about 8-10 mol-% DSPC; and 1.5-10 mol-% PEG lipid, wherein the remainder of the lipid component is cholesterol, and wherein the N/P ratio of the LNP composition is 3-8 ±0.2.

[00193] In some embodiments, the vector may be delivered systemically. In some embodiments, the vector may be delivered into the hepatic circulation.

[00194] This description and exemplary embodiments should not be taken as limiting. For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities, percentages, or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term "about," to the extent they are not already so modified. Accordingly, unless indicated to the

contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[00195] It is noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the," and any singular use of any word, include plural referents unless expressly and unequivocally limited to one referent. As used herein, the term "include" and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

EXAMPLES

[00196] The following examples are provided to illustrate certain disclosed embodiments and are not to be construed as limiting the scope of this disclosure in any way.

Example 1. Materials and Methods

In vitro transcription ("IVT") of nuclease mRNA

[00197] Capped and polyadenylated *Streptococcus pyogenes* ("Spy") Cas9 mRNA containing N1-methyl pseudo-U was generated by *in vitro* transcription using a linearized plasmid DNA template and T7 RNA polymerase. Plasmid DNA containing a T7 promoter, a sequence for transcription according to SEQ ID NO: 1 or 2, and a 100 nt poly (A/T) region was linearized by incubating at 37°C for 2 hours with XbaI with the following conditions: 200 ng/μL plasmid, 2 U/μL XbaI (NEB), and 1x reaction buffer. The XbaI was inactivated by heating the reaction at 65°C for 20 min. The linearized plasmid was purified from enzyme and buffer salts using a silica maxi spin column (Epoch Life Sciences) and analyzed by agarose gel to confirm linearization. The IVT reaction to generate Cas9 modified mRNA was incubated at 37°C for 4 hours in the following conditions: 50 ng/μL linearized plasmid; 2 mM each of GTP, ATP, CTP, and N1-methyl pseudo-UTP (Trilink); 10 mM ARCA (Trilink); 5 U/μL T7 RNA polymerase (NEB); 1 U/μL Murine RNase inhibitor (NEB); 0.004 U/μL Inorganic E. coli pyrophosphatase (NEB); and 1x reaction buffer. After the 4-hour incubation, TURBO DNase (ThermoFisher) was added to a final concentration of 0.01 U/μL, and the reaction was incubated for an additional 30 minutes to remove the DNA template. The Cas9 mRNA was purified from enzyme and nucleotides using a MegaClear Transcription Clean-up kit per the manufacturer's protocol (ThermoFisher). Alternatively, the mRNA was purified through a precipitation protocol, which in some cases was followed by

HPLC-based purification. Briefly, after the DNase digestion, the mRNA was precipitated by adding 0.21x vol of a 7.5 M LiCl solution and mixing, and the precipitated mRNA was pelleted by centrifugation. Once the supernatant was removed, the mRNA was reconstituted in water. The mRNA was precipitated again using ammonium acetate and ethanol. 5M Ammonium acetate was added to the mRNA solution for a final concentration of 2M along with 2x volume of 100% EtOH. The solution was mixed and incubated at -20 °C for 15 min. The precipitated mRNA was again pelleted by centrifugation, the supernatant was removed, and the mRNA was reconstituted in water. As a final step, the mRNA was precipitated using sodium acetate and ethanol. 1/10 volume of 3 M sodium acetate (pH 5.5) was added to the solution along with 2x volume of 100% EtOH. The solution was mixed and incubated at -20 °C for 15 min. The precipitated mRNA was again pelleted by centrifugation, the supernatant was removed, the pellet was washed with 70% cold ethanol and allowed to air dry. The mRNA was reconstituted in water. For HPLC purified mRNA, after the LiCl precipitation and reconstitution, the mRNA was purified by RP-IP HPLC (see, e.g., Kariko, et al. *Nucleic Acids Research*, 2011, Vol. 39, No. 21 e142). The fractions chosen for pooling were combined and desalted by sodium acetate/ethanol precipitation as described above. The transcript concentration was determined by measuring the light absorbance at 260 nm (Nanodrop), and the transcript was analyzed by capillary electrophoresis by Bioanlayzer (Agilent).

[00198] When SEQ ID NOs: 1 and 2 are referred to below with respect to RNAs, it is understood that Ts should be replaced with Us (which were N1-methyl pseudouridines as described above). Cas9 mRNAs used in the Examples include a 5' cap and a 3' poly-A tail, e.g., up to 100 nts, and are identified by SEQ ID NO.

[00199] SEQ ID NO: 1: Cas9 sequence 1 for transcription.

GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTAT
TCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTC
GGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAA
CACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA
CAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGA
ATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCA
CAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCG
GAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAG
AAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACAT
GATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCG
ACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAAC
GCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGA
AAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCAC
TGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTG

CAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCA
GTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCC
TGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGAC
GAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTA
CAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAA
GCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAA
CTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAG
CATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCT
ACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTAC
TACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGA
AACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCA
TCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGC
CTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGG
AATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCA
AGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGC
TTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCA
CGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCC
TGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTG
AAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACAC
AGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGA
CAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCAC
GACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAG
CCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGA
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[00200] SEQ ID NO: 2: Cas9 sequence 2 for transcription.

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Human TTR guide design and human TTR with cynomolgus monkey homology guide design

[00201] Initial guide selection was performed *in silico* using a human reference genome (e.g., hg38) and user defined genomic regions of interest (e.g., TTR protein coding exons), for identifying PAMs in the regions of interest. For each identified PAM, analyses were performed and statistics reported. gRNA molecules were further selected and rank ordered based on a number of criteria (e.g., GC content, predicted on-target activity, and potential off-target activity).

A total of 68 guide RNAs were designed toward TTR (ENSG00000118271) targeting the protein coding regions within Exon 1, 2, 3 and 4. Of the total 68 guides, 33 were 100% homologous in cynomolgus monkey ("cyno"). In addition, for 10 of the human TTR guides which were not perfectly homologous in cyno, "surrogate" guides were designed and made in

parallel to perfectly match the corresponding cyno target sequence. These "surrogate" or "tool" guides may be screened in cyno, e.g., to approximate the activity and function of the homologous human guide sequence. Guide sequences and corresponding genomic coordinates are provided (Table 1). All of the guide RNAs were made as dual guide RNAs, and a subset of the guide sequences were made as modified single guide RNA (Table 2). Guide ID alignment across dual guide RNA (dgRNA) IDs, modified single guide RNA (sgRNA) IDs, the number of mismatches to the cyno genome as well as the cyno exact matched IDs are provided (Table 3). Where dgRNAs are used in the experiments detailed throughout the Examples, SEQ ID NO: 270 was used.

Cas9 mRNA and guide RNA delivery in vitro

[00202] *HEK293_Cas9 cell line.* The human embryonic kidney adenocarcinoma cell line HEK293 constitutively expressing Spy Cas9 ("HEK293_Cas9") was cultured in DMEM media supplemented with 10% fetal bovine serum and 500 μg/ml G418. Cells were plated at a density of 10,000 cells/well in a 96-well plate 24 hours prior to transfection. Cells were transfected with Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) per the manufacturer's protocol. Cells were transfected with a lipoplex containing individual crRNA (25 nM), trRNA (25 nM), Lipofectamine RNAiMAX (0.3 μL/well) and OptiMem.

[00203] *HUH7 cell line.* The human hepatocellular carcinoma cell line HUH7 (Japanese Collection of Research Bioresources Cell Bank, Cat. JCRB0403) was cultured in DMEM media supplemented with 10% fetal bovine serum. Cells were plated on at a density of 15,000 cells/well in a 96-well plate 20 hours prior to transfection. Cells were transfected with Lipofectamine MessengerMAX (ThermoFisher, Cat. LMRNA003) per the manufacturer's protocol. Cells were sequentially transfected with a lipoplex containing Spy Cas9 mRNA (100 ng), MessengerMAX (0.3 μL/well) and OptiMem followed by a separate lipoplex containing individual crRNA (25 nM), tracer RNA (25 nM), MessengerMAX (0.3 μL/well) and OptiMem.

[00204] *HepG2 cell line.* The human hepatocellular carcinoma cell line HepG2 (American Type Culture Collection, Cat. HB-8065) was cultured in DMEM media supplemented with 10% fetal bovine serum. Cells were counted and plated on Bio-coat collagen I coated 96-well plates (ThermoFisher, Cat. 877272) at a density of 10,000 cells/well in a 96-well plate 24 hours prior to transfection. Cells were transfected with Lipofectamine 2000 (ThermoFisher, Cat. 11668019) per the manufacturer's protocol. Cells were sequentially transfected with lipoplex containing Spy Cas9 mRNA (100 ng), Lipofectamine 2000 (0.2

μL/well) and OptiMem followed by a separate lipoplex containing individual crRNA (25 nM), tracer RNA (25 nM), Lipofectamine 2000 (0.2 μL/well) and OptiMem.

[00205] *Primary liver hepatocytes*. Primary human liver hepatocytes (PHH) and primary cynomolgus liver hepatocytes (PCH) (Gibco) were cultured per the manufacturer's protocol (Invitrogen, protocol 11.28.2012). In brief, the cells were thawed and resuspended in hepatocyte thawing medium with supplements (Gibco, Cat. CM7000) followed by centrifugation at 100 g for 10 minutes for human and 80g for 4 minutes for cyno. The supernatant was discarded and the pelleted cells resuspended in hepatocyte plating medium plus supplement pack (Invitrogen, Cat. A1217601 and CM3000). Cells were counted and plated on Bio-coat collagen I coated 96-well plates (ThermoFisher, Cat. 877272) at a density of 33,000 cells/well for human or 60,000 cells/well for cyno (or 65,000 cells/well when assaying effects on TTR protein, described further below). Plated cells were allowed to settle and adhere for 6 or 24 hours in a tissue culture incubator at 37°C and 5% CO_2 atmosphere. After incubation cells were checked for monolayer formation and media was replaced with hepatocyte culture medium with serum-free supplement pack (Invitrogen, Cat. A1217601 and CM4000).

[00206] Lipofectamine RNAiMax (ThermoFisher, Cat. 13778150) based transfections were conducted as per the manufacturer's protocol. Cells were sequentially transfected with a lipoplex containing Spy Cas9 mRNA (100 ng), Lipofectamine RNAiMax (0.4 μL/well) and OptiMem followed by a separate lipoplex containing crRNA (25 nM) and tracer RNA (25 nM) or sgRNA (25nM), Lipofectamine RNAiMax (0.4 μL/well) and OptiMem.

[00207] Ribonucleotide formation was performed prior to electroporation or transfection of Spy Cas9 protein loaded with guide RNAs (RNPs) onto cells. For dual guide (dgRNAs), individual crRNA and trRNA was pre-annealed by mixing equivalent amounts of reagent and incubating at 95°C for 2 min and cooling to room temperature. Single guide (sgRNAs) were boiled at 95°C for 2 min and cooling to room temperature. The boiled dgRNA or sgRNA was incubated with Spy Cas9 protein in Optimem for 10 minutes at room temperature to form a ribonucleoprotein (RNP) complex.

[00208] For RNP electroporation into primary human and cyno hepatocytes, the cells are thawed and resuspended in Lonza electroporation Primary Cell P3 buffer at a concentration of 2500 cells per μL for human hepatocytes and 3500 cells per μL for cyno hepatocytes. A volume of 20 μL of resuspended cells and 5 μL of RNP are mixed together per guide. 20 μL of the mixture is placed into a Lonza electroporation plate. The cells were electroporated using the Lonza nucleofector with the preset protocol EX-147. Post electroporation, the cells

are transferred into a Biocoat plate containing pre-warmed maintenance media and placed in a tissue culture incubator at 37°C and 5% CO_2.

[00209] For RNP lipoplex transfections, cells were transfected with Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) per the manufacturer's protocol. Cells were transfected with an RNP containing Spy Cas9 (10nM), individual guide (10 nM), tracer RNA (10 nM), Lipofectamine RNAiMAX (1.0 μL/well) and OptiMem. RNP formation was performed as described above.

[00210] LNPs were formed either by by microfluidic mixing of the lipid and RNA solutions using a Precision Nanosystems NanoAssemblr™ Benchtop Instrument, per the manufacturer's protocol, or cross-flow mixing.

[00211] LNP formulation - NanoAssemblr

[00212] In general, the lipid nanoparticle components were dissolved in 100% ethanol with the lipid component of various molar ratios. The RNA cargos were dissolved in 25 mM citrate, 100 mM NaCl, pH 5.0, resulting in a concentration of RNA cargo of approximately 0.45 mg/mL. The LNPs were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 4.5 or about 6, with the ratio of mRNA to gRNA at 1:1 by weight.

[00213] The LNPs were formed by microfluidic mixing of the lipid and RNA solutions using a Precision Nanosystems NanoAssemblr™ Benchtop Instrument, according to the manufacturer's protocol. A 2:1 ratio of aqueous to organic solvent was maintained during mixing using differential flow rates. After mixing, the LNPs were collected, diluted in water (approximately 1:1 v/v), held for 1 hour at room temperature, and further diluted with water (approximately 1:1 v/v) before final buffer exchange. The final buffer exchange into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS) was completed with PD-10 desalting columns (GE). If required, formulations were concentrated by centrifugation with Amicon 100 kDa centrifugal filters (Millipore). The resulting mixture was then filtered using a 0.2 μm sterile filter. The final LNP was stored at -80 °C until further use.

LNP Formulation – Cross-Flow

[00214] For LNPs prepared using the cross-flow technique, the LNPs were formed by impinging jet mixing of the lipid in ethanol with two volumes of RNA solutions and one volume of water. The lipid in ethanol is mixed through a mixing cross with the two volumes of RNA solution. A fourth stream of water is mixed with the outlet stream of the cross through an inline tee. (*See* WO2016010840 FIG.2.) The LNPs were held for 1 hour at room temperature, and further diluted with water (approximately 1:1 v/v). Diluted LNPs were concentrated using tangential flow filtration on a flat sheet cartridge (Sartorius, 100kD

MWCO) and then buffer exchanged by diafiltration into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS). Alternatively, the final buffer exchange into TSS was completed with PD-10 desalting columns (GE). If required, formulations were concentrated by centrifugation with Amicon 100 kDa centrifugal filters (Millipore). The resulting mixture was then filtered using a 0.2 μm sterile filter. The final LNP was stored at 4°C or -80°C until further use.

Formulation Analytics

[00215] Dynamic Light Scattering ("DLS") is used to characterize the polydispersity index ("pdi") and size of the LNPs of the present disclosure. DLS measures the scattering of light that results from subjecting a sample to a light source. PDI, as determined from DLS measurements, represents the distribution of particle size (around the mean particle size) in a population, with a perfectly uniform population having a PDI of zero. Average particle size and polydispersity are measured by dynamic light scattering (DLS) using a Malvern Zetasizer DLS instrument. LNP samples were diluted 30X in PBS prior to being measured by DLS. Z-average diameter which is an intensity based measurement of average particle size was reported along with number average diameter and pdi. A Malvern Zetasizer instrument is also used to measure the zeta potential of the LNP. Samples are diluted 1:17 (50uL into 800uL) in 0.1X PBS, pH 7.4 prior to measurement.

[00216] A fluorescence-based assay (Ribogreen®, ThermoFisher Scientific) is used to determine total RNA concentration and free RNA. Encapsulation efficiency is calclulated as (Total RNA - Free RNA)/Total RNA. LNP samples are diluted appropriately with 1x TE buffer containing 0.2% Triton-X 100 to determine total RNA or 1x TE buffer to determine free RNA. Standard curves are prepared by utilizing the starting RNA solution used to make the formulations and diluted in 1x TE buffer +/- 0.2% Triton-X 100. Diluted RiboGreen® dye (according to the manufacturer's instructions) is then added to each of the standards and samples and allowed to incubate for approximately 10 minutes at room temperature, in the absence of light. A SpectraMax M5 Microplate Reader (Molecular Devices) is used to read the samples with excitation, auto cutoff and emission wavelengths set to 488 nm, 515 nm, and 525 nm respectively. Total RNA and free RNA are determined from the appropriate standard curves.

[00217] Encapsulation efficiency is calclulated as (Total RNA - Free RNA)/Total RNA. The same procedure may be used for determining the encapsulation efficiency of a DNA-based cargo component. For single-strand DNA Oligreen Dye may be used, and for double-strand DNA, Picogreen Dye.

[00218] Typically, when preparing LNPs, encapsulation was >80%, particle size was <120 nm, and pdi was <0.2.

***LNP Delivery* In Vivo**

[00219] Unless otherwise noted, CD-1 female mice, ranging from 6-10 weeks of age were used in each study. Animals were weighed and grouped according to body weight for preparing dosing solutions based on group average weight. LNPs were dosed via the lateral tail vein in a volume of 0.2 mL per animal (approximately 10 mL per kilogram body weight). The animals were observed at approximately 6 hours post dose for adverse effects. Body weight was measured at twenty-four hours post-administration, and animals were euthanized at various time points by exsanguination via cardiac puncture under isoflourane anesthesia. Blood was collected into serum separator tubes or into tubes containing buffered sodium citrate for plasma as described herein. For studies involving in vivo editing, liver tissue was collected from the median lobe or from three independent lobes (e.g., the right median, left median, and left lateral lobes) from each animal for DNA extraction and analysis.

Transthyretin (TTR) ELISA analysis used in animal studies

[00220] Blood was collected and the serum was isolated as indicated. The total mouse TTR serum levels were determined using a Mouse Prealbumin (Transthyretin) ELISA Kit (Aviva Systems Biology, Cat. OKIA00111); rat TTR serum levels were measured using a rat specific ELISA kit (Aviva Systems Biology catalog number OKIA00159); human TTR serum levels were measured using a human specific ELISA kit (Aviva Systems Biology catalog number OKIA00081); each according to manufacture's protocol. Briefly, sera were serial diluted with kit sample diluent to a final dilution of 10,000-fold, or 5,000-fold when measuring human TTR in mouse sera. 100ul of the prepared standard curve or diluted serum samples were added to the ELISA plate, incubated for 30 minutes at room temperature then washed 3 times with provided wash buffer. 100uL of detection antibody was then added to each well and incubated for 20 minutes at room temperature followed by 3 washes. 100uL of substrate is added then incubated for 10 minutes at room temperature before the addition of 100uL stop solution. The absorbance of the contents was measured on the Spectramax M5 plate reader with analysis using SoftmaxPro version 7.0 software. Serum TTR levels were quantitated off the standard curve using 4 parameter logistic fit and expressed as ug/mL of serum or percent knockdown relative control (vehicle treated) animals.

Genomic DNA isolation

[00221] Transfected cells were harvested post-transfection at 24, 48, or 72 hours. The genomic DNA was extracted from each well of a 96-well plate using 50 μL/well BuccalAmp DNA Extraction solution (Epicentre, Cat. QE09050) per manufacturer's protocol. All DNA samples were subjected to PCR and subsequent NGS analyses, as described herein.

Next-generation sequencing ("NGS") analysis

[00222] To quantitatively determine the efficiency of editing at the target location in the genome, sequencing was utilized to identify the presence of insertions and deletions introduced by gene editing.

[00223] Primers were designed around the target site within the gene of interest (e.g. *TTR*), and the genomic area of interest was amplified.

[00224] Additional PCR was performed per the manufacturer's protocols (Illumina) to add chemistry for sequencing. The amplicons were sequenced on an Illumina MiSeq instrument. The reads were aligned to a reference genome (e.g., the human reference genome (hg38), the cynomologus reference genome (mf5), the rat reference genome (rn6), or the mouse reference genome (mm10)) after eliminating those having low quality scores. The resulting files containing the reads were mapped to the reference genome (BAM files), where reads that overlapped the target region of interest were selected and the number of wild type reads versus the number of reads which contain an insertion, substitution, or deletion was calculated.

[00225] The editing percentage (e.g., the "editing efficiency" or "percent editing" or "indel frequency") is defined as the total number of sequence reads with insertions/deletions ("indels") or substitutions over the total number of sequence reads, including wild type.

Analysis of secreted transthyretin ("TTR") protein by Western Blot

[00226] Secreted levels of TTR protein in media were determined using western blotting methods. HepG2 cells were transfected as previously described with select guides from Table 1. Media changes were performed every 3 days post transfection. Six days post-transfection, the media was removed, and cells were washed once with media that did not contain fetal bovine serum (FBS). Media without serum was added to the cells and incubated at 37°C. After 4hrs the media was removed and centrifuged to pellet any debris; cell number for each well was estimated based on the values obtained from a CTG assay on remaining cells and comparison to the plate average. After centrifugation, the media was transferred to a new plate and stored at -20°C. An acetone precipitation of the media was performed to precipitate any protein that had been secreted into the media. Four volumes of ice cold acetone were added to one volume of media. The solution was mixed well and kept at -20°C for 90min.

The acetone:media mixture was centrifuged at 15,000xg and 4°C for 15min. The supernatant was discarded and the retained pellet was air dried to eliminate any residual acetone. The pellet was resuspended in 15µL RIPA buffer (Boston Bio Products, Cat. BP-115) plus freshly added protease inhibitor mixture consisting of complete protease inhibitor cocktail (Sigma, Cat. 11697498001) and 1mM DTT. Lysates were mixed with Laemmli buffer and denatured at 95°C for 10 minutes. Western blots were run using the NuPage system on 12% Bis-Tris gels (ThermoFisher) per the manufacturer's protocol followed by wet transfer onto 0.45 µm nitrocellulose membrane (Bio-Rad, Cat. 1620115). Blots were blocked using 5% Dry Milk in TBS for 30 minutes on a lab rocker at room temperature. Blots were rinsed with TBST and probed with rabbit α-TTR monoclonal antibody (Abcam, Cat. Ab75815) at 1:1000 in TBST. Alpha-1 antitrypsin was used as a loading control (Sigma, Cat. HPA001292) at 1:1000 in TBST and incubated simultaneously with the TTR primary antibody. Blots were sealed in a bag and kept overnight at 4°C on a lab rocker. After incubation, blots were rinsed 3 times for 5min each in TBST and probed with secondary antibodies to Rabbit (ThermoFisher, Cat. PISA535571) at 1:25,000 in TBST for 30min at room temperature. After incubation, blots were rinsed 3 times for 5min each in TBST and 2 times with PBS. Blots were visualized and analyzed using a Licor Odyssey system.

Analysis of intracellular TTR by Western Blot

[00227] The hepatocellular carcinoma cell line, HUH7, was transfected as previously described with select guides from Table 1. Six-days post-transfection, the media was removed and the cells were lysed with 50 µL/well RIPA buffer (Boston Bio Products, Cat. BP-115) plus freshly added protease inhibitor mixture consisting of complete protease inhibitor cocktail (Sigma, Cat. 11697498001), 1 mM DTT, and 250 U/ml Benzonase (EMD Millipore, Cat. 71206-3). Cells were kept on ice for 30 minutes at which time NaCl (1 M final concentration) was added. Cell lysates were thoroughly mixed and retained on ice for 30 minutes. The whole cell extracts ("WCE") were transferred to a PCR plate and centrifuged to pellet debris. A Bradford assay (Bio-Rad, Cat. 500-0001) was used to assess protein content of the lysates. The Bradford assay procedure was completed per the manufacturer's protocol. Extracts were stored at minus 20°C prior to use. Western blots were performed to assess intracellular TTR protein levels. Lysates were mixed with Laemmli buffer and denatured at 95°C for 10min. Western blots were run using the NuPage system on 12% Bis-Tris gels (ThermoFisher) per the manufacturer's protocol followed by wet transfer onto 0.45 µm nitrocellulose membrane (Bio-Rad, Cat. 1620115). After transfer membranes were rinsed thoroughly with water and stained with Ponceau S solution (Boston Bio Products, Cat. ST-

180) to confirm complete and even transfer. Blots were blocked using 5% Dry Milk in TBS for 30 minutes on a lab rocker at room temperature. Blots were rinsed with TBST and probed with rabbit α-TTR monoclonal antibody (Abcam, Cat. Ab75815) at 1:1000 in TBST. β-actin was used as a loading control (ThermoFisher, Cat. AM4302) at 1:2500 in TBST and incubated simultaneously with the TTR primary antibody. Blots were sealed in a bag and kept overnight at 4°C on a lab rocker. After incubation, blots were rinsed 3 times for 5 minutes each in TBST and probed with secondary antibodies to Mouse and Rabbit (ThermoFisher, Cat. PI35518 and PISA535571) at 1:25,000 each in TBST for 30min at room temperature. After incubation, blots were rinsed 3 times for 5min each in TBST and 2 times with PBS. Blots were visualized and analyzed using a Licor Odyssey system.

Example 2. Screening of dgRNA sequences

[00228] ***Cross screening of TTR dgRNAs in multiple cell types***

[00229] Guides in dgRNA format targeting human *TTR* and the cynomologus matched sequences were delivered to HEK293_Cas9, HUH7 and HepG2 cell lines, as well as primary human hepatocytes and primary cynomolgus monkey hepatocytes as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on highest % edit. The screening data for the guide sequences in Table 1 in all five cell lines are listed below (Table 4 through 11).

[00230] Table 4 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the *TTR* crRNAs in the human kidney adenocarcinoma cell line, HEK293_Cas9, which constitutively over expresses Spy Cas9 protein.

Table 4: TTR editing data in Hek_Cas9 cells transfected with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003335	26.59	4.73	4.73	0.65	21.87	4.09
CR003336	29.09	4.57	3.31	0.24	25.78	4.32
CR003337	42.72	1.72	5.24	1.62	37.48	0.70
CR003338	52.42	3.28	4.76	0.03	47.66	3.30
CR003339	56.37	4.13	49.39	3.23	6.98	0.91
CR003340	42.38	8.43	27.88	4.31	14.50	4.13
CR003341	20.04	5.26	6.73	1.86	13.31	3.41
CR003342	36.57	5.80	1.19	0.22	35.38	5.59
CR003343	24.36	1.51	4.82	0.43	19.53	1.39
CR003344	33.87	2.93	4.32	0.58	29.54	2.37

CR003345	35.02	7.05	19.00	3.58	16.01	3.48
CR003346	48.33	5.81	33.03	3.12	15.30	2.72
CR003347	21.45	5.57	0.95	0.33	20.50	5.26
CR003348	35.53	5.81	22.32	3.79	13.21	2.03
CR003349	13.19	4.46	8.03	2.81	5.16	1.66
CR003350	22.31	4.25	5.54	0.74	16.77	3.51
CR003351	49.67	3.77	28.42	1.69	21.24	2.22
CR003352	27.90	7.55	4.91	1.35	22.99	6.26
CR003353	25.03	5.16	3.71	0.75	21.32	4.42
CR003354	18.46	2.02	2.56	0.21	15.90	1.89
CR003355	30.60	2.53	6.99	0.80	23.61	1.75
CR003356	32.21	4.71	10.03	1.39	22.19	3.36
CR003357	43.23	6.71	5.38	0.87	37.85	5.88
CR003358	5.44	0.86	1.29	0.16	4.14	0.84
CR003359	37.75	7.50	18.35	3.73	19.40	3.78
CR003360	22.68	3.16	2.70	0.56	19.98	2.60
CR003361	34.45	8.97	8.66	1.66	25.78	7.32
CR003362	9.90	2.66	1.48	0.33	8.41	2.33
CR003363	31.03	10.74	14.77	4.21	16.26	6.54
CR003364	35.65	7.90	19.17	4.24	16.48	3.76
CR003365	36.43	6.20	11.83	1.88	24.61	4.45
CR003366	47.36	6.59	10.10	1.28	37.26	5.32
CR003367	47.11	15.43	28.44	9.11	18.67	6.33
CR003368	40.35	10.13	3.73	0.96	36.61	9.17
CR003369	33.10	7.26	9.06	1.12	24.04	6.16
CR003370	34.22	5.69	4.49	0.67	29.73	5.06
CR003371	25.60	8.33	3.84	1.41	21.76	6.92
CR003372	15.24	7.92	3.25	1.61	11.99	6.31
CR003373	13.55	2.40	1.31	0.21	12.25	2.19
CR003374	10.91	0.88	0.81	0.10	10.10	0.81
CR003375	11.63	3.18	0.78	0.17	10.85	3.05
CR003376	28.16	4.49	1.35	0.18	26.81	4.52
CR003377	24.70	4.44	2.71	0.54	21.99	3.91
CR003378	20.97	2.67	4.49	0.49	16.48	2.18
CR003379	26.32	2.91	5.34	0.61	20.98	2.30
CR003380	47.64	5.74	3.64	0.24	44.00	5.52
CR003381	22.04	5.74	3.82	1.26	18.23	4.64
CR003382	29.95	3.13	4.46	0.45	25.49	2.73
CR003383	40.47	0.64	25.12	0.45	15.35	0.66
CR003384	17.45	1.32	1.45	0.23	16.00	1.42
CR003385	26.19	5.62	7.36	1.57	18.82	4.06
CR003386	33.12	10.65	2.94	0.63	30.18	10.03
CR003387	24.68	5.93	7.75	1.99	16.92	3.94
CR003388	19.23	4.41	1.41	0.39	17.82	4.07

CR003389	34.18	5.09	10.30	2.12	23.87	3.02
CR003390	28.02	3.77	4.31	0.25	23.71	3.61
CR003391	44.81	4.67	0.61	0.07	44.19	4.63
CR003392	21.67	7.52	0.85	0.26	20.82	7.27

[00231] Table 5 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR crRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in the human hepatocellular carcinoma cell line, HUH7.

Table 5: TTR editing data in HUH7 cells transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003335	31.95	4.50	4.62	0.83	27.57	4.08
CR003336	30.05	4.25	4.14	1.07	26.56	3.55
CR003337	55.72	3.12	8.34	0.93	48.95	2.24
CR003338	75.64	2.03	10.22	1.42	67.06	2.79
CR003339	79.97	4.73	60.55	3.94	20.13	1.02
CR003340	46.93	7.12	33.33	6.01	14.23	1.65
CR003341	20.58	5.98	7.78	1.64	13.20	4.44
CR003342	45.14	7.16	1.23	0.91	44.66	7.68
CR003343	76.13	7.04	9.58	3.49	66.97	6.10
CR003344	64.02	3.33	10.76	1.35	54.40	2.71
CR003345	72.43	2.17	41.33	0.96	32.18	1.37
CR003346	18.07	1.02	13.17	1.39	6.97	3.06
CR003347	32.16	5.50	1.64	0.42	30.79	5.11
CR003348	57.14	10.98	36.08	6.97	22.71	4.42
CR003349	14.14	4.99	9.73	3.26	4.82	1.91
CR003350	52.91	7.61	13.43	2.00	41.64	6.03
CR003351	63.51	4.61	36.87	2.49	27.49	2.14
CR003352	39.68	9.53	7.62	7.42	32.79	7.37
CR003353	69.18	4.59	7.73	2.46	62.87	3.13
CR003354	12.27	3.38	1.25	0.40	11.46	3.23
CR003355	38.83	5.31	9.40	1.81	30.31	3.56
CR003356	49.63	5.55	18.98	2.67	31.31	3.04
CR003357	36.31	5.72	6.37	1.17	30.82	4.68
CR003358	36.50	6.17	10.53	1.56	26.60	4.49
CR003359	66.75	5.84	21.73	2.30	45.97	3.93
CR003360	58.62	8.73	5.01	0.60	55.13	8.19
CR003361	28.68	6.52	6.84	1.26	22.44	5.31
CR003362	26.43	0.83	3.43	0.32	23.76	0.85
CR003363	41.01	7.16	17.83	3.32	23.78	3.97
CR003364	47.13	10.61	24.68	5.15	23.03	5.74
CR003365	60.68	5.25	17.77	1.57	43.82	3.73

CR003366	69.98	8.84	20.77	3.10	50.32	5.69
CR003367	66.29	4.48	33.62	4.14	33.48	0.51
CR003368	31.57	11.73	3.08	0.92	29.69	11.32
CR003369	24.19	6.89	7.12	2.27	17.38	4.76
CR003370	39.16	11.59	4.83	1.79	35.55	10.35
CR003371	40.47	7.68	6.07	0.89	35.65	7.01
CR003372	21.52	6.02	4.89	1.66	17.25	4.58
CR003373	27.29	4.45	3.31	0.66	25.12	4.12
CR003374	3.10	0.68	0.45	0.24	2.87	0.54
CR003375	2.38	0.22	0.26	0.14	2.25	0.12
CR003376	19.42	5.60	1.37	0.45	18.55	5.28
CR003377	34.93	5.47	5.59	0.88	29.89	4.71
CR003378	40.73	4.63	9.73	1.85	32.27	2.91
CR003379	19.18	5.17	3.38	0.77	16.48	4.32
CR003380	31.76	5.81	3.29	0.57	29.29	5.42
CR003381	99.70	0.17	1.92	0.20	99.70	0.17
CR003382	34.47	5.71	0.14	0.16	34.47	5.71
CR003383	42.89	10.14	2.14	0.56	41.19	9.67
CR003384	17.03	1.95	0.84	0.30	16.29	1.84
CR003386	69.40	19.41	0.53	0.23	69.34	19.32
CR003387	25.64	3.69	0.23	0.07	25.55	3.62
CR003388	59.48	4.29	3.88	0.68	56.45	4.45
CR003389	62.32	1.97	13.19	1.18	50.90	1.02
CR003390	18.97	4.82	3.31	0.91	16.49	3.98
CR003391	61.31	13.21	2.10	0.51	59.70	12.76
CR003392	28.37	8.58	1.93	0.73	26.98	7.94

[00232] Table 6 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR and control crRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in the human hepatocellular carcinoma cell line, HepG2.

Table 6: TTR editing data in HepG2 cells transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR001261 (control)	49.16	7.45	16.46	3.46	32.71	4.06
CR001262 (control)	63.33	5.66	59.88	4.92	3.45	0.86
CR001263 (control)	39.19	6.98	37.59	8.01	1.60	1.92
CR001264 (control)	57.09	12.14	47.47	9.25	9.61	2.89

CR003335	37.19	2.12	32.96	1.67	4.23	0.59
CR003336	31.31	5.47	30.48	5.10	0.83	0.75
CR003337	61.93	2.68	59.28	2.11	2.65	1.39
CR003338	68.00	6.09	65.40	6.78	2.60	1.17
CR003339	68.21	7.67	12.37	1.47	55.84	6.31
CR003340	37.76	6.01	6.12	1.95	31.65	4.07
CR003341	15.60	5.49	9.94	3.38	5.66	2.13
CR003342	11.06	6.71	10.78	6.69	0.28	0.03
CR003343	45.41	15.20	40.05	10.79	5.36	5.20
CR003344	33.43	6.11	29.81	5.09	3.62	1.13
CR003345	10.58	9.25	6.12	5.38	4.45	3.87
CR003346	0.13	0.05	0.07	0.02	0.05	0.03
CR003347	22.57	10.94	21.08	11.19	1.49	0.90
CR003348	38.44	10.45	17.04	5.04	21.40	5.89
CR003349	8.36	2.19	4.46	1.75	3.91	0.76
CR003350	29.60	5.17	25.16	4.56	4.44	0.67
CR003351	57.54	5.67	31.98	2.63	25.57	3.08
CR003352	44.28	8.71	39.51	7.10	4.77	1.79
CR003353	60.40	11.37	56.71	9.95	3.68	1.45
CR003354	5.36	3.94	4.84	3.41	0.53	0.71
CR003355	15.80	5.38	12.36	4.23	3.44	1.16
CR003356	9.39	1.82	5.67	1.03	3.72	0.92
CR003357	45.83	10.66	42.37	8.47	3.46	2.28
CR003358	35.93	7.34	28.66	7.76	7.27	1.77
CR003359	64.44	14.90	48.79	14.32	15.65	1.94
CR003360	41.31	12.23	38.94	10.60	2.38	1.78
CR003361	14.05	4.79	11.47	4.35	2.58	0.43
CR003362	17.44	4.34	16.50	4.86	0.94	0.52
CR003363	42.65	9.90	28.58	6.95	14.07	3.01
CR003364	51.88	7.67	31.03	2.67	20.85	5.03
CR003365	46.88	15.78	35.77	13.49	11.11	2.30
CR003366	54.69	9.10	46.20	8.98	8.49	1.11
CR003367	45.55	8.19	24.28	6.57	21.27	1.62
CR003368	51.55	8.60	48.34	9.87	3.22	1.36
CR003369	22.62	4.01	17.11	4.47	5.51	2.52
CR003370	28.51	6.94	24.88	6.17	3.62	1.45
CR003371	15.91	4.17	14.07	4.02	1.84	0.22
CR003372	14.57	2.47	12.14	2.08	2.42	0.40
CR003373	17.69	8.41	15.92	6.44	1.77	1.97
CR003374	5.43	0.53	5.12	0.62	0.31	0.36
CR003375	2.06	0.04	1.96	0.06	0.10	0.03
CR003376	14.41	3.01	14.16	2.93	0.24	0.10
CR003377	16.30	2.85	15.29	2.59	1.02	0.59
CR003378	8.16	3.83	6.82	3.43	1.34	0.61
CR003379	19.74	4.24	17.70	4.30	2.04	0.33
CR003380	17.08	2.48	14.78	1.18	2.30	1.36

CR003381	6.81	3.48	6.18	3.82	0.63	0.44
CR003382	1.73	0.14	1.58	0.12	0.15	0.03
CR003383	6.35	1.67	6.19	1.68	0.16	0.04
CR003384	3.37	0.88	3.12	0.94	0.25	0.09
CR003385	53.94	9.41	46.32	10.66	7.62	1.29
CR003386	2.71	0.76	2.15	0.77	0.56	0.53
CR003387	1.39	0.15	1.27	0.17	0.12	0.02
CR003388	9.33	4.47	7.76	4.56	1.56	0.10
CR003389	31.84	6.09	27.27	5.96	4.57	1.21
CR003390	24.88	4.96	22.44	3.41	2.44	2.25
CR003391	48.78	14.41	48.28	14.44	0.50	0.52
CR003392	14.64	5.25	14.32	4.95	0.33	0.36
CR005298	42.65	10.94	21.29	8.16	21.36	2.87
CR005299	38.61	5.57	36.32	3.99	2.30	2.11
CR005300	64.34	9.55	53.20	6.59	11.15	3.33
CR005301	37.04	5.32	33.39	3.85	3.65	1.89
CR005302	33.21	2.19	30.93	2.43	2.29	0.24
CR005303	21.63	6.05	20.55	5.80	1.08	0.25
CR005304	62.82	3.28	8.07	1.22	54.75	4.27
CR005305	13.51	3.58	12.30	3.49	1.21	0.84
CR005306	24.07	5.24	21.20	5.03	2.87	1.10
CR005307	22.03	3.86	7.70	1.35	14.33	4.15

[00233] Table 7 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* dgRNAs electroporated with Spy Cas9 protein (RNP) in primary human hepatocytes.

Table 7: TTR editing data in primary human hepatocytes electroporated with Spy Cas9 protein loaded with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003335	72.20	4.53	69.70	4.36	2.50	0.30
CR003336	39.17	3.04	38.43	3.20	0.70	0.17
CR003337	54.27	2.70	53.23	3.05	1.30	0.26
CR003338	83.03	4.84	80.87	4.63	2.13	0.25
CR003339	43.00	2.66	8.93	1.86	34.07	1.72
CR003340	12.03	1.55	5.60	1.32	6.50	0.53
CR003341	11.43	0.71	7.03	0.50	4.40	1.21
CR003342	32.77	3.63	31.87	3.28	0.90	0.35
CR003343	77.10	2.21	75.63	2.01	1.50	0.36
CR003344	39.40	3.86	33.30	2.52	6.10	1.31
CR003345	48.07	6.24	34.53	2.95	13.57	3.74
CR003346	35.67	1.80	20.83	1.65	14.83	1.66
CR003347	82.30	5.93	81.97	5.98	0.43	0.15

CR003348	28.53	1.79	11.30	2.46	17.27	0.86
CR003349	4.10	0.17	2.33	0.46	1.87	0.25
CR003350	28.13	3.50	22.40	2.41	5.73	1.22
CR003351	51.77	5.11	30.83	3.32	20.97	2.43
CR003352	29.83	4.18	25.63	3.67	4.30	0.56
CR003353	84.83	4.68	82.23	4.05	2.63	0.74
CR003354	2.50	0.36	2.43	0.32	0.03	0.06
CR003355	12.53	1.54	10.60	2.36	1.97	1.17
CR003356	9.97	2.68	7.80	2.01	2.23	0.85
CR003357	36.23	4.02	35.47	4.11	0.77	0.61
CR003358	5.70	1.42	4.93	1.36	0.80	0.26
CR003359	63.77	7.07	56.33	5.81	7.50	1.35
CR003360	32.23	3.09	31.67	2.97	0.63	0.31
CR003361	4.10	0.36	3.73	0.42	0.37	0.06
CR003362	7.03	1.30	6.87	1.20	0.20	0.20
CR003363	9.43	8.22	7.80	6.86	1.63	1.44
CR003364	23.30	5.20	16.93	4.96	6.53	0.55
CR003365	42.37	3.88	35.57	1.88	6.83	2.00
CR003366	34.70	3.26	31.63	2.98	3.10	1.15
CR003367	39.20	5.31	22.93	4.14	16.37	1.46
CR003368	28.47	3.29	27.63	2.90	0.80	0.66
CR003369	3.67	1.16	3.30	1.06	0.40	0.20
CR003370	15.27	1.75	14.43	1.72	0.90	0.20
CR003371	16.20	2.13	14.47	2.37	1.87	0.81
CR003372	12.17	2.69	10.47	2.63	1.77	0.12
CR003373	0.87	0.21	0.83	0.25	0.07	0.12
CR003374	0.80	0.17	0.70	0.26	0.10	0.10
CR003375	1.33	1.10	1.27	1.08	0.07	0.06
CR003376	1.90	1.06	1.87	1.00	0.03	0.06
CR003377	10.23	1.53	10.13	1.51	0.10	0.10
CR003378	4.60	1.92	3.87	1.19	0.73	0.67
CR003379	6.57	1.00	6.30	0.70	0.27	0.31
CR003380	5.37	2.57	5.27	2.54	0.10	0.10
CR003381	6.20	2.74	5.83	2.61	0.50	0.10
CR003382	8.40	2.07	8.10	1.87	0.43	0.21
CR003383	8.57	0.75	3.37	0.67	5.27	0.46
CR003384	1.87	0.67	1.73	0.57	0.23	0.12
CR003385	40.87	6.86	38.43	6.41	2.53	0.45
CR003386	4.90	1.20	4.47	1.14	0.47	0.25
CR003387	1.87	0.25	1.70	0.26	0.20	0.10
CR003388	5.70	0.40	5.47	0.40	0.27	0.12
CR003389	27.67	2.76	27.20	2.88	0.50	0.36
CR003390	15.97	3.86	15.80	3.99	0.23	0.15
CR003391	29.77	3.85	29.57	3.85	0.27	0.06
CR003392	4.13	1.21	4.00	1.15	0.17	0.06
CR005298	39.90	2.92	22.37	3.04	17.57	0.42

CR005299	8.65	0.78	8.30	0.99	0.35	0.21
CR005300	57.47	1.69	53.47	1.86	4.10	0.92
CR005301	25.37	1.65	24.00	2.26	1.60	0.82
CR005302	61.10	5.20	60.10	4.77	1.00	0.46
CR005303	53.57	8.52	53.07	8.36	0.53	0.47
CR005304	67.00	5.80	5.53	1.37	61.63	6.98
CR005305	3.83	0.78	3.53	0.61	0.40	0.17
CR005306	9.43	1.63	8.07	2.17	1.37	0.72
CR005307	8.17	1.20	5.20	0.87	3.00	0.82

[00234] Table 8 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* and control dgRNAs transfected with Spy Cas9 protein (RNP) in primary human hepatocytes.

Table 8: TTR editing data in primary human hepatocytes transfected with Spy Cas9 loaded with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR001261	32.51	1.00	12.50	0.47	20.01	0.59
CR001262	50.09	1.48	45.25	1.69	4.83	0.31
CR001263	15.25	2.41	14.83	2.37	0.42	0.10
CR001264	45.30	3.48	23.87	2.09	21.43	1.68
CR003335	51.14	4.27	49.51	4.04	1.63	0.25
CR003336	30.70	2.41	30.11	2.48	0.58	0.11
CR003337	49.43	4.75	47.54	4.49	1.88	0.47
CR003338	61.34	3.55	59.13	3.44	2.22	0.11
CR003339	45.06	9.83	8.85	1.65	36.21	8.34
CR003340	10.44	2.44	5.94	1.34	4.50	1.16
CR003341	19.66	3.67	14.64	3.31	5.02	0.37
CR003342	20.66	2.55	19.85	2.54	0.81	0.15
CR003343	43.25	4.47	41.61	4.26	1.63	0.33
CR003344	35.45	13.12	30.97	11.72	4.48	1.51
CR003345	28.90	6.33	21.00	5.23	7.91	1.81
CR003346	4.11	1.36	2.27	0.53	1.84	0.85
CR003347	66.35	4.48	66.11	4.51	0.24	0.08
CR003348	23.18	2.16	13.74	1.17	9.44	0.99
CR003349	10.83	1.57	9.00	1.41	1.83	0.32
CR003350	24.84	2.74	19.77	1.91	5.07	0.89
CR003351	40.28	1.31	23.92	0.70	16.36	0.78
CR003352	30.48	1.93	27.27	2.31	3.21	0.38
CR003353	61.54	4.13	59.38	4.04	2.16	0.11
CR003354	10.31	1.47	10.07	1.50	0.23	0.11
CR003355	19.11	0.92	17.69	0.79	1.42	0.44
CR003356	7.53	1.78	6.24	1.51	1.29	0.32

CR003357	49.35	2.53	48.45	2.54	0.90	0.13
CR003358	31.62	5.97	25.95	5.03	5.67	1.04
CR003359	59.47	6.05	50.96	5.69	8.51	0.54
CR003360	31.47	4.12	30.27	4.21	1.19	0.22
CR003361	13.08	1.48	12.52	1.45	0.56	0.18
CR003362	11.65	1.24	11.10	1.06	0.56	0.36
CR003363	27.65	2.84	21.47	2.39	6.18	0.61
CR003364	35.29	3.50	23.93	2.63	11.36	1.16
CR003365	47.78	3.67	40.24	3.12	7.54	0.72
CR003366	42.74	3.41	37.95	2.88	4.79	0.60
CR003367	31.19	4.60	16.06	2.66	15.13	1.94
CR003368	34.83	5.05	33.83	5.09	1.00	0.10
CR003369	12.98	0.26	11.67	0.21	1.31	0.11
CR003370	20.06	1.79	18.80	1.65	1.26	0.28
CR003371	18.80	2.73	17.23	2.34	1.57	0.43
CR003372	17.56	2.26	15.74	2.16	1.81	0.10
CR003373	3.64	0.29	3.44	0.30	0.19	0.07
CR003374	2.65	0.33	2.52	0.33	0.14	0.02
CR003375	5.04	0.66	4.93	0.66	0.11	0.01
CR003376	5.00	1.10	4.86	1.10	0.14	0.03
CR003377	12.77	2.00	12.45	1.84	0.31	0.18
CR003378	8.66	1.90	8.24	1.74	0.42	0.19
CR003379	16.86	2.62	16.51	2.62	0.34	0.08
CR003380	8.17	1.42	7.71	1.47	0.46	0.10
CR003381	7.15	0.73	6.88	0.67	0.27	0.07
CR003382	2.44	0.06	2.28	0.05	0.15	0.03
CR003383	4.76	0.40	4.52	0.42	0.24	0.09
CR003384	3.56	0.26	3.39	0.26	0.17	0.01
CR003385	41.15	6.06	38.15	5.59	3.00	0.48
CR003386	3.22	0.25	2.97	0.27	0.25	0.02
CR003387	1.79	0.11	1.68	0.09	0.11	0.04
CR003388	5.43	1.03	4.38	1.00	1.05	0.25
CR003389	19.87	4.39	19.19	4.52	0.68	0.24
CR003390	16.09	2.84	15.85	2.91	0.24	0.09
CR003391	34.72	8.29	34.46	8.35	0.26	0.06
CR003392	10.07	1.06	9.93	1.02	0.14	0.04
CR005298	32.07	1.02	21.12	1.02	10.95	0.15
CR005299	19.37	0.61	18.79	0.51	0.58	0.13
CR005300	57.23	6.24	53.62	5.44	3.61	0.87
CR005301	31.37	3.02	29.53	2.88	1.84	0.15
CR005302	48.29	5.22	47.32	5.32	0.97	0.14
CR005303	36.45	4.83	36.06	4.72	0.39	0.12
CR005304	49.45	6.85	4.32	0.31	45.13	6.74
CR005305	7.07	1.43	6.73	1.30	0.34	0.17
CR005306	18.81	1.82	16.24	1.57	2.57	0.35
CR005307	18.73	1.68	10.18	0.92	8.55	0.88

[00235] Table 9 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* and control dgRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in primary human hepatocytes.

Table 9: TTR editing data in primary human hepatocytes transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR001261	32.33	4.95	5.83	1.63	26.47	3.30
CR001262	41.50	4.71	34.43	3.31	7.13	1.42
CR001263	10.23	3.61	9.40	3.20	0.90	0.44
CR001264	42.80	0.50	11.90	1.32	30.90	1.80
CR003335	36.43	2.98	33.03	2.31	3.40	0.70
CR003336	16.93	3.78	16.20	3.41	0.80	0.44
CR003337	19.30	1.57	18.10	1.44	1.23	0.15
CR003338	36.30	9.55	33.73	9.27	2.73	0.49
CR003339	36.43	1.21	2.27	0.15	34.23	1.31
CR003340	24.97	2.78	1.83	0.23	23.17	2.66
CR003341	15.83	1.38	6.80	0.53	9.07	0.81
CR003342	22.10	1.27	20.60	0.57	1.50	0.71
CR003343	55.03	0.38	52.40	0.53	2.60	0.44
CR003344	31.50	1.30	22.40	1.31	9.20	0.10
CR003345	50.65	2.90	32.30	1.56	18.45	1.20
CR003346	19.97	1.94	5.63	0.55	14.33	1.72
CR003347	41.47	3.59	41.33	3.63	0.17	0.06
CR003348	18.00	0.87	2.30	0.66	15.80	0.61
CR003349	2.57	0.81	0.90	0.35	1.70	0.46
CR003350	26.63	4.25	16.33	2.45	10.33	1.75
CR003351	26.50	1.61	10.20	0.92	16.37	0.97
CR003352	16.80	5.03	11.73	3.86	5.07	1.14
CR003353	53.73	6.01	49.50	5.82	4.43	0.75
CR003354	2.97	0.95	2.87	0.85	0.13	0.12
CR003355	12.07	2.61	10.47	2.08	1.63	0.59
CR003356	7.27	0.72	4.70	0.53	2.67	0.21
CR003357	25.93	4.55	25.30	4.22	0.63	0.35
CR003358	3.90	0.79	2.73	0.45	1.17	0.51
CR003359	32.93	4.34	25.67	3.25	7.33	1.24
CR003360	14.90	4.85	14.13	4.66	0.90	0.52
CR003361	3.53	0.60	2.73	0.55	0.87	0.15
CR003362	6.60	1.47	6.17	1.45	0.47	0.21
CR003363	16.70	1.08	11.80	0.79	4.93	0.60
CR003364	15.63	2.45	6.73	0.81	8.93	1.70
CR003365	26.90	3.05	20.23	2.02	6.67	1.16

CR003366	24.53	1.26	20.47	1.45	4.07	0.23
CR003367	37.33	1.40	14.03	0.40	23.37	1.25
CR003368	11.10	1.91	10.53	1.90	0.60	0.10
CR003369	1.60	0.46	0.90	0.20	0.70	0.36
CR003370	2.83	0.57	2.33	0.40	0.50	0.17
CR003371	3.40	0.80	2.67	0.75	0.73	0.15
CR003372	1.77	0.75	1.13	0.57	0.63	0.23
CR003373	1.40	0.36	1.00	0.35	0.37	0.12
CR003374	0.27	0.21	0.27	0.21	0.03	0.06
CR003375	1.27	0.64	1.23	0.58	0.03	0.06
CR003376	2.83	0.81	2.73	0.81	0.13	0.06
CR003377	17.53	6.35	16.97	6.11	0.57	0.25
CR003378	9.80	1.37	8.50	1.21	1.37	0.15
CR003379	13.20	1.18	12.00	1.05	1.27	0.15
CR003380	2.93	0.58	2.47	0.57	0.47	0.15
CR003381	4.07	1.21	3.33	0.96	0.73	0.25
CR003382	0.97	0.25	0.97	0.25	0.00	0.00
CR003383	15.70	3.22	2.07	0.35	13.70	2.82
CR003384	1.70	0.62	1.50	0.56	0.20	0.10
CR003385	36.77	0.70	33.23	0.74	3.60	0.26
CR003386	8.27	1.63	8.20	1.57	0.13	0.06
CR003387	7.87	1.58	7.80	1.64	0.03	0.06
CR003388	12.97	1.30	11.87	1.21	1.17	0.25
CR003389	44.27	1.72	41.47	1.59	2.83	0.15
CR003390	20.23	2.08	18.73	1.92	1.60	0.17
CR003391	15.47	5.87	15.20	5.72	0.30	0.10
CR003392	2.43	0.55	2.37	0.59	0.07	0.06
CR005298	15.70	2.79	4.13	0.87	11.60	2.00
CR005299	9.43	0.68	8.93	0.68	0.60	0.00
CR005300	31.53	3.44	27.60	2.77	3.97	0.76
CR005301	6.77	1.44	5.47	0.96	1.40	0.61
CR005302	34.80	7.17	33.67	7.01	1.13	0.21
CR005303	35.50	5.90	35.00	5.81	0.50	0.10
CR005304	45.27	4.71	0.83	0.15	44.47	4.57
CR005305	7.53	1.06	5.93	1.10	1.60	0.10
CR005306	9.97	0.38	7.13	0.23	2.87	0.12
CR005307	12.90	2.43	3.67	0.61	9.30	1.80

[00236] Table 10 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* dgRNAs electroporated with Spy Cas9 protein (RNP) in primary cyno hepatocytes.

Table 10: TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003336	8.18	1.93	8.10	1.94	0.07	0.01
CR003337	24.94	5.80	24.10	4.71	0.84	1.10
CR003338	44.94	9.99	44.89	9.97	0.05	0.01
CR003339	8.95	0.89	4.93	0.64	4.02	0.25
CR003340	12.53	2.22	7.72	0.13	4.80	2.09
CR003341	8.43	10.53	7.66	9.91	0.77	0.63
CR003344	35.72	4.67	33.81	5.29	1.91	0.61
CR003345	52.92	3.26	30.74	0.78	22.19	2.48
CR003346	1.91	0.86	1.82	0.82	0.09	0.04
CR003347	72.41	0.38	72.15	0.73	0.25	0.34
CR003352	1.25	0.20	1.16	0.21	0.09	0.01
CR003353	4.75	0.43	4.67	0.47	0.08	0.04
CR003358	20.47	0.30	19.01	0.51	1.46	0.21
CR003359	46.17	1.14	40.66	2.00	5.51	0.86
CR003360	29.47	0.63	29.05	1.00	0.42	0.37
CR003361	4.53	0.14	4.46	0.18	0.08	0.04
CR003362	4.59	0.80	4.36	0.77	0.22	0.03
CR003363	15.64	1.92	13.24	2.65	2.39	0.73
CR003364	19.62	2.54	14.27	2.72	5.35	0.17
CR003365	10.31	1.81	9.33	1.80	0.97	0.01
CR003366	18.52	0.71	17.62	0.33	0.90	0.39
CR003368	18.56	3.89	18.30	3.77	0.26	0.11
CR003369	1.53	0.25	1.28	0.40	0.25	0.15
CR003370	2.52	0.64	2.40	0.63	0.12	0.01
CR003371	1.83	0.38	1.69	0.41	0.14	0.03
CR003372	2.15	0.30	1.83	0.33	0.32	0.04
CR003382	10.86	2.04	8.54	1.93	2.33	0.11
CR003383	8.86	2.30	4.31	0.69	4.55	1.61
CR003384	3.75	0.35	2.50	0.37	1.25	0.02
CR003385	30.96	1.61	26.84	2.20	4.12	0.59
CR003386	5.54	1.42	3.51	1.26	2.03	0.15
CR003387	4.72	0.03	4.55	0.08	0.17	0.11
CR003388	6.81	0.17	6.59	0.28	0.22	0.11
CR003389	18.83	4.99	18.05	4.92	0.78	0.07
CR003390	16.87	3.88	16.49	3.48	0.39	0.39
CR003391	36.44	1.09	35.73	1.37	0.71	0.28
CR003392	7.02	0.97	6.63	0.59	0.38	0.37
CR005299	13.48	2.96	13.23	2.74	0.26	0.22
CR005301	46.76	1.75	46.34	2.19	0.42	0.44
CR005302	1.34	0.19	1.26	0.19	0.08	0.00
CR005303	59.28	1.05	58.72	1.06	0.56	0.00

CR005305	11.28	0.39	11.13	0.39	0.15	0.00
CR005307	4.56	0.71	2.01	0.49	2.55	0.21

[00237] Table 11 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested cyno specific *TTR* dgRNAs electroporated with Spy Cas9 protein (RNP) on primary cyno hepatocytes.

Table 11: TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and cyno specific dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR000689	24.41	1.67	18.11	2.41	6.30	0.93
CR005364	27.70	0.74	0.58	0.29	27.11	0.60
CR005365	64.94	2.03	0.10	0.04	64.85	2.05
CR005366	77.00	1.17	0.33	0.27	76.67	0.99
CR005367	50.79	0.53	0.53	0.25	50.26	0.36
CR005368	27.60	2.07	0.33	0.45	27.27	2.32
CR005369	42.01	0.33	8.09	0.55	33.92	0.31
CR005370	63.52	3.21	0.59	0.33	62.93	2.88
CR005371	8.42	0.69	0.31	0.12	8.10	0.57
CR005372	17.98	1.39	0.83	0.77	17.16	0.71

Example 3. Screening of sgRNA sequences

Cross screening of TTR sgRNAs in multiple cell types

[00238] Guides in modified sgRNA format targeting human and/or cyno *TTR* were delivered to primary human hepatocytes and primary cyno hepatocytes as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on highest % edit. The screening data for the guide sequences in Table 2 in both cell lines are listed below (Table 12 through 15).

[00239] Table 12 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs transfected with Spy Cas9 protein (RNP) in primary human hepatocytes.

Table 12: TTR editing data in primary human hepatocytes transfected with Spy Cas9 protein and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion

G000480	81.80	1.98	77.15	2.19	4.70	0.28
G000481	46.90	1.71	27.77	3.88	19.43	4.76
G000482	66.67	2.35	56.57	4.14	10.10	1.85
G000483	47.90	6.56	19.57	3.37	28.50	3.25
G000484	62.97	0.90	29.23	0.21	33.83	0.95
G000485	56.07	3.37	53.07	2.84	3.13	0.60
G000486	69.73	6.86	9.83	1.93	59.93	5.63
G000487	67.30	2.75	65.27	3.41	2.07	1.06
G000488	61.27	1.95	26.30	1.55	35.00	1.30
G000489	60.17	2.75	51.07	3.18	9.43	0.45
G000490	55.90	7.88	46.13	7.55	9.80	0.69
G000491	74.30	1.55	70.27	2.37	4.33	0.72
G000492	60.97	5.81	57.90	4.64	3.13	1.35
G000493	41.40	3.08	38.90	3.29	2.67	0.35
G000494	62.23	3.30	61.47	3.25	0.77	0.31
G000495	50.80	1.85	45.80	1.25	5.37	0.64
G000496	72.33	1.63	44.73	2.14	27.67	1.46
G000497	59.67	1.40	51.10	1.14	8.73	0.71
G000498	72.80	3.75	60.17	3.12	12.70	0.72
G000499	66.40	3.55	65.23	3.72	1.17	0.38
G000500	65.53	1.21	62.00	1.11	3.83	0.40
G000501	60.93	1.91	55.13	1.43	6.00	0.56

[00240] Table 13 shows the average and standard deviation at 12.5 nM for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in primary human hepatocytes.

Table 13: TTR editing data in primary human hepatocytes transfected with Spy Cas9 mRNA and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000480	73.28	0.61	59.85	0.13	13.47	0.51
G000481	34.30	5.26	14.62	2.59	19.77	2.72
G000482	40.93	3.95	27.70	2.92	13.25	0.97
G000483	27.82	2.93	4.05	0.51	23.85	2.43
G000484	43.37	6.79	13.98	2.61	29.48	4.15
G000485	30.82	5.76	28.87	5.50	1.97	0.28
G000486	59.13	5.62	2.82	0.86	56.37	4.92
G000487	49.57	0.99	47.38	0.89	2.27	0.24
G000488	49.40	5.05	11.98	1.40	37.48	3.68
G000489	24.25	2.82	14.17	2.01	10.28	1.38
G000490	24.72	2.35	19.38	2.04	5.38	0.41
G000491	45.93	1.22	42.42	1.06	3.60	0.33
G000492	34.65	2.21	32.45	2.01	2.22	0.25

G000493	11.55	1.35	10.65	1.58	0.97	0.30
G000494	26.22	4.03	25.17	3.89	1.07	0.15
G000495	47.77	1.88	43.40	1.91	4.45	0.17
G000496	63.30	2.60	11.08	2.10	52.25	0.67
G000497	40.33	3.32	34.48	2.71	5.85	0.61
G000498	60.02	5.42	45.20	4.34	14.90	1.08
G000499	39.30	6.04	38.58	5.86	0.77	0.12
G000500	35.50	0.61	32.47	0.49	3.10	0.18
G000501	40.32	1.50	33.82	2.04	6.62	0.55
G000567	27.28	7.59	17.35	4.72	10.02	2.94
G000568	43.75	5.83	43.00	5.81	0.80	0.18
G000570	68.42	3.64	68.08	3.61	0.35	0.00
G000571	20.47	3.41	14.47	2.72	6.13	0.78
G000572	55.42	8.13	41.62	6.48	13.85	1.60

[00241] Table 14 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs electroporated with Spy Cas9 protein (RNP) on primary cyno hepatocytes. Note that guides G000480 and G000488 have one mismatch to cyno, which may compromise their editing efficiency in cyno cells.

Table 14: TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000480	10.20	0.56	9.83	0.81	0.37	0.25
G000481	69.13	8.62	33.73	2.67	35.50	11.23
G000482	75.17	2.34	55.23	2.00	20.03	0.85
G000485	22.93	0.95	22.00	0.82	1.07	0.21
G000486	79.90	0.79	11.90	0.85	68.07	0.35
G000488	9.63	0.50	5.37	0.38	4.27	0.35
G000489	67.53	1.15	53.53	1.56	14.17	0.64
G000490	61.67	0.72	54.47	1.10	7.27	1.23
G000491	66.20	1.11	64.37	0.47	1.90	0.70
G000493	50.13	0.74	48.07	1.69	2.10	0.98
G000494	81.53	0.71	79.57	0.49	2.07	0.67
G000498	91.37	1.48	68.50	1.64	22.87	1.50
G000499	83.40	0.36	82.00	0.20	1.43	0.55
G000500	45.20	3.66	42.60	3.80	2.63	0.25

[00242] Table 15 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested cyno specific *TTR* sgRNAs electroporated with Spy Cas9 protein (RNP) on primary cyno hepatocytes.

Table 15: TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and cyno specific sgRNAs (e.g., those having an analogous human gRNA, See Table 3)

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000502	95.10	0.96	13.97	1.69	81.27	2.60
G000503	58.53	2.40	52.07	1.68	6.50	2.46
G000504	77.17	0.96	69.73	1.29	7.53	0.57
G000505	95.53	1.06	95.50	1.01	0.10	0.10
G000506	89.43	1.36	86.90	1.64	3.07	0.42
G000507	71.17	3.22	67.03	2.39	4.60	1.65
G000508	45.63	3.01	41.57	2.95	4.17	0.91
G000509	93.03	0.81	43.60	1.30	49.73	1.76
G000510	90.80	0.53	89.13	0.40	1.77	0.12
G000511	62.77	1.63	60.87	1.55	2.00	0.35

Example 4. Screening of lipid nanoparticle (LNP) formulations containing Spy Ca9 mRNA and sgRNA

[00243] Cross screening of LNP formulated TTR sgRNAs with Spy Cas9 mRNA in primary human hepatocytes and primary cyno hepatocytes.

[00244] Lipid nanoparticle formulations of modified sgRNAs targeting human TTR and the cyno matched sgRNA sequences were tested on primary human hepatocytes and primary cyno hepatocytes in a dose response curve. Primary human and cyno hepatocytes were plated as described in Example 1. Both cell lines were incubated at 37°C, 5% CO_2 for 24 hours prior to treatment with LNPs. The LNPs used in the experiments detailed in Tables 16-19 were prepared using the Nanoassemblr™ procedure, each containing the specified sgRNA and Cas9 mRNA (SEQ ID NO:2), each having Lipid. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 45:44:9:2 molar ratio, respectively, and had a N:P ratio of 4.5. LNPs were incubated in hepatocyte maintenance media containing 6% cyno serum at 37°C for 5 minutes. Post incubation the LNPs were added onto the primary human or cyno hepatocytes in an 8 point 2-fold dose response curve starting at 100 ng mRNA. The cells were lysed 72 hours post treatment for NGS analysis as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on highest % editing at 12.5 ng mRNA input and 3.9 nM guide concentration. The dose response curve data for the guide

sequences in both cell lines is shown in Figs. 4 through 7. The % editing at 12.5 ng mRNA input and 3.9 nM guide concentration are listed below (Table 16 through 18).

[00245] Table 16 shows the average and standard deviation at 12.5 ng of cas9 mRNA for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs formulated in lipid nanoparticles with Spy Cas9 mRNA on primary human hepatocytes as dose response curves. G000570 exhibited an uncharacteristic dose response curve compared to the other sgRNAs which may be an artifact of the experiment. The data are shown graphically in FIG.4.

Table 16: TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO:2) and sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA Avg % Edit	Std Dev % Edit
G000480	59.33	0.73
G000481	24.37	0.37
G000482	19.10	2.64
G000483	7.37	0.67
G000484	16.67	1.23
G000485	14.23	2.36
G000486	61.33	2.59
G000487	17.37	0.95
G000488	44.80	3.00
G000489	16.85	0.06
G000490	10.53	1.90
G000491	31.60	2.33
G000492	15.87	0.44
G000493	7.33	0.73
G000494	6.37	1.07
G000495	23.97	1.66
G000496	30.73	3.76
G000497	15.10	3.30
G000498	24.43	1.30
G000499	16.07	1.67
G000500	23.57	2.44
G000501	32.30	2.49
G000567	48.95	1.06
G000568	54.60	3.68
G000570	88.30	1.84
G000572	55.45	1.20

[00246] Table 17 shows the average and standard deviation at 12.5 ng of mNRA and 3.9 nM guide concentration for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs formulated in lipid nanoparticles with Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG.5.

Table 17: TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO: 2) and sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA, Avg % Edit	Std Dev % Edit
G000480	0.73	0.15
G000481	49.20	1.39
G000482	26.13	5.33
G000483	0.73	0.60
G000484	0.10	0.00
G000485	1.43	1.02
G000489	31.87	2.40
G000490	15.23	1.08
G000491	6.37	0.38
G000492	0.70	0.28
G000493	7.63	1.14
G000494	14.30	1.06
G000495	0.73	0.06
G000497	0.23	0.06
G000498	37.90	1.42
G000499	14.63	0.70
G000500	10.47	0.32
G000501	1.37	0.31
G000567	0.10	0.00
G000568	9.25	0.21
G000570	17.30	0.85
G000571	20.20	2.26
G000572	30.60	0.42

[00247] Table 18 shows the average and standard deviation at 12.5 ng of mRNA and 3.9 nM guide concentration for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested cyno specific *TTR* sgRNAs formulated in lipid nanoparticles with Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG.6.

Table 18: TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO: 2) and cyno matched sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA % Edit	Std Dev % Edit
G000502	80.70	0.14
G000506	60.13	0.70
G000509	74.47	7.28
G000510	61.87	2.54

Cross screening of LNP formulated TTR sgRNAs with Spy Cas9 mRNA in primary human hepatocytes and primary cyno hepatocytes

[00248] Lipid nanoparticle formulations of modified sgRNAs targeting human TTR and the cyno matched sgRNA sequences were tested on primary human hepatocytes and primary cyno hepatocytes in a dose response curve. Primary human and cyno hepatocytes were plated as described in Example 1. Both cell lines were incubated at 37°C, 5% CO_2 for 24 hours prior to treatment with LNPs. The LNPs used in the experiments detailed in Tables 20-22 were prepared using the cross-flow procedure described above but purified using PD-10 columns (GE Healthcare Life Sciences) and concentrated using Amicon centrifugal filter units (Millipore Sigma), each containing the specificed sgRNA and Cas9 mRNA (SEQ ID NO:1). The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had a N:P ratio of 6.0. LNPs were incubated in hepatocyte maintenance media containing 6% cyno serum at 37°C, 5% CO_2 for 5 minutes. Post incubation the LNPs were added onto the primary human or cyno hepatocytes in an 8 point 3-fold dose response curve starting at 300 ng mRNA. The cells were lysed 72 hours post treatment for NGS analysis as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on EC50 values and maximum editing percent. The dose response curve data for the guide sequences in both cell lines is shown in Figs. 4 through 7. The EC 50 values and maximum editing percent are listed below (Table 19 through 22).

[00249] Table 19 shows the EC50 and maximum editing the tested human specific *TTR* sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary human hepatocytes as dose response curves. The data are shown graphically in FIG.4.

Table 19: TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA and human specific sgRNAs

GUIDE ID	EC50	Max Editing
G000480	0.10	98.69
G000481	1.43	87.05
G000482	0.65	97.02
G000483	1.88	77.39
G000484	0.95	94.14
G000488	0.72	95.83
G000489	1.38	86.33
G000490	1.52	94.16
G000493	2.42	63.95
G000494	1.28	75.70
G000499	0.63	96.31
G000500	0.39	88.70
G000568	0.78	95.72
G000570	0.23	98.22
G000571	2.21	71.28
G000572	0.42	97.94

[00250] Table 20 shows the EC50 and maximum editing the tested human specific *TTR* sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG. 16.

Table 20: TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA and human specific sgRNAs

GUIDE ID	EC50	Max Editing
G000480	5.28	20.32
G000481	0.93	95.07
G000482	0.89	97.47
G000483	4.40	56.52
G000484	3.47	0.22
G000488	11.56	21.63

G000489	1.79	89.21
G000490	3.09	90.76
G000493	4.97	61.15
G000494	2.77	60.84
G000499	2.00	74.94
G000500	4.42	58.04
G000567	1.76	97.06
G000568	1.87	87.93
G000570	2.00	96.73
G000571	1.55	97.03
G000572	0.79	100.31

[00251] Table 21 shows the EC50 and maximum editing the tested cyno matched *TTR* sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary human hepatocytes as dose response curves. The data are shown graphically in FIG.17.

Table 21: TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA and cyno specific sgRNAs

GUIDE ID	EC50	Max Editing
G000502	0.70	91.50
G000504	5.16	7.16
G000505	3.57	13.48
G000506	1.26	89.49

[00252] Table 22 shows the EC50 and maximum editing the tested cyno matched *TTR* sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG. 18.

Table 22: TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA and cyno specific sgRNAs

GUIDE ID	EC50	Max Editing
G000502	0.26	100.05
G000503	2.26	83.41
G000504	1.42	98.04
G000505	1.10	99.97
G000506	0.66	99.18

Example 5. Off-Target analysis of TTR dgRNAs and sgRNAs

Off-target analysis of TTR guides

[00253] An oligo insertion based assay (See, e.g., Tsai et al., Nature Biotechnology 33, 187–197; 2015) was used to determine potential off-target genomic sites cleaved by Cas9 targeting *TTR*. Forty-five dgRNAs from Table 1 (and two control guides with known off-target profiles) were screened in the HEK293_Cas9 cells. The human embryonic kidney adenocarcinoma cell line HEK293 constitutively expressing Spy Cas9 ("HEK293_Cas9") was cultured in DMEM media supplemented with 10% fetal bovine serum and 500 µg/ml G418. Cells were plated at a density of 30,000 cells/well in a 96-well plate 24 hours prior to transfection. Cells were transfected with Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) per the manufacturer's protocol. Cells were transfected with a lipoplex containing individual crRNA (15 nM), trRNA (15 nM), and donor oligo with (10 nM) Lipofectamine RNAiMAX (0.3 µL/well) and OptiMem. Cells were lysed 24 hours post transfection and genomic DNA was extracting using Zymo's Quick gDNA 96 Extraction kit (catalog # D3012) following the manufacturer's recommended protocol. The gDNA was quantified using the Qubit High Sensitivity dsDNA kit (Life Technologies). Libraries were prepared per the previously described method in Tsai et al, 2015 with minor modifications. Sequencing was performed on Illumina's MiSeq and HiSeq 2500. The assay identified potential off-target sites for some of the crRNAs which are plotted in FIG.2.

[00254] Table 23 shows the number of off-target integration sites detected in HekCas9 cells transfected with *TTR* dgRNAs along with a double stranded DNA oligo donor sequence.

Table 23: Number of off-target integration sites detected for TTR dgRNAs via an oligo insertion based assay

GUIDE ID	# Sites
CR003335	0
CR003336	2
CR003337	10
CR003338	2
CR003339	3
CR003340	0
CR003342	0
CR003343	2
CR003344	0

CR003345	0
CR003346	0
CR003347	1
CR003348	3
CR003351	1
CR003352	2
CR003353	2
CR003355	1
CR003356	4
CR003357	3
CR003359	6
CR003360	0
CR003363	4
CR003365	3
CR003366	1
CR003367	1
CR003368	2
CR003369	2
CR003377	0
CR003380	0
CR003382	34
CR003383	1
CR003385	3
CR003386	1
CR003387	6
CR003388	2
CR003389	2
CR003390	1
CR003391	0
CR003392	0
CR005298	0
CR005300	0
CR005301	0
CR005302	1
CR005303	1
CR005304	0

[00255] Additionally, a subset of the guides was assessed for off-target potential as modified sgRNAs in the Hek_Cas9 cells via the oligo based insertion method described above. The off-target results were plotted in FIG.4.

[00256] Table 24 shows the number of off-target integration sites detected in HekCas9 cells transfected with *TTR* sgRNAs along with a double stranded DNA oligo donor sequence.

Table 24: Number of off-target integration sites detected for TTR sgRNAs via an insertion detection method

GUIDE ID	# Sites
G000480	11
G000481	3
G000482	13
G000483	5
G000484	7
G000485	22
G000486	12
G000487	14
G000488	0
G000489	19
G000490	12
G000491	28
G000492	97
G000493	7
G000494	4
G000495	13
G000496	1
G000497	26
G000498	82
G000499	4
G000500	46
G000501	4
G000567	9
G000568	937
G000570	19
G000571	16
G000572	15

Example 6. Targeted sequencing for validating potential off-target sites

[00257] The HEK293_Cas9 cells used in Example 5 for detecting potential off-targets constitutively overexpress Cas9, leading to a higher number of potential off-target "hits" as compared to a transient delivery paradigm in various cell types. Further, when delivering sgRNAs (as opposed to dgRNAs), the number of potential off-target hits may be further inflated as sgRNA molecules are more stable than dgRNAs (especially when chemically modified). Accordingly, potential off-target sites identified by an oligo insertion method as used in Example 5 may be validated using targeted sequencing of the identified potential off-target sites.

[00258] In one approach, primary hepatocytes are treated with LNPs comprising Cas9 mRNA and a sgRNA of interest (e.g., a sgRNA having potential off-target sites for evaluation). The primary hepatocytes are then lysed and primers flanking the potential off-target site(s) are used to generate an amplicon for NGS analysis. Identification of indels at a certain level may validate potential off-target site, whereas the lack of indels found at the potential off-target site may indicate a false positive in the HEK293_Cas9 cell assay.

Example 7. Phenotypic Analysis

Western blot analysis of secreted TTR

[00259] The hepatocellular carcinoma cell line, HepG2, was transfected as described in Example 1 with select guides from Table 1 in triplicate. Two days post-transfection, one replicate was harvested for genomic DNA and analysis by NGS sequencing for editing efficiency. Five days post-transfection, media without serum was replaced on one replicate. After 4hrs the media was harvested for analysis of secreted TTR by WB as previously described. The data for % edit for each guide and reduction of extracellular *TTR* is provided in FIG.7.

Western blot analysis of intracellular TTR

[00260] The hepatocellular carcinoma cell line, HUH7, was transfected as described in Example 1 with crRNA comprising the guides from Table 1. The transfected pools of cells were retained in tissue culture and passaged for further analysis. At seven days post-transfection, cells were harvested and whole cell extracts (WCEs) were prepared and subjected to analysis by Western Blot as previously described.

[00261] WCEs were analyzed by Western Blot for reduction of *TTR* protein. Full length *TTR* protein has a predicted molecular weight of ~16 kD. A band at this molecular weight was observed in the control lanes in the Western Blot.

[00262] Percent reduction of *TTR* protein was calculated using the Licor Odyssey Image Studio Ver 5.2 software. GAPDH was used as a loading control and probed simultaneously with *TTR*. A ratio was calculated for the densitometry values for GAPDH within each sample compared to the total region encompassing the *TTR* band. Percent reduction of *TTR* protein was determined after the ratios were normalized to control lanes. Results are shown in FIG.8.

Example 8. LNP delivery to humanized TTR mice and mice having wt (murine) TTR.

[00263] Mice humanized with respect to the *TTR* gene were dosed with LNP formulations 701-704 containing the guides indicated in Table 25 (5 mice per formulation). These humanized *TTR* mice were engineered such that a region of the endogenous murine *TTR*

locus was deleted and replaced with an orthologous human *TTR* sequence so that the locus encodes a human TTR protein. For comparison, 6 mice with murine *TTR* were dosed with LNP700, containing a guide (G000282) targeting murine *TTR*. LNPs with Formulation Numbers 1-5 in Table 25 were prepared using the Nanoassemblr™ procedure as desctibed above while LNPs with Formulation Numbers 6-16 were prepared using the cross-flow procedure described above but purified using PD-10 columns (GE Healthcare Life Sciences) and concentrated using Amicon centrifugal filter units (Millipore Sigma). As negative controls, mice of the corresponding genotype were dosed with vehicle alone (Tris-saline-sucrose buffer (TSS)). The background of the humanized *TTR* mice administered LNPs with Formulation Numers 2-5 in Table 25 was 50% 129S6/SvEvTac 50% C57BL/6NTac; the background of the humanized *TTR* mice administered LNPs having Formulation Numbers 6-16 in Table 25 as well as the mice with murine *TTR* (administered LNP700, Formulation Number 1) was 75% C57BL/6NTac 25% 129S6/SvEvTac.

Table 25. LNP formulations for dosing humanized *TTR* mice.

Formulation Number	LNP	Guide	RNA concentration (mg/ml)	N:P Ratio	Molar Ratios (Lipid A, Cholesterol, DSPC, and PEG2k-DMG, respectively)
1	LNP700	G000282	0.53	4.5	45:44:9:2
2	LNP701	G000481	0.46	4.5	45:44:9:2
3	LNP702	G000489	0.61	4.5	45:44:9:2
4	LNP703	G000494	0.57	4.5	45:44:9:2
5	LNP704	G000499	0.59	4.5	45:44:9:2
6	LNP1148	G000481	0.73	4.5	45:44:9:2
7	LNP1152	G000499	0.45	6.0	50:38:9:3
8	LNP1153	G000482	0.53	6.0	50:38:9:3
9	LNP1155	G000571	0.70	6.0	50:38:9:3
10	LNP1156	G000572	0.58	6.0	50:38:9:3
11	LNP1157	G000480	0.84	6.0	50:38:9:3
12	LNP1159	G000488	0.79	6.0	50:38:9:3
13	LNP1160	G000493	0.71	6.0	50:38:9:3
14	LNP1161	G000500	0.66	6.0	50:38:9:3
15	LNP1162	G000567	0.69	6.0	50:38:9:3
16	LNP1163	G000570	0.66	6.0	50:38:9:3

[00264] LNPs having Formulation numbers 1-5 contained Cas9 mRNA of SEQ ID NO:2 and LNPs having Formulation Numbers 6-16 contained Cas9 mRNA of SEQ ID NO: 1, all in a 1:1 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in the molar ratios recited in Table 25, respectively. Dosing with LNPs having Formulation Numbers 1-5 was at 2 mg/kg (total RNA content) and dosing with LNPs having Formulation Numbers 6-16 was at 1 mg/kg (total RNA content). Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis. Liver editing results for Formulation Numbers 1-5 are shown in FIG.9 and indicate editing of the human *TTR* sequence with each of the four guides tested at a level >35% editing (mean values) with G000494 and G000499 providing values near 60%. Liver editing results for formulation numbers 6-8, 10-13, and 15-16 are shown in FIG.13 and Table 26, which show efficient editing of the human *TTR* sequence with each of the formulations tested. Greater than 38% editing was seen for all formulations, with several formulations providing editing values greater than 60%. Formulations 9 and 14 are not shown due to the design of the PCR amplicon and a resulting low number of sequencing reads.

[00265] The level of human TTR in serum was measured in the mice provided formulation numbers 6-8, 10-13, and 15-16. *See* FIG.14B. FIG.14A is a repeat of FIG.13 provided for comparison purposes. Knockdown of serum human TTR was detected for each formulation tested, which correlated with the amount of editing detected in liver (See FIG.14A vs 14B, Table 26).

Table 26

GUIDE ID	**% Editing**	**Serum TTR(%TSS)**
TSS (vehicle)	0.06	100
G481	61.28	10.52
G499	65.66	8.39
G482	70.86	4.65
G572	73.52	2.11

G480	77.34	3.48
G488	59.125	27.78
G493	38.55	49.73
G567	47.525	44.24
G570	45.5	41.73
G571	33.88	11.39
G500	44.44	34.28

[00266] In another set of experiments, humanized TTR mice were dosed with LNP formulations across a range of doses with guides G000480, G000488, G000489 and G000502. The formulations contained Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and having a N:P ratio of 6. Dosing was at 1, 0.3, 0.1, or 0.03 mg/kg (n=5/group). The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis and serum human TTR levels were measured as described above. Results for liver editing are shown in FIG.26A and serum human TTR levels in FIG.26B-C. A dose response for both editing and serum TTR levels was evident.

[00267] In another set of experiments, humanized TTR mice were dosed with LNP formulations across a range of doses with guides G000481, G000482, G000486 and G000499. The formulations contained Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had an N:P ratio of 6. Dosing was at 1, 0.3, or 0.1 mg/kg (n=5/group). The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. Liver editing results were determined using primers designed to amplify the

region of interest for NGS analysis and serum human TTR levels were measured as described above. Results for liver editing are shown in FIG.27A and serum human TTR levels in FIG.27B-C. A dose response for both editing and serum TTR levels was evident.

[00268] In another set of experiments, humanized TTR mice were dosed with LNP formulations across a range of doses with guides G000480, G000481, G000486, G000499 and G000502. The formulations contained Cas9 mRNA (SEQ ID NO: 1) in a 1:2 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had an N:P ratio of 6. Dosing was at 1, 0.3, or 0.1 mg/kg (n=5/group). The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis and serum human TTR levels were measured as described above. Results for liver editing are shown in FIG.28A and serum human TTR levels in FIG.28B-C. A dose response for both editing and serum TTR levels was evident.

[00269] In separate experiments using wild type CD-1 mice, an LNP formulation comprising guide G000502, which is cross homologous between mouse and cyno, was tested in a dose response study. The formulation contained Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide. The LNP contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 45:44:9:2 molar ratio, respectively, and having a N:P ratio of 6. Dosing was at 1, 0.3, 0.1, 0.03, or 0.01 mg/kg (n=5/group). Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis. Results for liver editing are shown in FIG.15A and serum mouse TTR levels in FIG.15B. A dose response for both editing and serum TTR levels was evident.

Example 9. LNP delivery to mice in multiple doses

[00270] Mice (females from Charles River Laboratory, aged approximately 6-7 weeks) were dosed with an LNP formulation LNP705, prepared using cross-flow and TFF procedures as described above containing G000282 ("G282") and Cas9 mRNA (SEQ ID NO: 2) in a 1:1 ratio by weight and a total RNA concentration of 0.5 mg/ml. The LNP had an N:P ratio of 4.5 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 45:44:9:2 molar ratio, respectively. Groups were dosed either once weekly up to one, two, three, or four weeks (QWx1-4) or once monthly up to two or three months (QMx2-3). Dosages were 0.5 mg/kg or 1 mg/kg (total RNA content). Control groups received a single dose on day 1 of 0.5, 1, or 2 mg/kg. Each group contained 5 mice. Serum TTR was analyzed by ELISA and at

necropsy the liver, spleen and muscle were each collected for NGS editing analysis. Groups are shown in Table 27. X = sacrifice and necropsy. MPK = mg/kg.

Table 27. Study Groups

Group	Duration/ Dose Regimen	Dose (MPK)	Total Dose (MPK) Given	Dose Day 1	Dose Day 8	Dose Day 15	Dose Day 22	NX Day 28	Dose Day 43	NX Day 49
1	4 Week Multi Dose/ QWx4	0 (TSS control)	0	X	X	X	X	X		
2	2 Month Multi Dose/ QMx3	1	3	X			X		X	X
3		0.5	1.5	X			X		X	X
4	1 Month Multi Dose/ QMx2	1	2	X			X	X		
5		0.5	1	X			X	X		
6	4 Week Multi Dose/ QWx4	1	4	X	X	X	X	X		
7		0.5	2	X	X	X	X	X		
8	3 Week Multi Dose/ QWx3	1	3		X	X	X	X		
9		0.5	1.5		X	X	X	X		
10	2 Week Multi Dose/ QWx2	1	2			X	X	X		
11		0.5	1			X	X	X		
12	Single Dose/ QWx1	1	1				X	X		
13		0.5	0.5				X	X		
14		2	2				Day 26	Day 32		

[00271] Table 28 and FIGS. 10A-11B show serum TTR level results (% KD = % knockdown). Table 29 and FIGS. 12A-C show liver editing results.

Table 28. Serum TTR Results.

Time Regimen	Dose	Serum TTR (µg/mL)	Serum TTR (% KD)
QWx4	TSS	1190.7	-
QMx3	0.5	245.01	79.42

QMx2	0.5	776.73	34.77
QWx4	0.5	347.43	70.82
QWx3	0.5	405.70	65.93
QWx2	0.5	432.25	63.70
QWx1	0.5	804.06	32.47
QMx3	1	91.95	92.28
QMx2	1	176.81	85.15
QWx4	1	119.52	89.96
QWx3	1	167.15	85.96
QWx2	1	130.98	89.00
QWx1	1	573.02	51.88
QWx1	2	219.07	81.60

Table 29. Liver Editing Results.

Time Regimen	Dose	Liver Editing (%)
QWx4	TSS	0.38
QMx3	0.5	48.18
QMx2	0.5	36.66
QWx4	0.5	56.03
QWx3	0.5	51.35
QWx2	0.5	34.77
QWx1	0.5	24.16
QMx3	1	63.40
QMx2	1	57.37
QWx4	1	62.89
QWx3	1	59.22
QWx2	1	60.12
QWx1	1	35.16
QWx1	2	60.57

[00272] The results show that it is possible to build up a cumulative dose and effect with multiple administrations over time, including at weekly or monthly intervals, to achieve increasing editing levels and % KD of TTR.

Example 10. RNA Cargo: varying mRNA and gRNA ratios

[00273] This study evaluated in vivo efficacy in mice of different ratios of gRNA to mRNA. CleanCap™ capped Cas9 mRNAs with the ORF of SEQ ID NO: 4, HSD 5' UTR, human albumin 3' UTR, a Kozak sequence, and a poly-A tail were made by IVT synthesis as indicated in Example 1 with N1-methylpseudouridine triphosphate in place of uridine triphosphate.

[00274] LNP formulations prepared from the mRNA described and G282 (SEQ ID NO: 124) as described in Example 1 with Lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio and with an N:P ratio of 6. The gRNA:Cas9 mRNA weight ratios of the formulations were as shown in FIG.19A and 19B.

[00275] For in vivo characterization, the LNPs were administered to mice at 0.1 mg total RNA (mg guide RNA + mg mRNA) per kg (n=5 per group). At 7-9 days post-dose, animals were sacrificed, blood and the liver were collected, and serum TTR and liver editing were measured as described in Example 1. Serum TTR and liver editing results are shown in FIG.19A and 19B. Negative control mice were dosed with TSS vehicle.

In addition, the above LNPs were administered to mice at a constant mRNA dose of 0.05 mg mRNA per kg (n=5 per group), while varying the gRNA dose from 0.06 mg per kg to 0.4 mg per kg. At 7-9 days post-dose, animals were sacrificed, blood and the liver were collected, and serum TTR and liver editing were measured. Serum TTR and liver editing results are shown in FIG.19C and FIG.19D. Negative control mice were dosed with TSS vehicle.

Example 11. Off-Target analysis of TTR sgRNAs in Primary Human Hepatocyes

[00276] Off-target analysis of sgRNAs targeting TTR was performed in primary human hepatocytes (PHH) as described in Example 5, with the following modifications. PHH were plated at a density of 33,000 cells per well on collagen-coated 96-well plates as described in Example 1. Twenty-four hours post plating, cells were washed with media and transfected using Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) as described in Example 1. Cells were transfected with a lipoplex containing 100 ng Cas9 mRNA, immediately followed by the addition of another lipoplex containing 25 nM of the sgRNA and 12.5 nM of the donor oligo (0.3 μL/well). Cells were lysed 48 hours post-transfection and gDNA was extracted and analyzed as further described in Example 5. The data is graphically represented in FIG.20.

[00277] Table 30 shows the number of off-target integration sites detected in PHH, and compares to the the number of sites that were detected in the HekCas9 cells used in Example 5. Fewer sites were detected in PHH for every guide tested as compared to the HekCas9 cell line, with no unique sites detected in PHH alone.

Table 30. Number of off-target integration sites detected for TTR sgRNAs in PHH via an oligo insertion based assay

GUIDE ID	# Sites in PHH	# Sites in HekCas9 cells (Example 5)
G000480	2	11
G000481	0	3
G000482	2	13
G000483	0	5
G000484	0	7
G000485	3	22
G000486	0	12
G000487	0	14
G000488	0	0
G000489	2	19
G000490	0	12
G000491	7	28
G000492	5	97
G000493	1	7
G000494	0	4
G000495	1	13
G000496	0	1
G000497	3	26
G000498	19	82
G000499	1	4
G000500	12	46
G000501	0	4
G000567	0	9
G000568	11	936
G000570	1	19
G000571	1	16
G000572	2	15

[00278] Following the identification of potential off-target sites in PHH via the oligo insertion assay, certain potential sites were further evaluated by targeted amplicon sequencing, e.g., as described in Example 6. In addition to the potential off-target sites identified by the oligo insertion strategy, additional potential off-target sites identified by *in silico* prediction were included in the analysis.

[00279] To this end, PHH were treated with LNPs comprising 100 ng of Cas9 mRNA (SEQ ID NO:1) and the gRNA of interest at 14.68 nM (in a 1:1 ratio by weight), as described in Example 4. The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. The LNPs were formulated with an N:P ratio of 6.0 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:2 molar ratio, respectively. Following LNP treatment, isolated genomic DNA was analyzed by NGS (e.g., as described in Examples 1 and 6) to determine whether indels could be detected at the potential off-target site, which would be indicative of a Cas9-mediated cleavage event. Tables 31 and 32 show the potential off-target sites that were evaluated for the gRNAs G000480 and G000486, respectively.

[00280] As shown in FIG.21A-B and 22A-B and Table 33 below, indels were detected at low levels for only two of the potential off-target sites identified by the oligo insertion assay for G000480, and only one for G000486. No indels were detected at any of the *in silico* predicted sites for either guide. Further, indels were only detected at these sites using a near-saturating dose of LNP, as the indel rates observed at the on-target sites for G000480 and G000486 were ~97% and ~91%, respectively (See Table 33). The genomic coordinates of these sites are also reported in Tables 31 and 32, and each correspond to sequences that do not code for any protein.

[00281] A dose response assay was then performed in order to determine the highest dose of LNP in which no off-targets were detected. PHH were treated with LNPs comprising either G000480 or G000486 as described in Example 4. The doses ranged across 11 points with respect to gRNA concentration (0.001 nM, 0.002 nM, 0.007 nM, 0.02 nM, 0.06 nM, 0.19 nM, 0.57 nM, 1.72 nM, 5.17 nM, 15.51 nM, and 46.55 nM). As represented by the dashed vertical line in FIG.21A-B and 22A-B, the highest concentrations (with respect to the concentration of gRNA) at which the potential off-target sites were no longer detected for G000480 and G000486 were 0.57 nM and 15.51 nM, respectively, which resulted in on-target indel rates of 84.60% and 89.50%, respectively.

Table 31. Identified potential off target sites via insertion detection and in silico prediction for G000480 evaluated via targeted amplicon sequencing

GUIDE ID	Off-target (OT) Site ID	Assay Used	Chromosomal Coordinates (hg38)	Strand
G000480	INS-OT.1	Insertion Detection	chr7:94767406-94767426	+

G000480	INS-OT.2	Insertion Detection	chr2:192658562-192658582	+
G000480	INS-OT.3	Insertion Detection	chr7:4834390-4834410	+
G000480	INS-OT.4	Insertion Detection	chr20:9216118-9216138	-
G000480	INS-OT.5	Insertion Detection	chr10:12547071-12547091	+
G000480	INS-OT.6	Insertion Detection	chr6:168377978-168377998	-
G000480	INS-OT.7	Insertion Detection	chr12:114144669-114144689	-
G000480	INS-OT.8	Insertion Detection	chr10:7376755-7376775	+
G000480	INS-OT.9	Insertion Detection	chr2:52950299-52950319	+
G000480	INS-OT.10	Insertion Detection	chr8:56579165-56579185	-
G000480	INS-OT.11	Insertion Detection	chr1:189992255-189992275	+
G000480	PRED-OT.1	*in silico* prediction	chr10:12547071-12547091	+
G000480	PRE-DOT.2	*in silico* prediction	chrX:119702782-119702802	+
G000480	PRED-OT.3	*in silico* prediction	chr1:116544586-116544606	+
G000480	PRED-OT.4	*in silico* prediction	chr6:88282884-88282904	+
G000480	PRED-OT.6	*in silico* prediction	chr5:121891868-121891888	+
G000480	PRED-OT.7	*in silico* prediction	chr3:52544945-52544965	+
G000480	PRED-OT.8	*in silico* prediction	chr15:36949639-36949659	+
G000480	PRED-OT.9	*in silico* prediction	chr5:33866486-33866506	+
G000480	PRED-OT.10	*in silico* prediction	chr5:159755754-159755774	+
G000480	PRED-OT.11	*in silico* prediction	chr5:31349859-31349879	+
G000480	PRED-OT.12	*in silico* prediction	chr11:79485652-79485672	+
G000480	PRED-OT.13	*in silico* prediction	chr15:29448864-29448884	+
G000480	PRED-OT.14	*in silico* prediction	chr5:171153565-171153585	+
G000480	PRED-OT.15	*in silico* prediction	chr9:84855273-84855293	+
G000480	PRED-OT.16	*in silico* prediction	chr6:159953060-159953080	+
G000480	PRED-OT.17	*in silico* prediction	chr16:51849024-51849044	+
G000480	PRED-OT.18	*in silico* prediction	chr3:24108809-24108829	+
G000480	PRED-OT.19	*in silico* prediction	chr18:41118310-41118330	+
G000480	PRED-OT.20	*in silico* prediction	chr10:108975241-108975261	+
G000480	PREDO-T.21	*in silico* prediction	chr1:44683633-44683653	+
G000480	PRED-OT.22	*in silico* prediction	chr2:196214849-196214869	+
G000480	PRED-OT.23	*in silico* prediction	chr9:117353544-117353564	+
G000480	PRED-OT.24	*in silico* prediction	chr1:55583322-55583342	+
G000480	PRED-OT.25	*in silico* prediction	chr12:28246827-28246847	+
G000480	PRED-OT.26	*in silico* prediction	chr4:54545361-54545381	+
G000480	PRED-OT.27	*in silico* prediction	chr13:22364836-22364856	+
G000480	PRED-OT.28	*in silico* prediction	chr13:80816049-80816069	+
G000480	PRED-OT.29	*in silico* prediction	chr7:39078622-39078642	+
G000480	PRED-OT.30	*in silico* prediction	chr2:59944386-59944406	+

"INS-OT.N" refers to an off-target site ID detected by oligo insertion, where N is an integer specified above; "PRED-OT.N refers to an off-target site ID predicted via *in silico* methods, where N is an integer specified above.

Table 32. Identified potential off target sites via insertion detection and in silico prediction for G000486 evaluated via targeted amplicon sequencing

GUIDE ID	Off-target (OT) Site ID	Assay Used	Chromosomal Coordinates (hg38)	Strand
G000486	INS-OT.1	Insertion Detection	chr14:77332157-77332177	+
G000486	INS-OT.2	Insertion Detection	chr14:54672059-54672079	-
G000486	INS-OT.3	Insertion Detection	chr4:108513169-108513189	-
G000486	INS-OT.4	Insertion Detection	chr5:91397023-91397043	-
G000486	INS-OT.5	Insertion Detection	chr9:116626135-116626155	-
G000486	INS-OT.6	Insertion Detection	chr6:73201226-73201246	+
G000486	INS-OT.7	Insertion Detection	chr16:89368352-89368372	-
G000486	INS-OT.8	Insertion Detection	chr7:56308371-56308391	-
G000486	INS-OT.9	Insertion Detection	chr21:43605667-43605687	+
G000486	INS-OT.10	Insertion Detection	chr5:26758030-26758050	+
G000486	INS-OT.11	Insertion Detection	chr17:30656428-30656448	+
G000486	INS-OT.12	Insertion Detection	chr8:130486452-130486472	+
G000486	PRED-OT.1	*in silico* prediction	chr11:44707064-44707084	+
G000486	PRED-OT.2	*in silico* prediction	chr5:50775396-50775416	+
G000486	PRED-OT.3	*in silico* prediction	chr4:141623949-141623969	+
G000486	PRED-OT.4	*in silico* prediction	chr1:223481186-223481206	+
G000486	PRED-OT.5	*in silico* prediction	chr6:39951487-39951507	+
G000486	PRED-OT.6	*in silico* prediction	chrY:5456047-5456067	+
G000486	PRED-OT.8	*in silico* prediction	chr6:129868719-129868739	+
G000486	PRED-OT.9	*in silico* prediction	chrX:80450312-80450332	+
G000486	PRED-OT.10	*in silico* prediction	chr7:27256771-27256791	+
G000486	PRED-OT.11	*in silico* prediction	chr3:181416528-181416548	+
G000486	PRED-OT12	*in silico* prediction	chr7:146425020-146425040	+
G000486	PRED-OT.13	*in silico* prediction	chr3:16980977-16980997	+
G000486	PRED-OT.14	*in silico* prediction	chr7:118161002-118161022	+
G000486	PRED-OT.15	*in silico* prediction	chr6:102220539-102220559	+
G000486	PRED-OT.16	*in silico* prediction	chr12:127278991-127279011	+
G000486	PRED-OT.17	*in silico* prediction	chr2:67686631-67686651	+
G000486	PRED-OT.18	*in silico* prediction	chr1:114467665-114467685	+
G000486	PRED-OT.19	*in silico* prediction	chr3:194514436-194514456	+
G000486	PRED-OT.20	*in silico* prediction	chr14:31767581-31767601	+
G000486	PRED-OT.21	*in silico* prediction	chr16:28706209-28706229	+
G000486	PRED-OT.22	*in silico* prediction	chr8:110526279-110526299	+
G000486	PRED-OT.23	*in silico* prediction	chr19:2899814-2899834	+
G000486	PRED-OT.25	*in silico* prediction	chr3:130760261-130760281	+
G000486	PRED-OT.26	*in silico* prediction	chr11:2506046-2506066	+
G000486	PRED-OT.27	*in silico* prediction	chr2:153918318-153918338	+
G000486	PRED-OT.28	*in silico* prediction	chr14:40590226-40590246	+
G000486	PRED-OT.29	*in silico* prediction	chr18:806650-806670	+
G000486	PRED-OT.30	*in silico* prediction	chr2:117707480-117707500	+

"INS-OT.N" refers to an off-target site ID detected by oligo insertion, where N is an integer specified above; "PRED-OT.N" refers to an off-target site ID predicted via *in silico* methods, where N is an integer specified.

Table 33. Detected Off Target sites in PHH treated with LNP containing 100 ng mRNA and 31.03 nM gRNA

GUIDE ID	Off-target (OT) Site ID	Site Type	Indel Frequency (using LNP with 100 ng Cas9 mRNA and 14.68 nM gRNA)	Indel Frequency std. dev.
G000480	n/a	On-Target	97.33%	1.10%
G000480	INS-OT.2	Off-Target	1.43%	0.40%
G000480	INS-OT.4	Off-Target	0.97%	0.25%
G000486	n/a	On-Target	91.33%	1.97%
G000486	INS-OT.4	Off-Target	0.47%	0.06%

Example 12. LNP delivery to humanized mouse model of ATTR

[00282] A well-established humanized transgenic mouse model of hereditary ATTR amyloidosis that expresses the V30M pathogenic mutant form of human TTR protein was used in this Example. This mouse model recapitulates the TTR deposition phenotype in tissues observed in ATTR patients, including within the peripheral nervous system and gastrointestinal (GI) tract (See Santos et al., Neurobiol Aging. 2010 Feb;31(2):280-9).

[00283] Mice (aged approximately 4-5 months) were dosed with LNP formulations prepared using the cross-flow and TFF procedures as described in Example 1. The LNPs were formulated with an N:P ratio of 6.0 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:2 molar ratio, respectively. The LNPs contained Cas9 mRNA (SEQ ID NO: 1) and either G000481 ("G481") or a non-targeting control guide G000395 ("G395"; SEQ ID NO: 273), in a 1:1 ratio of gRNA:mRNA by weight.

[00284] Mice were injected via the lateral tail vein as described in Example 1 with a single 1mg/kg (of total RNA content) dose of LNP with an n=10/group. At 8 weeks post treatment, the mice were euthanized for sample collection. Human TTR protein levels were measured in serum and cerebrospinal fluid (CSF) by ELISA as previously described by Butler et al., Amyloid. 2016 Jun;23(2):109-18. Liver tissue was assayed for editing levels as described in Example 1. Other tissues (stomach, colon, sciatic nerve, dorsal root ganglion (DRG)) were collected and processed for semi-quantitative immunohistochemistry as previously described

by Gonçalves et al., Amyloid. 2014 Sep; 21(3): 175–184. Statistical analysis for the immunohistochemistry data was performed using Mann Whitney test with a p-value<0.0001.

[00285] As shown in FIG.23A-B, robust editing (49.4%) of TTR was observed in livers of the humanized mice following the single dose of LNP comprising G481, with no editing detected in the control group. Analysis of the editing events demonstrated that 96.8% of the events were insertions, with the remainder deletions.

[00286] As shown in FIG.24A-B, TTR protein levels were decreased in plasma but not in CSF from the treated mice, with greater than 99% knockdown of TTR plasma levels observed ($p<0.001$).

[00287] The near complete knockdown of TTR observed in the plasma of treated animals correlated with the clearance of TTR protein amyloid deposition in the assayed tissues. As shown in FIG.25, control mice exhibited amyloid staining in tissues which resembles the pathophysiology observed in human subjects with ATTR. Decreasing circulating TTR by editing the HuTTR V30M locus resulted in a dramatic decrease of amyloid deposition in tissues. Approximately 85% or better reduction in TTR staining was observed across the treated tissues 8 weeks post-treatment (FIG.25).

Example 13. *TTR* mRNA knockdown in Primary Human Hepatocytes (PHH)

[00288] In one experiment, PHH were cultured and treated with LNPs comprising Cas9 mRNA (SEQ ID NO:1) and a gRNA of interest (See FIG.29, Table 34), as described in Example 4. The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. The LNPs were formulated with an N:P ratio of 6.0 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:2 molar ratio, respectively. The LNPs comprised a gRNA:mRNA ratio of 1:2, and the cells were treated at a dose of 300 ng (with respect to the amount of mRNA cargo delivered).

[00289] Ninety-six (96) hours following LNP treatment (with biological triplicates for each condition), mRNA was purified from PHH cells using the Dynabeads mRNA DIRECT Kit (ThermoFisher Scientific) according to the manufacturer's protocol. Reverse Transcription (RT) was performed with Maxima reverse transcriptase (ThermoFisher Scientific) and a poly-dT primer. The resulting cDNA was purified with Ampure XP Beads (Agencourt). For Quantitative PCR, 2% of the purified cDNA was amplified with Taqman Fast Advanced Mastermix and 3 Taqman probe sets, TTR (Assay ID: Hs00174914_m1),

GAPDH (Assay ID: Hs02786624_g1), and PPIB (Assay ID: Hs00168719_m1). The assays were run on the QuantStudio 7 Flex Real Time PCR System according to the manufacturer's instructions (Life Technologies). Relative expression of *TTR* mRNA was calculated by normalizing to the endogenous controls (GAPDH and PPIB) individually, and then averaged.

[00290] As shown in FIG.29 and reproduced numerically in Table 34 below, each of the LNP formulations tested resulted in knockdown of *TTR* mRNA, as compared to the negative (untreated) control. The groups in FIG.29 and Table 34 are identified by the gRNA ID used in each LNP preparation. Relative expression of *TTR* mRNA is plotted in FIG.29, whereas the percent knockdown of *TTR* mRNA is provided in Table 34.

Table 34.

GUIDE ID	Avg % Knockdown	Std Dev
G000480	95.19	1.68
G000481	91.39	2.39
G000482	82.31	4.51
G000483	68.78	13.45
G000484	75.22	9.05
G000488	92.77	3.76
G000489	91.85	2.77
G000490	78.34	5.76
G000493	87.53	4.54
G000494	91.15	3.63
G000499	91.38	1.71
G000500	92.90	3.15
G000567	90.89	5.39
G000568	53.44	20.20
G000570	93.38	2.66
G000571	96.17	2.07
G000572	55.92	24.53

[00291] In a separate experiment, *TTR* mRNA knockdown was evaluated following treatment with LNPs comprising G000480, G000486, and G000502. The LNPs were formulated and PHH were cultured and treated with the LNPs, each as described in the experiment above in this Example with the exception that the cells were treated at a dose of 100 ng (with respect to the amount of mRNA cargo delivered).

[00292] Ninety-six (96) hours following LNP treatment (single treatment for each condition), mRNA was purified from PHH cells using the Dynabeads mRNA DIRECT Kit (ThermoFisher Scientific) according to the manufacturer's protocol. Reverse Transcription (RT) was performed with the High Capacity cDNA Reverse Transcription Kit (ThermoFisher

Scientific) according to the manufacturer's instructions. For Quantitative PCR, 2% of the cDNA was amplified with Taqman Fast Advanced Mastermix and 3 Taqman probe sets, TTR (Assay ID: Hs00174914_m1), GAPDH (Assay ID: Hs02786624_g1), and PPIB (Assay ID: Hs00168719_m1). The assays were run on the QuantStudio 7 Flex Real Time PCR System according to the manufacturer's instructions (Life Technologies). Relative expression of *TTR* mRNA was calculated by normalizing to the endogenous controls (GAPDH and PPIB) individually, and then averaged.

[00293] As shown in FIG.30 and reproduced numerically in Table 35 below, each of the LNP formulations tested resulted in knockdown of *TTR* mRNA, as compared to the negative (untreated) control. The groups in FIG.30 and Table 35 are identified by the gRNA ID used in each LNP preparation. Relative expression of *TTR* mRNA is plotted in FIG.30, whereas the percent knockdown of *TTR* mRNA is provided in Table 35.

Table 35.

GUIDE ID	Avg % Knockdown	Std Dev
G000480	95.61	0.92
G000486	97.36	0.63
G000502	90.94	2.63

Sequence Table

[00294] The following sequence table provides a listing of sequences disclosed herein. It is understood that if a DNA sequence (comprising Ts) is referenced with respect to an RNA, then Ts should be replaced with Us (which may be modified or unmodified depending on the context), and vice versa.

Description	Sequence	SEQ ID No.
Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTT GCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACT GGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAA GGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCAT CAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGA AGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAA CAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGA CGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAA GAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATA CCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAG CACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGAT CAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAG CGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTT CGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAG CGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCC GGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGG ACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCT	1

	GCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACA GATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGA CGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGC ACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCT GACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGA AATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGG AGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGAT GGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAG AAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGG AGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAA GGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAA GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGG AGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCT GCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCAC AGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAA GCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTT CAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAA GAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATT CAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAA GGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCT GACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGAC ATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAG ATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGA CAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGA AGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACA CATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGAC AGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGA AAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACA GAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACT GGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAA CGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGA CCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGT CCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAG AAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGT CAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCAC ACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGA ACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGAT CACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGA CGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAAT CAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAAC AGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGA CTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAAT CGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTT CAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGAT CGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTT CGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAA GACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAG AAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTA CGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGG AATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCT GGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCC GAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAG CGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGA AGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGA CGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGA CGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCC	

	GATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCT GGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAG ATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCAT CACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGG AGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAA AAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAG CTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTA AAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	
Cas9 transcript comprising Cas9 ORF correspondin g to SEQ ID NO: 205 using codons with generally high expression in humans	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCCATGCCTAAGAAAAAGCGGAAGGTCGACGGGGA TAAGAAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGC AGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTCCTGGG GAACACCGATAGACACAGCATCAAGAAAAATCTCATCGGAGCCCTGCTGTT TGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCGAGGCG ACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAGATCTTTTC GAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACCGCCTGGAAGAATC TTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTTGGAAA CATCGTCGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTACCATCT GCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCTCAGATTGATCTA CTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGATCGAAGG CGATCTGAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTCAACTGGT GCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCTAGCGGCGT CGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCCTCGA AAACCTGATCGCACAGCTGCCGGGAGAGAAAAAGAACGGACTTTTCGGCAA CTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAATTTTGA CCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGACGACGA CTTGGACAATTTGCTGGCACAAATTGGCGATCAGTACGCGGATCTGTTCCT TGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTGCGCGT GAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAAGCGGTA CGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGACAGCA ACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAATGGGTA CGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGAGTTCTATAAGTTCAT CAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTCAAGCT GAACAGGGAGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGGATCCAT TCCCCACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGA GGACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAAAATTCT GACGTTCCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGCAATTCGCG CTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTGGAATTT CGAGGAAGTTGTGGATAAGGGAGCTTCGGCACAAAGCTTCATCGAACGAAT GACCAACTTCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAGCACAG CCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAAGTGAAATA CGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGTCCGGAGAACAGAAGAA AGCAATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCGTCAAGCA GCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTGGAAAT CAGCGGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCATGATCT CCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAACGAGGA CATCCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCGCGAGAT GATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAGGTCAT GAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAA GCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTGGATTT CCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAATTGATCCACGA CGACAGCCTGACCTTTAAGGAGGACATCCAAAAAGCACAAGTGTCCGGACA GGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCCGGCGAT TAAGAAGGGAATTCTGCAAACTGTGAAGGTGGTCGACGAGCTGGTGAAGGT CATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCCCGAGAAAA CCAGACTACCCAGAAGGGCCAGAAAAACTCCCGCGAAAGGATGAAGCGGAT CGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCACCCGGT GGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTTGCAAAA TGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTGTCTGA	2

	TTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGACTCGAT CGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCAGATAA TGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGGCAGCT CCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAGC CGAGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAAACGGCA GCTGGTCGAGACTCGGCAGATTACCAAGCACGTGGCGCAGATCTTGGACTC CCGCATGAACACTAAATACGACGAGAACGATAAGCTCATCCGGGAAGTGAA GGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGAAGGACTTTCA GTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGACGCATA CCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACCCTAAACTTGA ATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAGATGAT AGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTCTTTTA CTCAAACATCATGAACTTTTTCAAGACTGAAATTACGCTGGCCAATGGAGA AATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAAATCGT GTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCTCTATGCC GCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTTTCAAA GGAATCGATCCTCCCAAAGAGAAATAGCGACAAGCTCATTGCACGCAAGAA AGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTCGCATA CTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAAAAGCTCAA ATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGATCCTCGTTCGA GAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGTGAAGAA GGATCTGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGAAAATGG TCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGAGCT GGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGA AAAACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTCGTGGA GCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTC AAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCGGCCTA CAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATTATCCA CTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTTCAAGTACTTCGA TACTACTATCGATCGCAAAAGATACACGTCCACCAAGGAAGTTCTGGACGC GACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATCGATCT GTCGCAGCTGGGTGGCGATTGATAGTCTAGCCATCACATTTAAAAGCATCT CAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCA TCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACAT AAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAA ATGGAAAGAACCTCGAG	
modified sgRNA sequence ("N" may be any natural or non-natural nucleotide)	mN*mN*mN*NNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	3
30/30/39 poly-A sequence	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCGAAAAAAAAAAAAAAAAAA AAAAAAAAAAAACCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAA	4
CR003335 gRNA targeting Human TTR (Exon 1)	CUGCUCCUCCUCUGCCUUGC	5
CR003336 gRNA targeting Human TTR (Exon 1)	CCUCCUCUGCCUUGCUGGAC	6
CR003337 gRNA	CCAGUCCAGCAAGGCAGAGG	7

targeting Human TTR (Exon 1)		
CR003338 gRNA targeting Human TTR (Exon 1)	AUACCAGUCCAGCAAGGCAG	8
CR003339 gRNA targeting Human TTR (Exon 1)	ACACAAAUACCAGUCCAGCA	9
CR003340 gRNA targeting Human TTR (Exon 1)	UGGACUGGUAUUUGUGUCUG	10
CR003341 gRNA targeting Human TTR (Exon 1)	CUGGUAUUUGUGUCUGAGGC	11
CR003342 gRNA targeting Human TTR (Exon 2)	CUUCUCUACACCCAGGGCAC	12
CR003343 gRNA targeting Human TTR (Exon 2)	CAGAGGACACUUGGAUUCAC	13
CR003344 gRNA targeting Human TTR (Exon 2)	UUUGACCAUCAGAGGACACU	14
CR003345 gRNA targeting Human TTR (Exon 2)	UCUAGAACUUUGACCAUCAG	15
CR003346 gRNA targeting Human TTR (Exon 2)	AAAGUUCUAGAUGCUGUCCG	16
CR003347 gRNA targeting Human TTR (Exon 2)	CAUUGAUGGCAGGACUGCCU	17
CR003348 gRNA	AGGCAGUCCUGCCAUCAAUG	18

targeting Human TTR (Exon 2)		
CR003349 gRNA targeting Human TTR (Exon 2)	UGCACGGCCACAUUGAUGGC	19
CR003350 gRNA targeting Human TTR (Exon 2)	CACAUGCACGGCCACAUUGA	20
CR003351 gRNA targeting Human TTR (Exon 2)	AGCCUUUCUGAACACAUGCA	21
CR003352 gRNA targeting Human TTR (Exon 2)	GAAAGGCUGCUGAUGACACC	22
CR003353 gRNA targeting Human TTR (Exon 2)	AAAGGCUGCUGAUGACACCU	23
CR003354 gRNA targeting Human TTR (Exon 2)	ACCUGGGAGCCAUUUGCCUC	24
CR003355 gRNA targeting Human TTR (Exon 2)	CCCAGAGGCAAAUGGCUCCC	25
CR003356 gRNA targeting Human TTR (Exon 2)	GCAACUUACCCAGAGGCAAA	26
CR003357 gRNA targeting Human TTR (Exon 2)	UUCUUUGGCAACUUACCCAG	27
CR003358 gRNA targeting Human TTR (Exon 3)	AUGCAGCUCUCCAGACUCAC	28
CR003359 gRNA	AGUGAGUCUGGAGAGCUGCA	29

targeting Human TTR (Exon 3)		
CR003360 gRNA targeting Human TTR (Exon 3)	GUGAGUCUGGAGAGCUGCAU	30
CR003361 gRNA targeting Human TTR (Exon 3)	GCUGCAUGGGCUCACAACUG	31
CR003362 gRNA targeting Human TTR (Exon 3)	GCAUGGGCUCACAACUGAGG	32
CR003363 gRNA targeting Human TTR (Exon 3)	ACUGAGGAGGAAUUUGUAGA	33
CR003364 gRNA targeting Human TTR (Exon 3)	CUGAGGAGGAAUUUGUAGAA	34
CR003365 gRNA targeting Human TTR (Exon 3)	UGUAGAAGGGAUAUACAAAG	35
CR003366 gRNA targeting Human TTR (Exon 3)	AAAUAGACACCAAAUCUUAC	36
CR003367 gRNA targeting Human TTR (Exon 3)	AGACACCAAAUCUUACUGGA	37
CR003368 gRNA targeting Human TTR (Exon 3)	AAGUGCCUUCCAGUAAGAUU	38
CR003369 gRNA targeting Human TTR (Exon 3)	CUCUGCAUGCUCAUGGAAUG	39
CR003370 gRNA	CCUCUGCAUGCUCAUGGAAU	40

targeting Human TTR (Exon 3)		
CR003371 gRNA targeting Human TTR (Exon 3)	ACCUCUGCAUGCUCAUGGAA	41
CR003372 gRNA targeting Human TTR (Exon 3)	UACUCACCUCUGCAUGCUCA	42
CR003373 gRNA targeting Human TTR (Exon 4)	GUAUUCACAGCCAACGACUC	43
CR003374 gRNA targeting Human TTR (Exon 4)	GCGGCGGGGGCCGGAGUCGU	44
CR003375 gRNA targeting Human TTR (Exon 4)	AAUGGUGUAGCGGCGGGGGC	45
CR003376 gRNA targeting Human TTR (Exon 4)	CGGCAAUGGUGUAGCGGCGG	46
CR003377 gRNA targeting Human TTR (Exon 4)	GCGGCAAUGGUGUAGCGGCG	47
CR003378 gRNA targeting Human TTR (Exon 4)	GGCGGCAAUGGUGUAGCGGC	48
CR003379 gRNA targeting Human TTR (Exon 4)	GGGCGGCAAUGGUGUAGCGG	49
CR003380 gRNA targeting Human TTR (Exon 4)	GCAGGGCGGCAAUGGUGUAG	50
CR003381 gRNA	GGGGCUCAGCAGGGCGGCAA	51

targeting Human TTR (Exon 4)		
CR003382 gRNA targeting Human TTR (Exon 4)	GGAGUAGGGGCUCAGCAGGG	52
CR003383 gRNA targeting Human TTR (Exon 4)	AUAGGAGUAGGGGCUCAGCA	53
CR003384 gRNA targeting Human TTR (Exon 4)	AAUAGGAGUAGGGGCUCAGC	54
CR003385 gRNA targeting Human TTR (Exon 4)	CCCCUACUCCUAUUCCACCA	55
CR003386 gRNA targeting Human TTR (Exon 4)	CCGUGGUGGAAUAGGAGUAG	56
CR003387 gRNA targeting Human TTR (Exon 4)	GCCGUGGUGGAAUAGGAGUA	57
CR003388 gRNA targeting Human TTR (Exon 4)	GACGACAGCCGUGGUGGAAU	58
CR003389 gRNA targeting Human TTR (Exon 4)	AUUGGUGACGACAGCCGUGG	59
CR003390 gRNA targeting Human TTR (Exon 4)	GGGAUUGGUGACGACAGCCG	60
CR003391 gRNA targeting Human TTR (Exon 4)	GGCUGUCGUCACCAAUCCCA	61
CR003392 gRNA	AGUCCCUCAUUCCUUGGGAU	62

targeting Human TTR (Exon 4)		
CR005298 gRNA targeting Human TTR (Exon 1)	UCCACUCAUUCUUGGCAGGA	63
CR005299 gRNA targeting Human TTR (Exon 4)	AGCCGUGGUGGAAUAGGAGU	64
CR005300 gRNA targeting Human TTR (Exon 1)	UCACAGAAACACUCACCGUA	65
CR005301 gRNA targeting Human TTR (Exon 1)	GUCACAGAAACACUCACCGU	66
CR005302 gRNA targeting Human TTR (Exon 2)	ACGUGUCUUCUCUACACCCA	67
CR005303 gRNA targeting Human TTR (Exon 2)	UGAAUCCAAGUGUCCUCUGA	68
CR005304 gRNA targeting Human TTR (Exon 2)	GGCCGUGCAUGUGUUCAGAA	69
CR005305 gRNA targeting Human TTR (Exon 3)	UAUAGGAAAACCAGUGAGUC	70
CR005306 gRNA targeting Human TTR (Exon 3)	AAAUCUUACUGGAAGGCACU	71
CR005307 gRNA targeting Human TTR (Exon 4)	UGUCUGUCUUCUCUCAUAGG	72
CR000689 gRNA	ACACAAAUACCAGUCCAGCG	73

targeting Cyno TTR		
CR005364 gRNA targeting Cyno TTR	AAAGGCUGCUGAUGAGACCU	74
CR005365 gRNA targeting Cyno TTR	CAUUGACAGCAGGACUGCCU	75
CR005366 gRNA targeting Cyno TTR	AUACCAGUCCAGCGAGGCAG	76
CR005367 gRNA targeting Cyno TTR	CCAGUCCAGCGAGGCAGAGG	77
CR005368 gRNA targeting Cyno TTR	CCUCCUCUGCCUCGCUGGAC	78
CR005369 gRNA targeting Cyno TTR	AAAGUUCUAGAUGCCGUCCG	79
CR005370 gRNA targeting Cyno TTR	ACUUGUCUUCUCUAUACCCA	80
CR005371 gRNA targeting Cyno TTR	AAGUGACUUCCAGUAAGAUU	81
CR005372 gRNA targeting Cyno TTR	AAAAGGCUGCUGAUGAGACC	82
	Not Used	83
	Not Used	84
	Not Used	85
	Not Used	86
G000480 sgRNA modified sequence targeting Human TTR	mA*mA*mA*GGCUGCUGAUGACACCUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	87
G000481 sgRNA modified sequence targeting Human TTR	mU*mC*mU*AGAACUUUGACCAUCAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	88

G000482 sgRNA modified sequence targeting Human TTR	mU*mG*mU*AGAAGGGAUAUACAAAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	89
G000483 sgRNA modified sequence targeting Human TTR	mU*mC*mC*ACUCAUUCUUGGCAGGAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	90
G000484 sgRNA modified sequence targeting Human TTR	mA*mG*mA*CACCAAAUCUUACUGGAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	91
G000485 sgRNA modified sequence targeting Human TTR	mC*mC*mU*CCUCUGCCUUGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	92
G000486 sgRNA modified sequence targeting Human TTR	mA*mC*mA*CAAAUACCAGUCCAGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	93
G000487 sgRNA modified sequence targeting Human TTR	mU*mU*mC*UUUGGCAACUUACCCAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	94
G000488 sgRNA modified sequence targeting Human TTR	mA*mA*mA*GUUCUAGAUGCUGUCCGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	95
G000489 sgRNA modified sequence targeting Human TTR	mU*mU*mU*GACCAUCAGAGGACACUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	96
G000490 sgRNA modified sequence targeting Human TTR	mA*mA*mA*UAGACACCAAAUCUUACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	97
G000491 sgRNA modified sequence targeting Human TTR	mA*mU*mA*CCAGUCCAGCAAGGCAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	98

G000492 sgRNA modified sequence targeting Human TTR	mC*mU*mU*CUCUACACCCAGGGCACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	99
G000493 sgRNA modified sequence targeting Human TTR	mA*mA*mG*UGCCUUCCAGUAAGAUUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	100
G000494 sgRNA modified sequence targeting Human TTR	mG*mU*mG*AGUCUGGAGAGCUGCAUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	101
G000495 sgRNA modified sequence targeting Human TTR	mC*mA*mG*AGGACACUUGGAUUCACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	102
G000496 sgRNA modified sequence targeting Human TTR	mG*mG*mC*CGUGCAUGUGUUCAGAAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	103
G000497 sgRNA modified sequence targeting Human TTR	mC*mU*mG*CUCCUCCUCUGCCUUGCGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	104
G000498 sgRNA modified sequence targeting Human TTR	mA*mG*mU*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	105
G000499 sgRNA modified sequence targeting Human TTR	mU*mG*mA*AUCCAAGUGUCCUCUGAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	106
G000500 sgRNA modified sequence targeting Human TTR	mC*mC*mA*GUCCAGCAAGGCAGAGGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	107
G000501 sgRNA modified sequence targeting Human TTR	mU*mC*mA*CAGAAACACUCACCGUAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	108

G000567 sgRNA modified sequence targeting Human TTR	mG*mA*mA*AGGCUGCUGAUGACACCGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	109
G000568 sgRNA modified sequence targeting Human TTR	mG*mG*mC*UGUCGUCACCAAUCCCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	110
G000570 sgRNA modified sequence targeting Human TTR	mC*mA*mU*UGAUGGCAGGACUGCCUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	111
G000571 sgRNA modified sequence targeting Human TTR	mG*mU*mC*ACAGAAACACUCACCGUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	112
G000572 sgRNA modified sequence targeting Human TTR	mC*mC*mC*CUACUCCUAUUCCACCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	113
G000502 sgRNA modified sequence targeting Cyno TTR	mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	114
G000503 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*AGGCUGCUGAUGAGACCGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	115
G000504 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*GGCUGCUGAUGAGACCUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	116
G000505 sgRNA modified sequence targeting Cyno TTR	mC*mA*mU*UGACAGCAGGACUGCCUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	117
G000506 sgRNA modified sequence targeting Cyno TTR	mA*mU*mA*CCAGUCCAGCGAGGCAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	118

G000507 sgRNA modified sequence targeting Cyno TTR	mC*mC*mA*GUCCAGCGAGGCAGAGGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	119
G000508 sgRNA modified sequence targeting Cyno TTR	mC*mC*mU*CCUCUGCCUCGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	120
G000509 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*GUUCUAGAUGCCGUCCGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	121
G000510 sgRNA modified sequence targeting Cyno TTR	mA*mC*mU*UGUCUUCUCUAUACCCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	122
G000511 sgRNA modified sequence targeting Cyno TTR	mA*mA*mG*UGACUUCCAGUAAGAUUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	123
G000282 sgRNA modified sequence targeting Mouse TTR	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	124
	Not used	125 to 200
DNA coding sequence of Cas9 using the thymidine analog of the minimal uridine codons listed in Table 3, with start and stop codons	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGA TGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTC CTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTG CTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCA AGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATC TTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAA GAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTC GGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTAC CACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTG ATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAG CTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGA CTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTC GGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAAC TTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGAC GACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTG TTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTG AGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAG AGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGA CAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAG	201

	TTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTC AAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGA AGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGA CAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAG ATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAAC AGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGG AACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAA AGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAG CACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTC AAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAG AAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTC AAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTC GAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCAC GACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAAC GAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGA GAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAG GTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGC AGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTG GACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATC CACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCG GCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTC AAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAG AGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACAC CCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTG CAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTG AGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGAC AGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGA CAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAG AGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTG GACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAA GTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGAC TTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGAC GCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAG CTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAG ATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTC TTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAAC GGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAA ATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGC ATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTC AGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGA AAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTC GCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAG CTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGC TTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTC AAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAA AACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAAC GAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCAC TACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTC GTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAA TTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGC GCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATC ATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTAC TTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTG GACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATC GACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGTCTAG	

DNA coding sequence of Cas9 using codons with generally high expression in humans	ATGGATAAGAAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGT TGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTC CTGGGGAACACCGATAGACACAGCATCAAGAAAAATCTCATCGGAGCCCTG CTGTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCG AGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAGATC TTTTCGAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACCGCCTGGAA GAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTT GGAAACATCGTCGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTAC CATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCTCAGATTG ATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGATC GAAGGCGATCTGAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTCAA CTGGTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCTAGC GGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGC CTCGAAAACCTGATCGCACAGCTGCCGGGAGAGAAAAAGAACGGACTTTTC GGCAACTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAAT TTTGACCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGAC GACGACTTGGACAATTTGCTGGCACAAATTGGCGATCAGTACGCGGATCTG TTCCTTGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTG CGCGTGAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAAG CGGTACGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGA CAGCAACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAAT GGGTACGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGAGTTCTATAAG TTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTC AAGCTGAACAGGGAGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGGA TCCATTCCCCACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGC CAGGAGGACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAAA ATTCTGACGTTCCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGCAAT TCGCGCTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTGG AATTTCGAGGAAGTTGTGGATAAGGGAGCTTCGGCACAAAGCTTCATCGAA CGAATGACCAACTTCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAG CACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAAGTG AAATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGTCCGGAGAACAG AAGAAAGCAATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCGTC AAGCAGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTG GAAATCAGCGGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCAT GATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAAC GAGGACATCCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCGC GAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAG GTCATGAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCC CGCAAGCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTG GATTTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAATTGATC CACGACGACAGCCTGACCTTTAAGGAGGACATCCAAAAAGCACAAGTGTCC GGACAGGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCCG GCGATTAAGAAGGGAATTCTGCAAACTGTGAAGGTGGTCGACGAGCTGGTG AAGGTCATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCCCGA GAAAACCAGACTACCCAGAAGGGCCAGAAAAACTCCCGCGAAAGGATGAAG CGGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCAC CCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTTG CAAAATGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTG TCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGAC TCGATCGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCA GATAATGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGG CAGCTCCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACT AAAGCCGAGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAAA CGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACGTGGCGCAGATCTTG GACTCCCGCATGAACACTAAATACGACGAGAACGATAAGCTCATCCGGGAA GTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGAAGGAC TTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGAC GCATACCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACCCTAAA CTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAG	202

	ATGATAGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTC TTTTACTCAAACATCATGAACTTTTTCAAGACTGAAATTACGCTGGCCAAT GGAGAAATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAA ATCGTGTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCTCT ATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTT TCAAAGGAATCGATCCTCCCAAAGAGAAATAGCGACAAGCTCATTGCACGC AAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTC GCATACTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAAAAG CTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGATCCTCG TTCGAGAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGTG AAGAAGGATCTGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGAA AATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAAT GAGCTGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCAC TACGAAAAACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTC GTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAG TTTTCAAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCG GCCTACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATT ATCCACTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTTCAAGTAC TTCGATACTACTATCGATCGCAAAAGATACACGTCCACCAAGGAAGTTCTG GACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATC GATCTGTCGCAGCTGGGTGGCGATGGCGGTGGATCTCCGAAAAAGAAGAGA AAGGTGTAATGA	
Amino acid sequence of Cas9 with one nuclear localization signal (1xNLS) as the C-terminal 7 amino acids	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKR KV	203
Cas9 mRNA ORF using minimal uridine codons, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG	204

	AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC	

	AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGUCUAG	
Cas9 mRNA ORF using codons with generally high expression in humans, with start and stop codons	AUGGAUAAGAAGUACUCAAUCGGGCUGGAUAUCGGAACUAAUUCCGUGGGU UGGGCAGUGAUCACGGAUGAAUACAAAGUGCCGUCCAAGAAGUUCAAGGUC CUGGGGAACACCGAUAGACACAGCAUCAAGAAAAAUCUCAUCGGAGCCCUG CUGUUUGACUCCGGCGAAACCGCAGAAGCGACCCGGCUCAAACGUACCGCG AGGCGACGCUACACCCGGCGGAAGAAUCGCAUCUGCUAUCUGCAAGAGAUC UUUUCGAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACCGCCUGGAA GAAUCUUUCCUGGUGGAGGAGGACAAGAAGCAUGAACGGCAUCCUAUCUUU GGAAACAUCGUCGACGAAGUGGCGUACCACGAAAAGUACCCGACCAUCUAC CAUCUGCGGAAGAAGUUGGUUGACUCAACUGACAAGGCCGACCUCAGAUUG AUCUACUUGGCCCUCGCCCAUAUGAUCAAAUUCCGCGGACACUUCCUGAUC GAAGGCGAUCUGAACCCUGAUAACUCCGACGUGGAUAAGCUUUUCAUUCAA CUGGUGCAGACCUACAACCAACUGUUCGAAGAAAACCCAAUCAAUGCUAGC GGCGUCGAUGCCAAGGCCAUCCUGUCCGCCCGGCUGUCGAAGUCGCGGCGC CUCGAAAACCUGAUCGCACAGCUGCCGGGAGAGAAAAAGAACGGACUUUUC GGCAACUUGAUCGCUCUCUCACUGGGACUCACUCCCAAUUUCAAGUCCAAU UUUGACCUGGCCGAGGACGCGAAGCUGCAACUCUCAAAGGACACCUACGAC GACGACUUGGACAAUUUGCUGGCACAAAUUGGCGAUCAGUACGCGGAUCUG UUCCUUGCCGCUAAGAACCUUUCGGACGCAAUCUUGCUGUCCGAUAUCCUG CGCGUGAACACCGAAAUAACCAAAGCGCCGCUUAGCGCCUCGAUGAUUAAG CGGUACGACGAGCAUCACCAGGAUCUCACGCUGCUCAAAGCGCUCGUGAGA CAGCAACUGCCUGAAAAGUACAAGGAGAUCUUCUUCGACCAGUCCAAGAAU GGGUACGCAGGGUACAUCGAUGGAGGCGCUAGCCAGGAAGAGUUCUAUAAG UUCAUCAAGCCAAUCCUGGAAAAGAUGGACGGAACCGAAGAACUGCUGGUC AAGCUGAACAGGGAGGAUCUGCUCCGGAAACAGAGAACCUUUGACAACGGA UCCAUUCCCCACCAGAUCCAUCUGGGUGAGCUGCACGCCAUCUUGCGGCGC CAGGAGGACUUUUACCCAUUCCUCAAGGACAACCGGGAAAAGAUCGAGAAA AUUCUGACGUUCCGCAUCCCGUAUUACGUGGGCCCACUGGCGCGCGGCAAU UCGCGCUUCGCGUGGAUGACUAGAAAAUCAGAGGAAACCAUCACUCCUUGG AAUUUCGAGGAAGUUGUGGAUAAGGGAGCUUCGGCACAAAGCUUCAUCGAA CGAAUGACCAACUUCGACAAGAAUCUCCCAAACGAGAAGGUGCUUCCUAAG CACAGCCUCCUUUACGAAUACUUCACUGUCUACAACGAACUGACUAAAGUG AAAUACGUUACUGAAGGAAUGAGGAAGCCGGCCUUUCUGUCCGGAGAACAG AAGAAAGCAAUUGUCGAUCUGCUGUUCAAGACCAACCGCAAGGUGACCGUC AAGCAGCUUAAAGAGGACUACUUCAAGAAGAUCGAGUGUUUCGACUCAGUG GAAAUCAGCGGGGUGGAGGACAGAUUCAACGCUUCGCUGGGAACCUAUCAU GAUCUCCUGAAGAUCAUCAAGGACAAGGACUUCCUUGACAACGAGGAGAAC GAGGACAUCCUGGAAGAUAUCGUCCUGACCUUGACCCUUUUCGAGGAUCGC GAGAUGAUCGAGGAGAGGCUUAAGACCUACGCUCAUCUCUUCGACGAUAAG GUCAUGAAACAACUCAAGCGCCGCCGGUACACUGGUUGGGGCCGCCUCUCC CGCAAGCUGAUCAACGGUAUUCGCGAUAAACAGAGCGGUAAAACUAUCCUG GAUUUCCUCAAAUCGGAUGGCUUCGCUAAUCGUAACUUCAUGCAAUUGAUC CACGACGACAGCCUGACCUUUAAGGAGGACAUCCAAAAAGCACAAGUGUCC GGACAGGGAGACUCACUCCAUGAACACAUCGCGAAUCUGGCCGGUUCGCCG GCGAUUAAGAAGGGAAUUCUGCAAACUGUGAAGGUGGUCGACGAGCUGGUG AAGGUCAUGGGACGGCACAAACCGGAGAAUAUCGUGAUUGAAAUGGCCCGA GAAAACCAGACUACCCAGAAGGGCCAGAAAAACUCCCGCGAAAGGAUGAAG CGGAUCGAAGAAGGAAUCAAGGAGCUGGGCAGCCAGAUCCUGAAAGAGCAC CCGGUGGAAAACACGCAGCUGCAGAACGAGAAGCUCUACCUGUACUAUUUG	205

	CAAAAUGGACGGGACAUGUACGUGGACCAAGAGCUGGACAUCAAUCGGUUG UCUGAUUACGACGUGGACCACAUCGUUCCACAGUCCUUUCUGAAGGAUGAC UCGAUCGAUAACAAGGUGUUGACUCGCAGCGACAAGAACAGAGGGAAGUCA GAUAAUGUGCCAUCGGAGGAGGUCGUGAAGAAGAUGAAGAAUUACUGGCGG CAGCUCCUGAAUGCGAAGCUGAUUACCCAGAGAAAGUUUGACAAUCUCACU AAAGCCGAGCGCGGCGGACUCUCAGAGCUGGAUAAGGCUGGAUUCAUCAAA CGGCAGCUGGUCGAGACUCGGCAGAUUACCAAGCACGUGGCGCAGAUCUUG GACUCCCGCAUGAACACUAAAUACGACGAGAACGAUAAGCUCAUCCGGGAA GUGAAGGUGAUUACCCUGAAAAGCAAACUUGUGUCGGACUUUCGGAAGGAC UUUCAGUUUUACAAAGUGAGAGAAAUCAACAACUACCAUCACGCGCAUGAC GCAUACCUCAACGCUGUGGUCGGUACCGCCCUGAUCAAAAAGUACCCUAAA CUUGAAUCGGAGUUUGUGUACGGAGACUACAAGGUCUACGACGUGAGGAAG AUGAUAGCCAAGUCCGAACAGGAAAUCGGGAAAGCAACUGCGAAAUACUUC UUUUACUCAAACAUCAUGAACUUUUUCAAGACUGAAAUUACGCUGGCCAAU GGAGAAAUCAGGAAGAGGCCACUGAUCGAAACUAACGGAGAAACGGGCGAA AUCGUGUGGGACAAGGGCAGGGACUUCGCAACUGUUCGCAAAGUGCUCUCU AUGCCGCAAGUCAAUAUUGUGAAGAAAACCGAAGUGCAAACCGGCGGAUUU UCAAAGGAAUCGAUCCUCCCAAAGAGAAAUAGCGACAAGCUCAUUGCACGC AAGAAAGACUGGGACCCGAAGAAGUACGGAGGAUUCGAUUCGCCGACUGUC GCAUACUCCGUCCUCGUGGUGGCCAAGGUGGAGAAGGGAAAGAGCAAAAAG CUCAAAUCCGUCAAAGAGCUGCUGGGGAUUACCAUCAUGGAACGAUCCUCG UUCGAGAAGAACCCGAUUGAUUUCCUCGAGGCGAAGGGUUACAAGGAGGUG AAGAAGGAUCUGAUCAUCAAACUCCCCAAGUACUCACUGUUCGAACUGGAA AAUGGUCGGAAGCGCAUGCUGGCUUCGGCCGGAGAACUCCAAAAAGGAAAU GAGCUGGCCUUGCCUAGCAAGUACGUCAACUUCCUCUAUCUUGCUUCGCAC UACGAAAAACUCAAAGGGUCACCGGAAGAUAACGAACAGAAGCAGCUUUUC GUGGAGCAGCACAAGCAUUAUCUGGAUGAAAUCAUCGAACAAAUCUCCGAG UUUUCAAAGCGCGUGAUCCUCGCCGACGCCAACCUCGACAAAGUCCUGUCG GCCUACAAUAAGCAUAGAGAUAAGCCGAUCAGAGAACAGGCCGAGAACAUU AUCCACUUGUUCACCCUGACUAACCUGGGAGCCCCAGCCGCCUUCAAGUAC UUCGAUACUACUAUCGAUCGCAAAAGAUACACGUCCACCAAGGAAGUUCUG GACGCGACCCUGAUCCACCAAAGCAUCACUGGACUCUACGAAACUAGGAUC GAUCUGUCGCAGCUGGGUGGCGAUGGCGGUGGAUCUCCGAAAAAGAAGAGA AAGGUGUAAUGA	
Cas9 nickase (D10A) amino acid sequence	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE	206

	FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKR KV	
Cas9 nickase (D10A) mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC	207

	UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGUCUAG	
dCas9 (D10A H840A) amino acid sequence	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKR KV	208
dCas9 (D10A H840A) mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA	209

	GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC	

	GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGUCUAG	
Cas9 mRNA coding sequence using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA	210

	AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAG GUC	
Cas9 nickase coding sequence using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC	211

	AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAG GUC	
dCas9 coding sequence using	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG	212

minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG	

	CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAG GUC	
Amino acid sequence of Cas9 (without NLS)	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	213
Cas9 mRNA ORF encoding SEQ ID NO: 213 using minimal uridine codons, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC	214

	UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC	
	GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG	
	UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG	
	AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG	
	AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA	
	CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC	
	GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG	
	UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC	
	AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA	
	AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA	
	CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG	
	AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC	
	AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG	
	AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA	
	AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG	
	CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC	
	AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG	
	AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC	
	AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC	
	GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC	
	GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC	
	GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA	
	GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG	
	GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC	
	AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG	
	GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC	
	CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC	
	GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG	
	GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC	
	AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA	
	GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG	
	AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC	
	CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG	
	CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG	
	AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC	
	AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC	
	GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA	
	CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA	
	AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG	
	AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG	
	GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA	
	GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC	
	UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC	
	GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG	
	CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG	
	AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC	
	UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC	
	GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA	
	AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC	
	AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC	
	AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA	
	AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC	
	GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG	
	CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC	
	UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC	
	AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA	
	AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC	
	GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC	
	UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC	
	GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA	
	UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC	

	GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACUAG	
Cas9 coding sequence encoding SEQ ID NO: 213 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC	215

	AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGAC	
Amino acid sequence of Cas9 nickase (without NLS)	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	216
Cas9 nickase mRNA ORF encoding SEQ ID NO: 216 using	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC	217

minimal uridine codons as listed in Table 3, with start and stop codons	UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA	

	AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACUAG	
Cas9 nickase coding sequence encoding SEQ ID NO: 216 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA	218

	AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGAC	
Amino acid sequence of dCas9 (without NLS)	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE	219

	FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	
dCas9 mRNA ORF encoding SEQ ID NO: 219 using minimal uridine codons as listed in Table 3, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC	220

	GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACUAG	
dCas9 coding sequence encoding SEQ ID NO: 219 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC	221

	AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGC	
Amino acid sequence of Cas9 with two nuclear localization signals (2xNLS) as the C-terminal amino acids	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK	222

	RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGSGSPKKKR KVDGSPKKKRKVDSG	
Cas9 mRNA ORF encoding SEQ ID NO: 222 using minimal uridine codons, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG	223

	AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGUCCCGAAGAAGAAGAGAAAGGUC GGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGCCCGAAGAAG AAGAGAAAGGUCGACAGCGGAUAG	
Cas9 coding sequence encoding SEQ ID NO: 222 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC	224

	AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAG GUCCCGAAGAAGAAGAGAAAGGUC GGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGCCCGAAGAAG AAGAGAAAGGUCGACAGCGGA	

Amino acid sequence of Cas9 nickase with two nuclear localization signals as the C-terminal amino acids	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGSGSPKKKR KVDGSPKKKRKVDSG	225
Cas9 nickase mRNA ORF encoding SEQ ID NO: 25 using minimal uridine codons as listed in Table 3, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC	226

	AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACGGAAGCGGAAGCCCGAAGAAGAAGAGA AAGGUCGACGGAAGCCCGAAGAAGAAGAGAAAGGUCGACAGCGGAUAG	
Cas9 nickase coding sequence encoding SEQ ID NO: 25 using minimal uridine codons (no start or	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA	227

stop codons; suitable for inclusion in fusion protein coding sequence)	GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC	

	GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGAC GGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGCCCGAAGAAG AAGAGAAAGGUCGACAGCGGA	
Amino acid sequence of dCas9 with two nuclear localization signals as the C-terminal amino acids	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGSGSPKKKR KVDGSPKKKRKVDSG	228
dCas9 mRNA ORF encoding SEQ ID NO: 228 using minimal uridine codons, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG	229

	AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC	

	GACCUGAGCCAGCUGGGAGGAGAC GGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGCCCGAAGAAG AAGAGAAAGGUCGACAGCGGAUAG	
dCas9 coding sequence encoding SEQ ID NO: 228 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA	230

	UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGACGGAAGCGGAAGCCCGAAGAAGAAGAGAAAG GUCGACGGAAGCCCGAAGAAGAAGAGAAAGGUCGACAGCGGA	
T7 Promoter	TAATACGACTCACTATA	231
Human beta-globin 5′ UTR	ACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACC	232
Human beta-globin 3′ UTR	GCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAG TCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTC TGCCTAATAAAAAACATTTATTTTCATTGC	233
Human alpha-globin 5′ UTR	CATAAACCCTGGCGCGCTCGCGGCCCGGCACTCTTCTGGTCCCCACAGACT CAGAGAGAACCCACC	234
Human alpha-globin 3′ UTR	GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCC CTCCTCCCCTTCCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAG TGGGCGGC	235
Xenopus laevis beta-globin 5′ UTR	AAGCTCAGAATAAACGCTCAACTTTGGCC	236
Xenopus laevis beta-globin 3′ UTR	ACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAATACCAACT TACACTTTACAAAATGTTGTCCCCCAAAATGTAGCCATTCGTATCTGCTCC TAATAAAAAGAAAGTTTCTTCACATTCT	237
Bovine Growth Hormone 5′ UTR	CAGGGTCCTGTGGACAGCTCACCAGCT	238
Bovine Growth Hormone 3′ UTR	TTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGA AGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA	239
Mus musculus hemoglobin alpha, adult chain 1 (Hba-a1), 3′UTR	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTG CACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAG	240
HSD17B4 5′ UTR	TCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCA GGCCTTATTC	241

G282 single guide RNA targeting the mouse TTR gene	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	242
	Not used	243
Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 204, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCCATGGACAAGAAGTACAGCATCGGACTGGACAT CGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCC GAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAA GAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAAC AAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAAT CTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAG CTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCA CGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGA AAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGA CAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTT CAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGT CGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGA AAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAG ACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGA AAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGAC ACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCT GAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGG AGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAAT CCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCT GAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACT GCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTT CTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAG CCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGG AACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCA GAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACT GCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAA CAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGG ACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGA AGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAG CGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAA CGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTA CAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGC ATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGAC AAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGAT CGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGC AAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTT CCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACT GACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGC ACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACAC AGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCA GAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAG AAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACAT CCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGC AAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAA GGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACAT CGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAG CCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAA GCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGA ACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCA GAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGA CAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAA GATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAG AAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGA	244

	CAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAA GCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAA CGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGT CAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAA CTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACT GATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAA GGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAA GGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGAC AGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAAC AAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAAC AGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGA AGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAG CGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGG ATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGA AAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCAC AATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGC AAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGG AGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTT CCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAA CGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAAT CATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAA CCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAG AGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGC ACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACAC AAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGG ACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGG AAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAAAAGCAT CTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATT CATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAAC ATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAA AAATGGAAAGAACCTCGAG	
Alternative Cas9 ORF with 19.36% U content	ATGGATAAGAAGTACTCGATCGGGCTGGATATCGGAACTAATTCCGTGGGT TGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTC CTGGGGAACACCGATAGACACAGCATCAAGAAGAATCTCATCGGAGCCCTG CTGTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCG AGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAAATC TTTTCGAACGAAATGGCAAAGGTGGACGACAGCTTCTTCCACCGCCTGGAA GAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTT GGAAACATCGTGGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTAC CATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCTCAGATTG ATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGATC GAAGGCGATCTGAACCCTGATAACTCCGACGTGGATAAGCTGTTCATTCAA CTGGTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCCAGC GGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGC CTCGAAAACCTGATCGCACAGCTGCCGGGAGAGAAGAAGAACGGACTTTTC GGCAACTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAAT TTTGACCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGAC GACGACTTGGACAATTTGCTGGCACAAATTGGCGATCAGTACGCGGATCTG TTCCTTGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTG CGCGTGAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAAG CGGTACGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGA CAGCAACTGCCTGAAAAGTACAAGGAGATTTTCTTCGACCAGTCCAAGAAT GGGTACGCAGGGTACATCGATGGAGGCGCCAGCCAGGAAGAGTTCTATAAG TTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTC AAGCTGAACAGGGAGGATCTGCTCCGCAAACAGAGAACCTTTGACAACGGA AGCATTCCACACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGC CAGGAGGACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAAA ATTCTGACGTTCCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGCAAT TCGCGCTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTGG	245

	AATTTCGAGGAAGTTGTGGATAAGGGAGCTTCGGCACAATCCTTCATCGAA CGAATGACCAACTTCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAG CACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAAGTG AAATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGAGCGGAGAACAG AAGAAAGCGATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCGTC AAGCAGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTG GAAATCAGCGGAGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCAT GATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAAC GAGGACATCCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCGC GAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAG GTCATGAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCC CGCAAGCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTG GATTTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAGTTGATC CACGACGACAGCCTGACCTTTAAGGAGGACATCCAGAAAGCACAAGTGAGC GGACAGGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCCG GCGATTAAGAAGGGAATCCTGCAAACTGTGAAGGTGGTGGACGAGCTGGTG AAGGTCATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCCCGA GAAAACCAGACTACCCAGAAGGGCCAGAAGAACTCCCGCGAAAGGATGAAG CGGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCAC CCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTTG CAAAATGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTG TCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGAC TCCATCGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCA GATAATGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGG CAGCTCCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACT AAAGCCGAGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAAA CGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACGTGGCGCAGATCCTG GACTCCCGCATGAACACTAAATACGACGAGAACGATAAGCTCATCCGGGAA GTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGAAGGAC TTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGAC GCATACCTCAACGCTGTGGTCGGCACCGCCCTGATCAAGAAGTACCCTAAA CTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAG ATGATAGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTC TTTTACTCAAACATCATGAACTTCTTCAAGACTGAAATTACGCTGGCCAAT GGAGAAATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAA ATCGTGTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCTCT ATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTT TCAAAGGAATCGATCCTCCCAAAGAGAAATAGCGACAAGCTCATTGCACGC AAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTC GCATACTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAGAAG CTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGATCCTCG TTCGAGAAGAACCCGATTGATTTCCTGGAGGCGAAGGGTTACAAGGAGGTG AAGAAGGATCTGATCATCAAACTGCCCAAGTACTCACTGTTCGAACTGGAA AATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAGAAAGGAAAT GAGCTGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCAC TACGAGAAACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTC GTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAG TTTTCAAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCG GCCTACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATT ATCCACTTGTTCACCCTGACTAACCTGGGAGCTCCAGCCGCCTTCAAGTAC TTCGATACTACTATCGACCGCAAAAGATACACGTCCACCAAGGAAGTTCTG GACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATC GATCTGTCGCAGCTGGGTGGCGATGGTGGCGGTGGATCCTACCCATACGAC GTGCCTGACTACGCCTCCGGAGGTGGTGGCCCCAAGAAGAAACGGAAGGTG TGATAG	
Cas9 transcript with 5′ UTR of HSD, ORF correspondin	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTT GCAGGCCTTATTCGGATCTGCCACCATGGATAAGAAGTACTCGATCGGGCT GGATATCGGAACTAATTCCGTGGGTTGGGCAGTGATCACGGATGAATACAA AGTGCCGTCCAAGAAGTTCAAGGTCCTGGGGAACACCGATAGACACAGCAT CAAGAAGAATCTCATCGGAGCCCTGCTGTTTGACTCCGGCGAAACCGCAGA	246

g to SEQ ID NO: 245, Kozak sequence, and 3′ UTR of ALB	AGCGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAA TCGCATCTGCTATCTGCAAGAAATCTTTTCGAACGAAATGGCAAAGGTGGA CGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAA GAAGCATGAACGGCATCCTATCTTTGGAAACATCGTGGACGAAGTGGCGTA CCACGAAAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACTC AACTGACAAGGCCGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGAT CAAATTCCGCGGACACTTCCTGATCGAAGGCGATCTGAACCCTGATAACTC CGACGTGGATAAGCTGTTCATTCAACTGGTGCAGACCTACAACCAACTGTT CGAAGAAAACCCAATCAATGCCAGCGGCGTCGATGCCAAGGCCATCCTGTC CGCCCGGCTGTCGAAGTCGCGGCGCCTCGAAAACCTGATCGCACAGCTGCC GGGAGAGAAGAAGAACGGACTTTTCGGCAACTTGATCGCTCTCTCACTGGG ACTCACTCCCAATTTCAAGTCCAATTTTGACCTGGCCGAGGACGCGAAGCT GCAACTCTCAAAGGACACCTACGACGACGACTTGGACAATTTGCTGGCACA AATTGGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGGA CGCAATCTTGCTGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAGC GCCGCTTAGCGCCTCGATGATTAAGCGGTACGACGAGCATCACCAGGATCT CACGCTGCTCAAAGCGCTCGTGAGACAGCAACTGCCTGAAAAGTACAAGGA GATTTTCTTCGACCAGTCCAAGAATGGGTACGCAGGGTACATCGATGGAGG CGCCAGCCAGGAAGAGTTCTATAAGTTCATCAAGCCAATCCTGGAAAAGAT GGACGGAACCGAAGAACTGCTGGTCAAGCTGAACAGGGAGGATCTGCTCCG CAAACAGAGAACCTTTGACAACGGAAGCATTCCACACCAGATCCATCTGGG TGAGCTGCACGCCATCTTGCGGCGCCAGGAGGACTTTTACCCATTCCTCAA GGACAACCGGGAAAAGATCGAGAAAATTCTGACGTTCCGCATCCCGTATTA CGTGGGCCCACTGGCGCGCGGCAATTCGCGCTTCGCGTGGATGACTAGAAA ATCAGAGGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATAAGGG AGCTTCGGCACAATCCTTCATCGAACGAATGACCAACTTCGACAAGAATCT CCCAAACGAGAAGGTGCTTCCTAAGCACAGCCTCCTTTACGAATACTTCAC TGTCTACAACGAACTGACTAAAGTGAAATACGTTACTGAAGGAATGAGGAA GCCGGCCTTTCTGAGCGGAGAACAGAAGAAAGCGATTGTCGATCTGCTGTT CAAGACCAACCGCAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAA GAAGATCGAGTGTTTCGACTCAGTGGAAATCAGCGGAGTGGAGGACAGATT CAACGCTTCGCTGGGAACCTATCATGATCTCCTGAAGATCATCAAGGACAA GGACTTCCTTGACAACGAGGAGAACGAGGACATCCTGGAAGATATCGTCCT GACCTTGACCCTTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGAC CTACGCTCATCTCTTCGACGATAAGGTCATGAAACAACTCAAGCGCCGCCG GTACACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAACGGTATTCGCGA TAAACAGAGCGGTAAAACTATCCTGGATTTCCTCAAATCGGATGGCTTCGC TAATCGTAACTTCATGCAGTTGATCCACGACGACAGCCTGACCTTTAAGGA GGACATCCAGAAAGCACAAGTGAGCGGACAGGGAGACTCACTCCATGAACA CATCGCGAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGAATCCTGCAAAC TGTGAAGGTGGTGGACGAGCTGGTGAAGGTCATGGGACGGCACAAACCGGA GAATATCGTGATTGAAATGGCCCGAGAAAACCAGACTACCCAGAAGGGCCA GAAGAACTCCCGCGAAAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCT GGGCAGCCAGATCCTGAAAGAGCACCCGGTGGAAAACACGCAGCTGCAGAA CGAGAAGCTCTACCTGTACTATTTGCAAAATGGACGGGACATGTACGTGGA CCAAGAGCTGGACATCAATCGGTTGTCTGATTACGACGTGGACCACATCGT TCCACAGTCCTTTCTGAAGGATGACTCCATCGATAACAAGGTGTTGACTCG CAGCGACAAGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGAGGTCGT GAAGAAGATGAAGAATTACTGGCGGCAGCTCCTGAATGCGAAGCTGATTAC CCAGAGAAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGGACTCTCAGA GCTGGATAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAGAT TACCAAGCACGTGGCGCAGATCCTGGACTCCCGCATGAACACTAAATACGA CGAGAACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAA ACTTGTGTCGGACTTTCGGAAGGACTTTCAGTTTTACAAAGTGAGAGAAAT CAACAACTACCATCACGCGCATGACGCATACCTCAACGCTGTGGTCGGCAC CGCCCTGATCAAGAAGTACCCTAAACTTGAATCGGAGTTTGTGTACGGAGA CTACAAGGTCTACGACGTGAGGAAGATGATAGCCAAGTCCGAACAGGAAAT CGGGAAAGCAACTGCGAAATACTTCTTTTACTCAAACATCATGAACTTCTT CAAGACTGAAATTACGCTGGCCAATGGAGAAATCAGGAAGAGGCCACTGAT CGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGGCAGGGACTT CGCAACTGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATATTGTGAAGAA	

	AACCGAAGTGCAAACCGGCGGATTTTCAAAGGAATCGATCCTCCCAAAGAG AAATAGCGACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAAGTA CGGAGGATTCGATTCGCCGACTGTCGCATACTCCGTCCTCGTGGTGGCCAA GGTGGAGAAGGGAAAGAGCAAGAAGCTCAAATCCGTCAAAGAGCTGCTGGG GATTACCATCATGGAACGATCCTCGTTCGAGAAGAACCCGATTGATTTCCT GGAGGCGAAGGGTTACAAGGAGGTGAAGAAGGATCTGATCATCAAACTGCC CAAGTACTCACTGTTCGAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTC GGCCGGAGAACTCCAGAAAGGAAATGAGCTGGCCTTGCCTAGCAAGTACGT CAACTTCCTCTATCTTGCTTCGCACTACGAGAAACTCAAAGGGTCACCGGA AGATAACGAACAGAAGCAGCTTTTCGTGGAGCAGCACAAGCATTATCTGGA TGAAATCATCGAACAAATCTCCGAGTTTTCAAAGCGCGTGATCCTCGCCGA CGCCAACCTCGACAAAGTCCTGTCGGCCTACAATAAGCATAGAGATAAGCC GATCAGAGAACAGGCCGAGAACATTATCCACTTGTTCACCCTGACTAACCT GGGAGCTCCAGCCGCCTTCAAGTACTTCGATACTACTATCGACCGCAAAAG ATACACGTCCACCAAGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCAT CACTGGACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGGTGGCGATGG TGGCGGTGGATCCTACCCATACGACGTGCCTGACTACGCCTCCGGAGGTGG TGGCCCCAAGAAGAAACGGAAGGTGTGATAGCTAGCCATCACATTTAAAAG CATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTT ATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAA AACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAAT AAAAAATGGAAAGAACCTCGAG	
Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 245, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCTATGGATAAGAAGTACTCGATCGGGCTGGATAT CGGAACTAATTCCGTGGGTTGGGCAGTGATCACGGATGAATACAAAGTGCCC GTCCAAGAAGTTCAAGGTCCTGGGGAACACCGATAGACACAGCATCAAGAA GAATCTCATCGGAGCCCTGCTGTTTGACTCCGGCGAAACCGCAGAAGCGAC CCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAATCGCAT CTGCTATCTGCAAGAAATCTTTTCGAACGAAATGGCAAAGGTGGACGACAG CTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAAGAAGCA TGAACGGCATCCTATCTTTGGAAACATCGTGGACGAAGTGGCGTACCACGA AAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACTCAACTGA CAAGGCCGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGATCAAATT CCGCGGACACTTCCTGATCGAAGGCGATCTGAACCCTGATAACTCCGACGT GGATAAGCTGTTCATTCAACTGGTGCAGACCTACAACCAACTGTTCGAAGA AAACCCAATCAATGCCAGCGGCGTCGATGCCAAGGCCATCCTGTCCGCCCG GCTGTCGAAGTCGCGGCGCCTCGAAAACCTGATCGCACAGCTGCCGGGAGA GAAGAAGAACGGACTTTTCGGCAACTTGATCGCTCTCTCACTGGGACTCAC TCCCAATTTCAAGTCCAATTTTGACCTGGCCGAGGACGCGAAGCTGCAACT CTCAAAGGACACCTACGACGACGACTTGGACAATTTGCTGGCACAAATTGG CGATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGGACGCAAT CTTGCTGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAGCGCCGCT TAGCGCCTCGATGATTAAGCGGTACGACGAGCATCACCAGGATCTCACGCT GCTCAAAGCGCTCGTGAGACAGCAACTGCCTGAAAAGTACAAGGAGATTTT CTTCGACCAGTCCAAGAATGGGTACGCAGGGTACATCGATGGAGGCGCCAG CCAGGAAGAGTTCTATAAGTTCATCAAGCCAATCCTGGAAAAGATGGACGG AACCGAAGAACTGCTGGTCAAGCTGAACAGGGAGGATCTGCTCCGCAAACA GAGAACCTTTGACAACGGAAGCATTCCACACCAGATCCATCTGGGTGAGCT GCACGCCATCTTGCGGCGCCAGGAGGACTTTTACCCATTCCTCAAGGACAA CCGGGAAAAGATCGAGAAAATTCTGACGTTCCGCATCCCGTATTACGTGGG CCCACTGGCGCGCGGCAATTCGCGCTTCGCGTGGATGACTAGAAAATCAGA GGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATAAGGGAGCTTC GGCACAATCCTTCATCGAACGAATGACCAACTTCGACAAGAATCTCCCAAA CGAGAAGGTGCTTCCTAAGCACAGCCTCCTTTACGAATACTTCACTGTCTA CAACGAACTGACTAAAGTGAAATACGTTACTGAAGGAATGAGGAAGCCGGC CTTTCTGAGCGGAGAACAGAAGAAAGCGATTGTCGATCTGCTGTTCAAGAC CAACCGCAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAAGAAGAT CGAGTGTTTCGACTCAGTGGAAATCAGCGGAGTGGAGGACAGATTCAACGC TTCGCTGGGAACCTATCATGATCTCCTGAAGATCATCAAGGACAAGGACTT CCTTGACAACGAGGAGAACGAGGACATCCTGGAAGATATCGTCCTGACCTT	247

	GACCCTTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGACCTACGC TCATCTCTTCGACGATAAGGTCATGAAACAACTCAAGCGCCGCCGGTACAC TGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAACGGTATTCGCGATAAACA GAGCGGTAAAACTATCCTGGATTTCCTCAAATCGGATGGCTTCGCTAATCG TAACTTCATGCAGTTGATCCACGACGACAGCCTGACCTTTAAGGAGGACAT CCAGAAAGCACAAGTGAGCGGACAGGGAGACTCACTCCATGAACACATCGC GAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGAATCCTGCAAACTGTGAA GGTGGTGGACGAGCTGGTGAAGGTCATGGGACGGCACAAACCGGAGAATAT CGTGATTGAAATGGCCCGAGAAAACCAGACTACCCAGAAGGGCCAGAAGAA CTCCCGCGAAAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCTGGGCAG CCAGATCCTGAAAGAGCACCCGGTGGAAAACACGCAGCTGCAGAACGAGAA GCTCTACCTGTACTATTTGCAAAATGGACGGGACATGTACGTGGACCAAGA GCTGGACATCAATCGGTTGTCTGATTACGACGTGGACCACATCGTTCCACA GTCCTTTCTGAAGGATGACTCCATCGATAACAAGGTGTTGACTCGCAGCGA CAAGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGAGGTCGTGAAGAA GATGAAGAATTACTGGCGGCAGCTCCTGAATGCGAAGCTGATTACCCAGAG AAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGGACTCTCAGAGCTGGA TAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAGATTACCAA GCACGTGGCGCAGATCCTGGACTCCCGCATGAACACTAAATACGACGAGAA CGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAAACTTGT GTCGGACTTTCGGAAGGACTTTCAGTTTTACAAAGTGAGAGAAATCAACAA CTACCATCACGCGCATGACGCATACCTCAACGCTGTGGTCGGCACCGCCCT GATCAAGAAGTACCCTAAACTTGAATCGGAGTTTGTGTACGGAGACTACAA GGTCTACGACGTGAGGAAGATGATAGCCAAGTCCGAACAGGAAATCGGGAA AGCAACTGCGAAATACTTCTTTTACTCAAACATCATGAACTTCTTCAAGAC TGAAATTACGCTGGCCAATGGAGAAATCAGGAAGAGGCCACTGATCGAAAC TAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGGCAGGGACTTCGCAAC TGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATATTGTGAAGAAAACCGA AGTGCAAACCGGCGGATTTTCAAAGGAATCGATCCTCCCAAAGAGAAATAG CGACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAAGTACGGAGG ATTCGATTCGCCGACTGTCGCATACTCCGTCCTCGTGGTGGCCAAGGTGGA GAAGGGAAAGAGCAAGAAGCTCAAATCCGTCAAAGAGCTGCTGGGGATTAC CATCATGGAACGATCCTCGTTCGAGAAGAACCCGATTGATTTCCTGGAGGC GAAGGGTTACAAGGAGGTGAAGAAGGATCTGATCATCAAACTGCCCAAGTA CTCACTGTTCGAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTCGGCCGG AGAACTCCAGAAAGGAAATGAGCTGGCCTTGCCTAGCAAGTACGTCAACTT CCTCTATCTTGCTTCGCACTACGAGAAACTCAAAGGGTCACCGGAAGATAA CGAACAGAAGCAGCTTTTCGTGGAGCAGCACAAGCATTATCTGGATGAAAT CATCGAACAAATCTCCGAGTTTTCAAAGCGCGTGATCCTCGCCGACGCCAA CCTCGACAAAGTCCTGTCGGCCTACAATAAGCATAGAGATAAGCCGATCAG AGAACAGGCCGAGAACATTATCCACTTGTTCACCCTGACTAACCTGGGAGC TCCAGCCGCCTTCAAGTACTTCGATACTACTATCGACCGCAAAAGATACAC GTCCACCAAGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCATCACTGG ACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGGTGGCGATGGTGGCGG TGGATCCTACCCATACGACGTGCCTGACTACGCCTCCGGAGGTGGTGGCCC CAAGAAGAAACGGAAGGTGTGATAGCTAGCCATCACATTTAAAAGCATCTC AGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCAT CTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATA AATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAA TGGAAAGAACCTCGAG	
	Not used	248
Cas9 transcript comprising Kozak sequence with Cas9 ORF using codons with generally	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTT GCAGGCCTTATTCGGATCCGCCACCATGCCTAAGAAAAAGCGGAAGGTCGA CGGGGATAAGAAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGG TTGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGT CCTGGGGAACACCGATAGACACAGCATCAAGAAAAATCTCATCGGAGCCCT GCTGTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGC GAGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAGAT CTTTTCGAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACCGCCTGGA AGAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTT	249

high expression in humans	TGGAAACATCGTCGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTA CCATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCTCAGATT GATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGAT CGAAGGCGATCTGAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTCA ACTGGTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCTAG CGGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCG CCTCGAAAACCTGATCGCACAGCTGCCGGGAGAGAAAAAGAACGGACTTTT CGGCAACTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAA TTTTGACCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGA CGACGACTTGGACAATTTGCTGGCACAAATTGGCGATCAGTACGCGGATCT GTTCCTTGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCT GCGCGTGAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAA GCGGTACGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAG ACAGCAACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAA TGGGTACGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGAGTTCTATAA GTTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGT CAAGCTGAACAGGGAGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGG ATCCATTCCCCACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCG CCAGGAGGACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAA AATTCTGACGTTCCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGCAA TTCGCGCTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTG GAATTTCGAGGAAGTTGTGGATAAGGGAGCTTCGGCACAAAGCTTCATCGA ACGAATGACCAACTTCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAA GCACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAAGT GAAATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGTCCGGAGAACA GAAGAAAGCAATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCGT CAAGCAGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGT GGAAATCAGCGGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCA TGATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAA CGAGGACATCCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCG CGAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAA GGTCATGAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTC CCGCAAGCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCT GGATTTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAATTGAT CCACGACGACAGCCTGACCTTTAAGGAGGACATCCAAAAAGCACAAGTGTC CGGACAGGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCC GGCGATTAAGAAGGGAATTCTGCAAACTGTGAAGGTGGTCGACGAGCTGGT GAAGGTCATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCCCG AGAAAACCAGACTACCCAGAAGGGCCAGAAAAACTCCCGCGAAAGGATGAA GCGGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCA CCCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTT GCAAAATGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTT GTCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGA CTCGATCGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTC AGATAATGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCG GCAGCTCCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCAC TAAAGCCGAGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAA ACGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACGTGGCGCAGATCTT GGACTCCCGCATGAACACTAAATACGACGAGAACGATAAGCTCATCCGGGA AGTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGAAGGA CTTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGA CGCATACCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACCCTAA ACTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAA GATGATAGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTT CTTTTACTCAAACATCATGAACTTTTTCAAGACTGAAATTACGCTGGCCAA TGGAGAAATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGA AATCGTGTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCTC TATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATT TTCAAAGGAATCGATCCTCCCAAAGAGAAATAGCGACAAGCTCATTGCACG CAAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGT CGCATACTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAAAA	

	GCTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGATCCTC GTTCGAGAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGT GAAGAAGGATCTGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGA AAATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAA TGAGCTGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCA CTACGAAAAACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTT CGTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGA GTTTTCAAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTC GGCCTACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACAT TATCCACTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTTCAAGTA CTTCGATACTACTATCGATCGCAAAAGATACACGTCCACCAAGGAAGTTCT GGACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGAT CGATCTGTCGCAGCTGGGTGGCGATTGATAGTCTAGCCATCACATTTAAAA GCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCT TATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAA AAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAA TAAAAAATGGAAAGAACCTCGAG	
Cas9 ORF with splice junctions removed; 12.75% U content	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGA TGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTC CTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTG CTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCA AGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATC TTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACcggCTGGAA GAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTC GGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTAC CACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTG ATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAG CTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGA CTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTC GGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAAC TTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGAC GACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTG TTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTG AGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAG AGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGA CAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAG TTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTC AAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGA AGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGA CAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAG ATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAAC AGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGG AACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAA AGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAG CACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTC AAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAG AAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTC AAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTC GAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCAC GACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAAC GAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGA GAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAG GTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGC AGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTG GACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATC CACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCG	250

	GCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTC AAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAG AGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACAC CCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTG CAaAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTG AGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGAC AGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGA CAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAG AGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTG GACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAA GTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGAC TTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGAC GCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAG CTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAG ATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTC TTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAAC GGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAA ATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGC ATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTC AGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGA AAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTC GCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAG CTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGC TTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTC AAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAA AACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAAC GAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCAC TACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTC GTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAA TTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGC GCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATC ATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTAC TTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTG GACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATC GACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGTCTAG	
Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 250, Kozak sequence, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTT GCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACT GGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAA GGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCAT CAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGA AGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAA CAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGA CGACAGCTTCTTCCACcggCTGGAAGAAAGCTTCCTGGTCGAAGAAGACAA GAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATA CCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAG CACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGAT CAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAG CGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTT CGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAG CGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCC GGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGG ACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCT GCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACA GATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGA CGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGC ACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCT	251

	GACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGA AATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGG AGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGAT GGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAG AAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGG AGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAA GGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAA GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGG AGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCT GCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCAC AGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAA GCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTT CAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAA GAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATT CAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAA GGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCT GACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGAC ATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAG ATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGA CAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGA AGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACA CATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGAC AGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGA AAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACA GAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACT GGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAA CGAAAAGCTGTACCTGTACTACCTGCAaAACGGAAGAGACATGTACGTCGA CCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGT CCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAG AAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGT CAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCAC ACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGA ACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGAT CACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGA CGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAAT CAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAAC AGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGA CTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAAT CGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTT CAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGAT CGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTT CGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAA GACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAG AAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTA CGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGG AATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCT GGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCC GAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAG CGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGA AGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGA CGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGA CGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCC GATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCT GGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAG ATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCAT CACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGG	

	AGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAA AAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAG CTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTA AAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	
Cas9 ORF with minimal uridine codons frequently used in humans in general; 12.75% U content	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGC TGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTG CTGGGCAACACCGACAGACACAGCATCAAGAAGAACCTGATCGGCGCCCTG CTGTTCGACAGCGGCGAGACCGCCGAGGCCACCAGACTGAAGAGAACCGCC AGAAGAAGATACACCAGAAGAAAGAACAGAATCTGCTACCTGCAGGAGATC TTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAG GAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGAGACACCCCATCTTC GGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTAC CACCTGAGAAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTGAGACTG ATCTACCTGGCCCTGGCCCACATGATCAAGTTCAGAGGCCACTTCCTGATC GAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAG CTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGC GGCGTGGACGCCAAGGCCATCCTGAGCGCCAGACTGAGCAAGAGCAGAAGA CTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTC GGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAAC TTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGAC GACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTG TTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTG AGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCATGATCAAG AGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGAGA CAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAAC GGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAG TTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTG AAGCTGAACAGAGAGGACCTGCTGAGAAAGCAGAGAACCTTCGACAACGGC AGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGAGAAGA CAGGAGGACTTCTACCCCTTCCTGAAGGACAACAGAGAGAAGATCGAGAAG ATCCTGACCTTCAGAATCCCCTACTACGTGGGCCCCCTGGCCAGAGGCAAC AGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAGACCATCACCCCCTGG AACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAG AGAATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAG CACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTG AAGTACGTGACCGAGGGCATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAG AAGAAGGCCATCGTGGACCTGCTGTTCAAGACCAACAGAAAGGTGACCGTG AAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTG GAGATCAGCGGCGTGGAGGACAGATTCAACGCCAGCCTGGGCACCTACCAC GACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAGGAGAAC GAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACAGA GAGATGATCGAGGAGAGACTGAAGACCTACGCCCACCTGTTCGACGACAAG GTGATGAAGCAGCTGAAGAGAAGAAGATACACCGGCTGGGGCAGACTGAGC AGAAAGCTGATCAACGGCATCAGAGACAAGCAGAGCGGCAAGACCATCCTG GACTTCCTGAAGAGCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGC GGCCAGGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCC GCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTG AAGGTGATGGGCAGACACAAGCCCGAGAACATCGTGATCGAGATGGCCAGA GAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCAGAGAGAGAATGAAG AGAATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCAC CCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTG CAGAACGGCAGAGACATGTACGTGGACCAGGAGCTGGACATCAACAGACTG AGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGAC AGCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACAGAGGCAAGAGC GACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGAGA CAGCTGCTGAACGCCAAGCTGATCACCCAGAGAAAGTTCGACAACCTGACC AAGGCCGAGAGAGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAG AGACAGCTGGTGGAGACCAGACAGATCACCAAGCACGTGGCCCAGATCCTG	252

	GACAGCAGAATGAACACCAAGTACGACGAGAACGACAAGCTGATCAGAGAG GTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCAGAAAGGAC TTCCAGTTCTACAAGGTGAGAGAGATCAACAACTACCACCACGCCCACGAC GCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAG CTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGAGAAAG ATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTC TTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAAC GGCGAGATCAGAAAGAGACCCCTGATCGAGACCAACGGCGAGACCGGCGAG ATCGTGTGGGACAAGGGCAGAGACTTCGCCACCGTGAGAAAGGTGCTGAGC ATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTC AGCAAGGAGAGCATCCTGCCCAAGAGAAACAGCGACAAGCTGATCGCCAGA AAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTG GCCTACAGCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGAGCAAGAAG CTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGAGAAGCAGC TTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTG AAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAGCTGGAG AACGGCAGAAAGAGAATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAAC GAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCAC TACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTC GTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAG TTCAGCAAGAGAGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGC GCCTACAACAAGCACAGAGACAAGCCCATCAGAGAGCAGGCCGAGAACATC ATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTAC TTCGACACCACCATCGACAGAAAGAGATACACCAGCACCAAGGAGGTGCTG GACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACCAGAATC GACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGCCCCAAGAAGAAGAGA AAGGTGTGA	
Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 252, Kozak sequence, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTT GCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGCCT GGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAA GGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGACAGACACAGCAT CAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCGCCGA GGCCACCAGACTGAAGAGAACCGCCAGAAGAAGATACACCAGAAGAAAGAA CAGAATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGA CGACAGCTTCTTCCACAGACTGGAGGAGAGCTTCCTGGTGGAGGAGGACAA GAAGCACGAGAGACACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTA CCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAGCTGGTGGACAG CACCGACAAGGCCGACCTGAGACTGATCTACCTGGCCCTGGCCCACATGAT CAAGTTCAGAGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAG CGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTT CGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAG CGCCAGACTGAGCAAGAGCAGAAGACTGGAGAACCTGATCGCCCAGCTGCC CGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGG CCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCA GATCGGCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGA CGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGC CCCCCTGAGCGCCAGCATGATCAAGAGATACGACGAGCACCACCAGGACCT GACCCTGCTGAAGGCCCTGGTGAGACAGCAGCTGCCCGAGAAGTACAAGGA GATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGG CGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTGGAGAAGAT GGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACAGAGAGGACCTGCTGAG AAAGCAGAGAACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGG CGAGCTGCACGCCATCCTGAGAAGACAGGAGGACTTCTACCCCTTCCTGAA GGACAACAGAGAGAAGATCGAGAAGATCCTGACCTTCAGAATCCCCTACTA CGTGGGCCCCCTGGCCAGAGGCAACAGCAGATTCGCCTGGATGACCAGAAA GAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGG CGCCAGCGCCCAGAGCTTCATCGAGAGAATGACCAACTTCGACAAGAACCT GCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCAC CGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGAGAAA	253

	GCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTGTT CAAGACCAACAGAAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAA GAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACAGATT CAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAA GGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCT GACCCTGACCCTGTTCGAGGACAGAGAGATGATCGAGGAGAGACTGAAGAC CTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGAGAAGAAG ATACACCGGCTGGGGCAGACTGAGCAGAAAGCTGATCAACGGCATCAGAGA CAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTCGC CAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGA GGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGACAGCCTGCACGAGCA CATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGAC CGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCAGACACAAGCCCGA GAACATCGTGATCGAGATGGCCAGAGAGAACCAGACCACCCAGAAGGGCCA GAAGAACAGCAGAGAGAGAATGAAGAGAATCGAGGAGGGCATCAAGGAGCT GGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAA CGAGAAGCTGTACCTGTACTACCTGCAGAACGGCAGAGACATGTACGTGGA CCAGGAGCTGGACATCAACAGACTGAGCGACTACGACGTGGACCACATCGT GCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCAG AAGCGACAAGAACAGAGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGT GAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCCAAGCTGATCAC CCAGAGAAAGTTCGACAACCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGA GCTGGACAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAGACCAGACAGAT CACCAAGCACGTGGCCCAGATCCTGGACAGCAGAATGAACACCAAGTACGA CGAGAACGACAAGCTGATCAGAGAGGTGAAGGTGATCACCCTGAAGAGCAA GCTGGTGAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTGAGAGAGAT CAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCAC CGCCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGA CTACAAGGTGTACGACGTGAGAAAGATGATCGCCAAGAGCGAGCAGGAGAT CGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTT CAAGACCGAGATCACCCTGGCCAACGGCGAGATCAGAAAGAGACCCCTGAT CGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCAGAGACTT CGCCACCGTGAGAAAGGTGCTGAGCATGCCCCAGGTGAACATCGTGAAGAA GACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGAG AAACAGCGACAAGCTGATCGCCAGAAAGAAGGACTGGGACCCCAAGAAGTA CGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGG CATCACCATCATGGAGAGAAGCAGCTTCGAGAAGAACCCCATCGACTTCCT GGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCC CAAGTACAGCCTGTTCGAGCTGGAGAACGGCAGAAAGAGAATGCTGGCCAG CGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGT GAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGA GGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGAGAGTGATCCTGGCCGA CGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACAGAGACAAGCC CATCAGAGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCT GGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACAGAAAGAG ATACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCAT CACCGGCCTGTACGAGACCAGAATCGACCTGAGCCAGCTGGGCGGCGACGG CGGCGGCAGCCCCAAGAAGAAGAGAAAGGTGTGACTAGCCATCACATTTAA AAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAG CTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTA AAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	
Cas9 ORF with minimal uridine codons infrequently used in	ATGGACAAAAAATACAGCATAGGGCTAGACATAGGGACGAACAGCGTAGGG TGGGCGGTAATAACGGACGAATACAAAGTACCGAGCAAAAAATTCAAAGTA CTAGGGAACACGGACCGACACAGCATAAAAAAAAAACCTAATAGGGGCGCTA CTATTCGACAGCGGGGAAACGGCGGAAGCGACGCGACTAAAACGAACGGCG CGACGACGATACACGCGACGAAAAAACCGAATATGCTACCTACAAGAAATA TTCAGCAACGAAATGGCGAAAGTAGACGACAGCTTCTTCCACCGACTAGAA	254

humans in general; 12.75% U content	GAAAGCTTCCTAGTAGAAGAAGACAAAAAACACGAACGACACCCGATATTC GGGAACATAGTAGACGAAGTAGCGTACCACGAAAAATACCCGACGATATAC CACCTACGAAAAAAACTAGTAGACAGCACGGACAAAGCGGACCTACGACTA ATATACCTAGCGCTAGCGCACATGATAAAATTCCGAGGGCACTTCCTAATA GAAGGGGACCTAAACCCGGACAACAGCGACGTAGACAAACTATTCATACAA CTAGTACAAACGTACAACCAACTATTCGAAGAAAACCCGATAAACGCGAGC GGGGTAGACGCGAAAGCGATACTAAGCGCGCGACTAAGCAAAAGCCGACGA CTAGAAAACCTAATAGCGCAACTACCGGGGGAAAAAAAAAAACGGGCTATTC GGGAACCTAATAGCGCTAAGCCTAGGGCTAACGCCGAACTTCAAAAGCAAC TTCGACCTAGCGGAAGACGCGAAACTACAACTAAGCAAAGACACGTACGAC GACGACCTAGACAACCTACTAGCGCAAATAGGGGACCAATACGCGGACCTA TTCCTAGCGGCGAAAAACCTAAGCGACGCGATACTACTAAGCGACATACTA CGAGTAAACACGGAAATAACGAAAGCGCCGCTAAGCGCGAGCATGATAAAA CGATACGACGAACACCACCAAGACCTAACGCTACTAAAAGCGCTAGTACGA CAACAACTACCGGAAAAATACAAAGAAATATTCTTCGACCAAAGCAAAAAC GGGTACGCGGGGTACATAGACGGGGGGGCGAGCCAAGAAGAATTCTACAAA TTCATAAAACCGATACTAGAAAAAATGGACGGGACGGAAGAACTACTAGTA AAACTAAACCGAGAAGACCTACTACGAAAACAACGAACGTTCGACAACGGG AGCATACCGCACCAAATACACCTAGGGGAACTACACGCGATACTACGACGA CAAGAAGACTTCTACCCGTTCCTAAAAGACAACCGAGAAAAAATAGAAAAA ATACTAACGTTCCGAATACCGTACTACGTAGGGCCGCTAGCGCGAGGGAAC AGCCGATTCGCGTGGATGACGCGAAAAAGCGAAGAAACGATAACGCCGTGG AACTTCGAAGAAGTAGTAGACAAAGGGGCGAGCGCGCAAAGCTTCATAGAA CGAATGACGAACTTCGACAAAAACCTACCGAACGAAAAAGTACTACCGAAA CACAGCCTACTATACGAATACTTCACGGTATACAACGAACTAACGAAAGTA AAATACGTAACGGAAGGGATGCGAAAACCGGCGTTCCTAAGCGGGGAACAA AAAAAAGCGATAGTAGACCTACTATTCAAAACGAACCGAAAAGTAACGGTA AAACAACTAAAAGAAGACTACTTCAAAAAAATAGAATGCTTCGACAGCGTA GAAATAAGCGGGGTAGAAGACCGATTCAACGCGAGCCTAGGGACGTACCAC GACCTACTAAAAATAATAAAAGACAAAGACTTCCTAGACAACGAAGAAAAC GAAGACATACTAGAAGACATAGTACTAACGCTAACGCTATTCGAAGACCGA GAAATGATAGAAGAACGACTAAAAACGTACGCGCACCTATTCGACGACAAA GTAATGAAACAACTAAAACGACGACGATACACGGGGTGGGGGCGACTAAGC CGAAAACTAATAAACGGGATACGAGACAAACAAAGCGGGAAAACGATACTA GACTTCCTAAAAAGCGACGGGTTCGCGAACCGAAACTTCATGCAACTAATA CACGACGACAGCCTAACGTTCAAAGAAGACATACAAAAAGCGCAAGTAAGC GGGCAAGGGGACAGCCTACACGAACACATAGCGAACCTAGCGGGGAGCCCG GCGATAAAAAAAGGGATACTACAAACGGTAAAAGTAGTAGACGAACTAGTA AAAGTAATGGGGCGACACAAACCGGAAAACATAGTAATAGAAATGGCGCGA GAAAACCAAACGACGCAAAAAGGGCAAAAAAACAGCCGAGAACGAATGAAA CGAATAGAAGAAGGGATAAAAGAACTAGGGAGCCAAATACTAAAAGAACAC CCGGTAGAAAACACGCAACTACAAAACGAAAAACTATACCTATACTACCTA CAAAACGGGCGAGACATGTACGTAGACCAAGAACTAGACATAAACCGACTA AGCGACTACGACGTAGACCACATAGTACCGCAAAGCTTCCTAAAAGACGAC AGCATAGACAACAAAGTACTAACGCGAAGCGACAAAAACCGAGGGAAAAGC GACAACGTACCGAGCGAAGAAGTAGTAAAAAAAAATGAAAAACTACTGGCGA CAACTACTAAACGCGAAACTAATAACGCAACGAAAATTCGACAACCTAACG AAAGCGGAACGAGGGGGGCTAAGCGAACTAGACAAAGCGGGGTTCATAAAA CGACAACTAGTAGAAACGCGACAAATAACGAAACACGTAGCGCAAATACTA GACAGCCGAATGAACACGAAATACGACGAAAACGACAAACTAATACGAGAA GTAAAAGTAATAACGCTAAAAAGCAAACTAGTAAGCGACTTCCGAAAAGAC TTCCAATTCTACAAAGTACGAGAAATAAACAACTACCACCACGCGCACGAC GCGTACCTAAACGCGGTAGTAGGGACGGCGCTAATAAAAAAATACCCGAAA CTAGAAAGCGAATTCGTATACGGGGACTACAAAGTATACGACGTACGAAAA ATGATAGCGAAAAGCGAACAAGAAATAGGGAAAGCGACGGCGAAATACTTC TTCTACAGCAACATAATGAACTTCTTCAAAACGGAAATAACGCTAGCGAAC GGGGAAATACGAAAACGACCGCTAATAGAAACGAACGGGGAAACGGGGGAA ATAGTATGGGACAAAGGGCGAGACTTCGCGACGGTACGAAAAGTACTAAGC ATGCCGCAAGTAAACATAGTAAAAAAAACGGAAGTACAAACGGGGGGGTTC AGCAAAGAAAGCATACTACCGAAACGAAACAGCGACAAACTAATAGCGCGA AAAAAAGACTGGGACCCGAAAAAATACGGGGGGTTCGACAGCCCGACGGTA	

	GCGTACAGCGTACTAGTAGTAGCGAAAGTAGAAAAAGGGAAAAGCAAAAAA CTAAAAAGCGTAAAAGAACTACTAGGGATAACGATAATGGAACGAAGCAGC TTCGAAAAAAACCCGATAGACTTCCTAGAAGCGAAAGGGTACAAAGAAGTA AAAAAAGACCTAATAATAAAACTACCGAAATACAGCCTATTCGAACTAGAA AACGGGCGAAAACGAATGCTAGCGAGCGCGGGGGAACTACAAAAAGGGAAC GAACTAGCGCTACCGAGCAAATACGTAAACTTCCTATACCTAGCGAGCCAC TACGAAAAACTAAAAGGGAGCCCGGAAGACAACGAACAAAAACAACTATTC GTAGAACAACACAAACACTACCTAGACGAAATAATAGAACAAATAAGCGAA TTCAGCAAACGAGTAATACTAGCGGACGCGAACCTAGACAAAGTACTAAGC GCGTACAACAAACACCGAGACAAACCGATACGAGAACAAGCGGAAAACATA ATACACCTATTCACGCTAACGAACCTAGGGGCGCCGGCGGCGTTCAAATAC TTCGACACGACGATAGACCGAAAACGATACACGAGCACGAAAGAAGTACTA GACGCGACGCTAATACACCAAAGCATAACGGGGCTATACGAAACGCGAATA GACCTAAGCCAACTAGGGGGGGACGGGGGGGGGAGCCCGAAAAAAAAACGA AAAGTATGA	
Cas9 transcript with 5′ UTR of HSD, ORF correspondin g to SEQ ID NO: 254, Kozak sequence, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCCGCCACCATGGACAAAAAATACAGCATAGGGCT AGACATAGGGACGAACAGCGTAGGGTGGGCGGTAATAACGGACGAATACAA AGTACCGAGCAAAAAATTCAAAGTACTAGGGAACACGGACCGACACAGCAT AAAAAAAAACCTAATAGGGGCGCTACTATTCGACAGCGGGGAAACGGCGGA AGCGACGCGACTAAAACGAACGGCGCGACGACGATACACGCGACGAAAAAA CCGAATATGCTACCTACAAGAAATATTCAGCAACGAAATGGCGAAAGTAGA CGACAGCTTCTTCCACCGACTAGAAGAAAGCTTCCTAGTAGAAGAAGACAA AAAACACGAACGACACCCGATATTCGGGAACATAGTAGACGAAGTAGCGTA CCACGAAAAATACCCGACGATATACCACCTACGAAAAAAACTAGTAGACAG CACGGACAAAGCGGACCTACGACTAATATACCTAGCGCTAGCGCACATGAT AAAATTCCGAGGGCACTTCCTAATAGAAGGGGACCTAAACCCGGACAACAG CGACGTAGACAAACTATTCATACAACTAGTACAAACGTACAACCAACTATT CGAAGAAAACCCGATAAACGCGAGCGGGGTAGACGCGAAAGCGATACTAAG CGCGCGACTAAGCAAAAGCCGACGACTAGAAAACCTAATAGCGCAACTACC GGGGGAAAAAAAAAAACGGGCTATTCGGGAACCTAATAGCGCTAAGCCTAGG GCTAACGCCGAACTTCAAAAGCAACTTCGACCTAGCGGAAGACGCGAAACT ACAACTAAGCAAAGACACGTACGACGACGACCTAGACAACCTACTAGCGCA AATAGGGGACCAATACGCGGACCTATTCCTAGCGGCGAAAAACCTAAGCGA CGCGATACTACTAAGCGACATACTACGAGTAAACACGGAAATAACGAAAGC GCCGCTAAGCGCGAGCATGATAAAACGATACGACGAACACCACCAAGACCT AACGCTACTAAAAGCGCTAGTACGACAACAACTACCGGAAAAATACAAAGA AATATTCTTCGACCAAAGCAAAAACGGGTACGCGGGGTACATAGACGGGGG GGCGAGCCAAGAAGAATTCTACAAATTCATAAAACCGATACTAGAAAAAAT GGACGGGACGGAAGAACTACTAGTAAAACTAAACCGAGAAGACCTACTACG AAAACAACGAACGTTCGACAACGGGAGCATACCGCACCAAATACACCTAGG GGAACTACACGCGATACTACGACGACAAGAAGACTTCTACCCGTTCCTAAA AGACAACCGAGAAAAAATAGAAAAAATACTAACGTTCCGAATACCGTACTA CGTAGGGCCGCTAGCGCGAGGGAACAGCCGATTCGCGTGGATGACGCGAAA AAGCGAAGAAACGATAACGCCGTGGAACTTCGAAGAAGTAGTAGACAAAGG GGCGAGCGCGCAAAGCTTCATAGAACGAATGACGAACTTCGACAAAAACCT ACCGAACGAAAAAGTACTACCGAAACACAGCCTACTATACGAATACTTCAC GGTATACAACGAACTAACGAAAGTAAAATACGTAACGGAAGGGATGCGAAA ACCGGCGTTCCTAAGCGGGGAACAAAAAAAAGCGATAGTAGACCTACTATT CAAAACGAACCGAAAAGTAACGGTAAAACAACTAAAAGAAGACTACTTCAA AAAAATAGAATGCTTCGACAGCGTAGAAATAAGCGGGGTAGAAGACCGATT CAACGCGAGCCTAGGGACGTACCACGACCTACTAAAAATAATAAAAGACAA AGACTTCCTAGACAACGAAGAAAACGAAGACATACTAGAAGACATAGTACT AACGCTAACGCTATTCGAAGACCGAGAAATGATAGAAGAACGACTAAAAAC GTACGCGCACCTATTCGACGACAAAGTAATGAAACAACTAAAACGACGACG ATACACGGGGTGGGGGCGACTAAGCCGAAAACTAATAAACGGGATACGAGA CAAACAAAGCGGGAAAACGATACTAGACTTCCTAAAAAGCGACGGGTTCGC GAACCGAAACTTCATGCAACTAATACACGACGACAGCCTAACGTTCAAAGA AGACATACAAAAAGCGCAAGTAAGCGGGCAAGGGGACAGCCTACACGAACA CATAGCGAACCTAGCGGGGAGCCCGGCGATAAAAAAAGGGATACTACAAAC	255

	GGTAAAAGTAGTAGACGAACTAGTAAAAGTAATGGGGCGACACAAACCGGA AAACATAGTAATAGAAATGGCGCGAGAAAACCAAACGACGCAAAAAGGGCA AAAAAACAGCCGAGAACGAATGAAACGAATAGAAGAAGGGATAAAAGAACT AGGGAGCCAAATACTAAAAGAACACCCGGTAGAAAACACGCAACTACAAAA CGAAAAACTATACCTATACTACCTACAAAACGGGCGAGACATGTACGTAGA CCAAGAACTAGACATAAACCGACTAAGCGACTACGACGTAGACCACATAGT ACCGCAAAGCTTCCTAAAAGACGACAGCATAGACAACAAAGTACTAACGCG AAGCGACAAAAACCGAGGGAAAAGCGACAACGTACCGAGCGAAGAAGTAGT AAAAAAAATGAAAAACTACTGGCGACAACTACTAAACGCGAAACTAATAAC GCAACGAAAATTCGACAACCTAACGAAAGCGGAACGAGGGGGGCTAAGCGA ACTAGACAAAGCGGGGTTCATAAAACGACAACTAGTAGAAACGCGACAAAT AACGAAACACGTAGCGCAAATACTAGACAGCCGAATGAACACGAAATACGA CGAAAACGACAAACTAATACGAGAAGTAAAAGTAATAACGCTAAAAAGCAA ACTAGTAAGCGACTTCCGAAAAGACTTCCAATTCTACAAAGTACGAGAAAT AAACAACTACCACCACGCGCACGACGCGTACCTAAACGCGGTAGTAGGGAC GGCGCTAATAAAAAAATACCCGAAACTAGAAAGCGAATTCGTATACGGGGA CTACAAAGTATACGACGTACGAAAAATGATAGCGAAAAGCGAACAAGAAAT AGGGAAAGCGACGGCGAAATACTTCTTCTACAGCAACATAATGAACTTCTT CAAAACGGAAATAACGCTAGCGAACGGGGAAATACGAAAACGACCGCTAAT AGAAACGAACGGGGAAACGGGGGAAATAGTATGGGACAAAGGGCGAGACTT CGCGACGGTACGAAAAGTACTAAGCATGCCGCAAGTAAACATAGTAAAAAA AACGGAAGTACAAACGGGGGGGTTCAGCAAAGAAAGCATACTACCGAAACG AAACAGCGACAAACTAATAGCGCGAAAAAAAGACTGGGACCCGAAAAAATA CGGGGGGTTCGACAGCCCGACGGTAGCGTACAGCGTACTAGTAGTAGCGAA AGTAGAAAAAGGGAAAAGCAAAAAACTAAAAAGCGTAAAAGAACTACTAGG GATAACGATAATGGAACGAAGCAGCTTCGAAAAAAACCCGATAGACTTCCT AGAAGCGAAAGGGTACAAAGAAGTAAAAAAAGACCTAATAATAAAACTACC GAAATACAGCCTATTCGAACTAGAAAACGGGCGAAAACGAATGCTAGCGAG CGCGGGGGAACTACAAAAAGGGAACGAACTAGCGCTACCGAGCAAATACGT AAACTTCCTATACCTAGCGAGCCACTACGAAAAACTAAAAGGGAGCCCGGA AGACAACGAACAAAAACAACTATTCGTAGAACAACACAAACACTACCTAGA CGAAATAATAGAACAAATAAGCGAATTCAGCAAACGAGTAATACTAGCGGA CGCGAACCTAGACAAAGTACTAAGCGCGTACAACAAACACCGAGACAAACC GATACGAGAACAAGCGGAAAACATAATACACCTATTCACGCTAACGAACCT AGGGGCGCCGGCGGCGTTCAAATACTTCGACACGACGATAGACCGAAAACG ATACACGAGCACGAAAGAAGTACTAGACGCGACGCTAATACACCAAAGCAT AACGGGGCTATACGAAACGCGAATAGACCTAAGCCAACTAGGGGGGGACGG GGGGGGGAGCCCGAAAAAAAAACGAAAAGTATGACTAGCCATCACATTTAA AAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAG CTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTA AAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	
Cas9 transcript with AGG as first three nucleotides for use with CleanCap™, 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3′ UTR of ALB	AGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTT GCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACT GGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAA GGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCAT CAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGA AGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAA CAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGA CGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAA GAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATA CCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAG CACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGAT CAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAG CGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTT CGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAG CGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCC GGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGG ACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCT GCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACA	256

	GATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGA CGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGC ACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCT GACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGA AATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGG AGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGAT GGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAG AAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGG AGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAA GGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAA GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGG AGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCT GCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCAC AGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAA GCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTT CAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAA GAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATT CAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAA GGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCT GACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGAC ATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAG ATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGA CAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGA AGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACA CATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGAC AGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGA AAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACA GAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACT GGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAA CGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGA CCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGT CCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAG AAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGT CAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCAC ACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGA ACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGAT CACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGA CGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAAT CAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAAC AGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGA CTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAAT CGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTT CAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGAT CGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTT CGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAA GACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAG AAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTA CGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGG AATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCT GGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCC GAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAG CGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGA AGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGA CGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGA CGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCC GATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCT	

	GGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAG ATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCAT CACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGG AGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAA AAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAG CTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTA AAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	
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	ACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCG ACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACG TCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGC TGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAG AGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGC TGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCA GAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGG TCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGT TCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACC TGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAA GCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCG CAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACA GCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAA TCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCT GGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGG AAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGG ACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACA GCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGA GCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGG ACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAA GAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGG CACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAA AGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAAC AGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCA AGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACC TGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACA CAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAA CACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGA GCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCT AGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAA AGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGT AAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTC TTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAG	
Cas9 transcript with 5′ UTR from HBB, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3′ UTR of HBB	GGGACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACA CCGGATCTGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAA CAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCA AGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACC TGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGAC TGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCT ACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCT TCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAA GACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGT ACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGG CAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAG GACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACA AGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACC CGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGA GCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGA AGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGA ACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCA AGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACC AGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGC TGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCG CAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGA AGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCG ACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGG AAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAG	258

	AAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAA CATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACG CAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAG AAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGC TGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAA CAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAA AGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACG AACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCC TGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACA GAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAAT GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCC TGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACAC TGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACC TGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGAT GGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCG GAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACT TCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGA AGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACC TGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCG TCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCA TCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCA GAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGA TCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGT ACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGG ACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCT TCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGA ACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGA AGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGT TCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGG CAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACG TCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACA AGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCG ACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACC ACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCA AGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCT ACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAA CAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAA TCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACG GAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCA GAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCC AGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACA AGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCG ACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGG GAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCA TGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGG GATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCC TGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGT ACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAAC AGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCG AACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGG ACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAAC AGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGG CAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCA CAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGT ACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCC CGAAGAAGAAGAGAAAGGTCTAGCTAGCGCTCGCTTTCTTGCTGTCCAATT TCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATA	

	TTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTT TCATTGCCTCGAG	
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	TCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACC TGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAA GCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCG CAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACA GCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAA TCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCT GGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGG AAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGG ACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACA GCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGA GCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGG ACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAA GAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGG CACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAA AGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAAC AGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCA AGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACC TGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACA CAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAA CACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGA GCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCT AGCTAGCACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAAT ACCAACTTACACTTTACAAAATGTTGTCCCCCAAAATGTAGCCATTCGTAT CTGCTCCTAATAAAAAGAAAGTTTCTTCACATTCTCTCGAG	
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	CAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGC TGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCA GCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGC TGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACA TCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGA TCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGA AGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGC TGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCC TGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACG ACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGG GAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCA AGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCA TGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACC AGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCG AAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCG AAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACG GAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACT ACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCG ACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACG TCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGC TGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAG AGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGC TGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCA GAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGG TCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGT TCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACC TGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAA GCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCG CAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACA GCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAA TCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCT GGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGG AAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGG ACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACA GCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGA GCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGG ACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAA GAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGG CACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAA AGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAAC AGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCA AGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACC TGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACA CAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAA CACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGA GCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCT AGCTAGCACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAAT ACCAACTTACACTTTACAAAATGTTGTCCCCCAAAATGTAGCCATTCGTAT CTGCTCCTAATAAAAAGAAAGTTTCTTCACATTCTCTCGAG	
Cas9 transcript with AGG as first three nucleotides for use with CleanCap™, 5′ UTR from	AGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACT GGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAA GGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCAT CAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGA AGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAA CAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGA CGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAA	261

HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3' UTR of ALB	GAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATA CCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAG CACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGAT CAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAG CGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTT CGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAG CGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCC GGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGG ACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCT GCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACA GATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGA CGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGC ACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCT GACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGA AATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGG AGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGAT GGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAG AAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGG AGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAA GGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAA GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGG AGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCT GCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCAC AGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAA GCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTT CAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAA GAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATT CAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAA GGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCT GACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGAC ATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAG ATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGA CAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGA AGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACA CATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGAC AGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGA AAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACA GAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACT GGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAA CGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGA CCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGT CCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAG AAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGT CAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCAC ACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGA ACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGAT CACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGA CGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAAT CAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAAC AGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGA CTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAAT CGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTT CAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGAT CGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTT CGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAA GACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAG AAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTA CGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA	

	GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGG AATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCT GGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCC GAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAG CGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGA AGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGA CGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGA CGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCC GATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCT GGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAG ATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCAT CACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGG AGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAA AAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAG CTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTA AAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	
30/30/39 poly-A sequence	Not used	262
poly-A 100 sequence	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	263
G209 single guide RNA targeting the mouse TTR gene	AAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC	264
ORF encoding Neisseria meningitidis Cas9 using minimal uridine codons, with start and stop codons	ATGGCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATC GGAATCGCATCGGTCGGATGGGCAATGGTCGAAATCGACGAAGAAGAAAAC CCGATCAGACTGATCGACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTC CCGAAGACAGGAGACTCGCTGGCAATGGCAAGAAGACTGGCAAGATCGGTC AGAAGACTGACAAGAAGAAGAGCACACAGACTGCTGAGAACAAGAAGACTG CTGAAGAGAGAAGGAGTCCTGCAGGCAGCAAACTTCGACGAAAACGGACTG ATCAAGTCGCTGCCGAACACACCGTGGCAGCTGAGAGCAGCAGCACTGGAC AGAAAGCTGACACCGCTGGAATGGTCGGCAGTCCTGCTGCACCTGATCAAG CACAGAGGATACCTGTCGCAGAGAAAGAACGAAGGAGAAACAGCAGACAAG GAACTGGGAGCACTGCTGAAGGGAGTCGCAGGAAACGCACACGCACTGCAG ACAGGAGACTTCAGAACACCGGCAGAACTGGCACTGAACAAGTTCGAAAAG GAATCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACACATTCTCG AGAAAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCAGAAGGAA TTCGGAAACCCGCACGTCTCGGGAGGACTGAAGGAAGGAATCGAAACACTG CTGATGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTG GGACACTGCACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATAC ACAGCAGAAAGATTCATCTGGCTGACAAAGCTGAACAACCTGAGAATCCTG GAACAGGGATCGGAAAGACCGCTGACAGACACAGAAAGAGCAACACTGATG GACGAACCGTACAGAAAGTCGAAGCTGACATACGCACAGGCAAGAAAGCTG CTGGGACTGGAAGACACAGCATTCTTCAAGGGACTGAGATACGGAAAGGAC AACGCAGAAGCATCGACACTGATGGAAATGAAGGCATACCACGCAATCTCG AGAGCACTGGAAAAGGAAGGACTGAAGGACAAGAAGTCGCCGCTGAACCTG TCGCCGGAACTGCAGGACGAAATCGGAACAGCATTCTCGCTGTTCAAGACA GACGAAGACATCACAGGAAGACTGAAGGACAGAATCCAGCCGGAAATCCTG GAAGCACTGCTGAAGCACATCTCGTTCGACAAGTTCGTCCAGATCTCGCTG AAGGCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAAGAGATACGAC GAAGCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAAGAACACAGAA GAAAAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAACCCGGTC GTCCTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCAACGGAGTCGTCAGA AGATACGGATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGA AAGTCGTTCAAGGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAAACAGA AAGGACAGAGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTC	265

	GTCGGAGAACCGAAGTCGAAGGACATCCTGAAGCTGAGACTGTACGAACAG CAGCACGGAAAGTGCCTGTACTCGGGAAAGGAAATCAACCTGGGAAGACTG AACGAAAAGGGATACGTCGAAATCGACCACGCACTGCCGTTCTCGAGAACA TGGGACGACTCGTTCAACAACAAGGTCCTGGTCCTGGGATCGGAAAACCAG AACAAGGGAAACCAGACACCGTACGAATACTTCAACGGAAAGGACAACTCG AGAGAATGGCAGGAATTCAAGGCAAGAGTCGAAACATCGAGATTCCCGAGA TCGAAGAAGCAGAGAATCCTGCTGCAGAAGTTCGACGAAGACGGATTCAAG GAAAGAAACCTGAACGACACAAGATACGTCAACAGATTCCTGTGCCAGTTC GTCGCAGACAGAATGAGACTGACAGGAAAGGGAAAGAAGAGAGTCTTCGCA TCGAACGGACAGATCACAAACCTGCTGAGAGGATTCTGGGGACTGAGAAAG GTCAGAGCAGAAAACGACAGACACCACGCACTGGACGCAGTCGTCGTCGCA TGCTCGACAGTCGCAATGCAGCAGAAGATCACAAGATTCGTCAGATACAAG GAAATGAACGCATTCGACGGAAAGACAATCGACAAGGAAACAGGAGAAGTC CTGCACCAGAAGACACACTTCCCGCAGCCGTGGGAATTCTTCGCACAGGAA GTCATGATCAGAGTCTTCGGAAAGCCGGACGGAAAGCCGGAATTCGAAGAA GCAGACACACTGGAAAAGCTGAGAACACTGCTGGCAGAAAAGCTGTCGTCG AGACCGGAAGCAGTCCACGAATACGTCACACCGCTGTTCGTCTCGAGAGCA CCGAACAGAAAGATGTCGGGACAGGGACACATGGAAACAGTCAAGTCGGCA AAGAGACTGGACGAAGGAGTCTCGGTCCTGAGAGTCCCGCTGACACAGCTG AAGCTGAAGGACCTGGAAAAGATGGTCAACAGAGAAAGAGAACCGAAGCTG TACGAAGCACTGAAGGCAAGACTGGAAGCACACAAGGACGACCCGGCAAAG GCATTCGCAGAACCGTTCTACAAGTACGACAAGGCAGGAAACAGAACACAG CAGGTCAAGGCAGTCAGAGTCGAACAGGTCCAGAAGACAGGAGTCTGGGTC AGAAACCACAACGGAATCGCAGACAACGCAACAATGGTCAGAGTAGACGTC TTCGAAAAGGGAGACAAGTACTACCTGGTCCCGATCTACTCGTGGCAGGTC GCAAAGGGAATCCTGCCGGACAGAGCAGTCGTCCAGGGAAAGGACGAAGAA GACTGGCAGCTGATCGACGACTCGTTCAACTTCAAGTTCTCGCTGCACCCG AACGACCTGGTCGAAGTCATCACAAAGAAGGCAAGAATGTTCGGATACTTC GCATCGTGCCACAGAGGAACAGGAAACATCAACATCAGAATCCACGACCTG GACCACAAGATCGGAAAGAACGGAATCCTGGAAGGAATCGGAGTCAAGACA GCACTGTCGTTCCAGAAGTACCAGATCGACGAACTGGGAAAGGAAATCAGA CCGTGCAGACTGAAGAAGAGACCGCCGGTCAGATCCGGAAAGAGAACAGCA GACGGATCGGAATTCGAATCGCCGAAGAAGAAGAGAAAGGTCGAATGA	
ORF encoding Neisseria meningitidis Cas9 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGGA ATCGCATCGGTCGGATGGGCAATGGTCGAAATCGACGAAGAAGAAAACCCG ATCAGACTGATCGACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTCCCG AAGACAGGAGACTCGCTGGCAATGGCAAGAAGACTGGCAAGATCGGTCAGA AGACTGACAAGAAGAAGAGCACACAGACTGCTGAGAACAAGAAGACTGCTG AAGAGAGAAGGAGTCCTGCAGGCAGCAAACTTCGACGAAAACGGACTGATC AAGTCGCTGCCGAACACACCGTGGCAGCTGAGAGCAGCAGCACTGGACAGA AAGCTGACACCGCTGGAATGGTCGGCAGTCCTGCTGCACCTGATCAAGCAC AGAGGATACCTGTCGCAGAGAAAGAACGAAGGAGAAACAGCAGACAAGGAA CTGGGAGCACTGCTGAAGGGAGTCGCAGGAAACGCACACGCACTGCAGACA GGAGACTTCAGAACACCGGCAGAACTGGCACTGAACAAGTTCGAAAAGGAA TCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACACATTCTCGAGA AAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCAGAAGGAATTC GGAAACCCGCACGTCTCGGGAGGACTGAAGGAAGGAATCGAAACACTGCTG ATGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTGGGA CACTGCACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATACACA GCAGAAAGATTCATCTGGCTGACAAAGCTGAACAACCTGAGAATCCTGGAA CAGGGATCGGAAAGACCGCTGACAGACACAGAAAGAGCAACACTGATGGAC GAACCGTACAGAAAGTCGAAGCTGACATACGCACAGGCAAGAAAGCTGCTG GGACTGGAAGACACAGCATTCTTCAAGGGACTGAGATACGGAAAGGACAAC GCAGAAGCATCGACACTGATGGAAATGAAGGCATACCACGCAATCTCGAGA GCACTGGAAAAGGAAGGACTGAAGGACAAGAAGTCGCCGCTGAACCTGTCG CCGGAACTGCAGGACGAAATCGGAACAGCATTCTCGCTGTTCAAGACAGAC GAAGACATCACAGGAAGACTGAAGGACAGAATCCAGCCGGAAATCCTGGAA GCACTGCTGAAGCACATCTCGTTCGACAAGTTCGTCCAGATCTCGCTGAAG GCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAAGAGATACGACGAA GCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAAGAACACAGAAGAA AAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAACCCGGTCGTC	266

	CTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCAACGGAGTCGTCAGAAGA TACGGATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGAAAG TCGTTCAAGGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAAACAGAAAG GACAGAGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTCGTC GGAGAACCGAAGTCGAAGGACATCCTGAAGCTGAGACTGTACGAACAGCAG CACGGAAAGTGCCTGTACTCGGGAAAGGAAATCAACCTGGGAAGACTGAAC GAAAAGGGATACGTCGAAATCGACCACGCACTGCCGTTCTCGAGAACATGG GACGACTCGTTCAACAACAAGGTCCTGGTCCTGGGATCGGAAAACCAGAAC AAGGGAAACCAGACACCGTACGAATACTTCAACGGAAAGGACAACTCGAGA GAATGGCAGGAATTCAAGGCAAGAGTCGAAACATCGAGATTCCCGAGATCG AAGAAGCAGAGAATCCTGCTGCAGAAGTTCGACGAAGACGGATTCAAGGAA AGAAACCTGAACGACACAAGATACGTCAACAGATTCCTGTGCCAGTTCGTC GCAGACAGAATGAGACTGACAGGAAAGGGAAAGAAGAGAGTCTTCGCATCG AACGGACAGATCACAAACCTGCTGAGAGGATTCTGGGGACTGAGAAAGGTC AGAGCAGAAAACGACAGACACCACGCACTGGACGCAGTCGTCGTCGCATGC TCGACAGTCGCAATGCAGCAGAAGATCACAAGATTCGTCAGATACAAGGAA ATGAACGCATTCGACGGAAAGACAATCGACAAGGAAACAGGAGAAGTCCTG CACCAGAAGACACACTTCCCGCAGCCGTGGGAATTCTTCGCACAGGAAGTC ATGATCAGAGTCTTCGGAAAGCCGGACGGAAAGCCGGAATTCGAAGAAGCA GACACACTGGAAAAGCTGAGAACACTGCTGGCAGAAAAGCTGTCGTCGAGA CCGGAAGCAGTCCACGAATACGTCACACCGCTGTTCGTCTCGAGAGCACCG AACAGAAAGATGTCGGGACAGGGACACATGGAAACAGTCAAGTCGGCAAAG AGACTGGACGAAGGAGTCTCGGTCCTGAGAGTCCCGCTGACACAGCTGAAG CTGAAGGACCTGGAAAAGATGGTCAACAGAGAAAGAGAACCGAAGCTGTAC GAAGCACTGAAGGCAAGACTGGAAGCACACAAGGACGACCCGGCAAAGGCA TTCGCAGAACCGTTCTACAAGTACGACAAGGCAGGAAACAGAACACAGCAG GTCAAGGCAGTCAGAGTCGAACAGGTCCAGAAGACAGGAGTCTGGGTCAGA AACCACAACGGAATCGCAGACAACGCAACAATGGTCAGAGTAGACGTCTTC GAAAAGGGAGACAAGTACTACCTGGTCCCGATCTACTCGTGGCAGGTCGCA AAGGGAATCCTGCCGGACAGAGCAGTCGTCCAGGGAAAGGACGAAGAAGAC TGGCAGCTGATCGACGACTCGTTCAACTTCAAGTTCTCGCTGCACCCGAAC GACCTGGTCGAAGTCATCACAAAGAAGGCAAGAATGTTCGGATACTTCGCA TCGTGCCACAGAGGAACAGGAAACATCAACATCAGAATCCACGACCTGGAC CACAAGATCGGAAAGAACGGAATCCTGGAAGGAATCGGAGTCAAGACAGCA CTGTCGTTCCAGAAGTACCAGATCGACGAACTGGGAAAGGAAATCAGACCG TGCAGACTGAAGAAGAGACCGCCGGTCAGATCCGGAAAGAGAACAGCAGAC GGATCGGAATTCGAATCGCCGAAGAAGAAGAGAAAGGTCGAA	
Transcript comprising SEQ ID NO: 265 (encoding Neisseria meningitidis Cas9)	GGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTGGATCCGCCACCAT GGCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGG AATCGCATCGGTCGGATGGGCAATGGTCGAAATCGACGAAGAAGAAAACCC GATCAGACTGATCGACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTCCC GAAGACAGGAGACTCGCTGGCAATGGCAAGAAGACTGGCAAGATCGGTCAG AAGACTGACAAGAAGAAGAGCACACAGACTGCTGAGAACAAGAAGACTGCT GAAGAGAGAAGGAGTCCTGCAGGCAGCAAACTTCGACGAAAACGGACTGAT CAAGTCGCTGCCGAACACACCGTGGCAGCTGAGAGCAGCAGCACTGGACAG AAAGCTGACACCGCTGGAATGGTCGGCAGTCCTGCTGCACCTGATCAAGCA CAGAGGATACCTGTCGCAGAGAAAGAACGAAGGAGAAACAGCAGACAAGGA ACTGGGAGCACTGCTGAAGGGAGTCGCAGGAAACGCACACGCACTGCAGAC AGGAGACTTCAGAACACCGGCAGAACTGGCACTGAACAAGTTCGAAAAGGA ATCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACACATTCTCGAG AAAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCAGAAGGAATT CGGAAACCCGCACGTCTCGGGAGGACTGAAGGAAGGAATCGAAACACTGCT GATGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTGGG ACACTGCACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATACAC AGCAGAAAGATTCATCTGGCTGACAAAGCTGAACAACCTGAGAATCCTGGA ACAGGGATCGGAAAGACCGCTGACAGACACAGAAAGAGCAACACTGATGGA CGAACCGTACAGAAAGTCGAAGCTGACATACGCACAGGCAAGAAAGCTGCT GGGACTGGAAGACACAGCATTCTTCAAGGGACTGAGATACGGAAAGGACAA CGCAGAAGCATCGACACTGATGGAAATGAAGGCATACCACGCAATCTCGAG AGCACTGGAAAAGGAAGGACTGAAGGACAAGAAGTCGCCGCTGAACCTGTC GCCGGAACTGCAGGACGAAATCGGAACAGCATTCTCGCTGTTCAAGACAGA	267

	CGAAGACATCACAGGAAGACTGAAGGACAGAATCCAGCCGGAAATCCTGGA AGCACTGCTGAAGCACATCTCGTTCGACAAGTTCGTCCAGATCTCGCTGAA GGCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAAGAGATACGACGA AGCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAAGAACACAGAAGA AAAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAACCCGGTCGT CCTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCAACGGAGTCGTCAGAAG ATACGGATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGAAA GTCGTTCAAGGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAAACAGAAA GGACAGAGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTCGT CGGAGAACCGAAGTCGAAGGACATCCTGAAGCTGAGACTGTACGAACAGCA GCACGGAAAGTGCCTGTACTCGGGAAAGGAAATCAACCTGGGAAGACTGAA CGAAAAGGGATACGTCGAAATCGACCACGCACTGCCGTTCTCGAGAACATG GGACGACTCGTTCAACAACAAGGTCCTGGTCCTGGGATCGGAAAACCAGAA CAAGGGAAACCAGACACCGTACGAATACTTCAACGGAAAGGACAACTCGAG AGAATGGCAGGAATTCAAGGCAAGAGTCGAAACATCGAGATTCCCGAGATC GAAGAAGCAGAGAATCCTGCTGCAGAAGTTCGACGAAGACGGATTCAAGGA AAGAAACCTGAACGACACAAGATACGTCAACAGATTCCTGTGCCAGTTCGT CGCAGACAGAATGAGACTGACAGGAAAGGGAAAGAAGAGAGTCTTCGCATC GAACGGACAGATCACAAACCTGCTGAGAGGATTCTGGGGACTGAGAAAGGT CAGAGCAGAAAACGACAGACACCACGCACTGGACGCAGTCGTCGTCGCATG CTCGACAGTCGCAATGCAGCAGAAGATCACAAGATTCGTCAGATACAAGGA AATGAACGCATTCGACGGAAAGACAATCGACAAGGAAACAGGAGAAGTCCT GCACCAGAAGACACACTTCCCGCAGCCGTGGGAATTCTTCGCACAGGAAGT CATGATCAGAGTCTTCGGAAAGCCGGACGGAAAGCCGGAATTCGAAGAAGC AGACACACTGGAAAAGCTGAGAACACTGCTGGCAGAAAAGCTGTCGTCGAG ACCGGAAGCAGTCCACGAATACGTCACACCGCTGTTCGTCTCGAGAGCACC GAACAGAAAGATGTCGGGACAGGGACACATGGAAACAGTCAAGTCGGCAAA GAGACTGGACGAAGGAGTCTCGGTCCTGAGAGTCCCGCTGACACAGCTGAA GCTGAAGGACCTGGAAAAGATGGTCAACAGAGAAAGAGAACCGAAGCTGTA CGAAGCACTGAAGGCAAGACTGGAAGCACACAAGGACGACCCGGCAAAGGC ATTCGCAGAACCGTTCTACAAGTACGACAAGGCAGGAAACAGAACACAGCA GGTCAAGGCAGTCAGAGTCGAACAGGTCCAGAAGACAGGAGTCTGGGTCAG AAACCACAACGGAATCGCAGACAACGCAACAATGGTCAGAGTAGACGTCTT CGAAAAGGGAGACAAGTACTACCTGGTCCCGATCTACTCGTGGCAGGTCGC AAAGGGAATCCTGCCGGACAGAGCAGTCGTCCAGGGAAAGGACGAAGAAGA CTGGCAGCTGATCGACGACTCGTTCAACTTCAAGTTCTCGCTGCACCCGAA CGACCTGGTCGAAGTCATCACAAAGAAGGCAAGAATGTTCGGATACTTCGC ATCGTGCCACAGAGGAACAGGAAACATCAACATCAGAATCCACGACCTGGA CCACAAGATCGGAAAGAACGGAATCCTGGAAGGAATCGGAGTCAAGACAGC ACTGTCGTTCCAGAAGTACCAGATCGACGAACTGGGAAAGGAAATCAGACC GTGCAGACTGAAGAAGAGACCGCCGGTCAGATCCGGAAAGAGAACAGCAGA CGGATCGGAATTCGAATCGCCGAAGAAGAAGAGAAAGGTCGAATGATAGCT AGCTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGC CTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGA CCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTG CATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGC AGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATG CGGTGGGCTCTATGG	
Amino acid sequence of Neisseria meningitidis Cas9	MAAFKPNSINYILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRVFERAEV PKTGDSLAMARRLARSVRRLTRRRAHRLLRTRRLLKREGVLQAANFDENGL IKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADK ELGALLKGVAGNAHALQTGDFRTPAELALNKFEKESGHIRNQRSDYSHTFS RKDLQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKML GHCTFEPAEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDTERATLM DEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMKAYHAIS RALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLKDRIQPEIL EALLKHISFDKFVQISLKALRRIVPLMEQGKRYDEACAEIYGDHYGKKNTE EKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPARIHIETAREVG KSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKSKDILKLRLYEQ QHGKCLYSGKEINLGRLNEKGYVEIDHALPFSRTWDDSFNNKVLVLGSENQ NKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQRILLQKFDEDGFK	268

	ERNLNDTRYVNRFLCQFVADRMRLTGKGKKRVFASNGQITNLLRGFWGLRK VRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGEV LHQKTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTLEKLRTLLAEKLSS RPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSAKRLDEGVSVLRVPLTQL KLKDLEKMVNREREPKLYEALKARLEAHKDDPAKAFAEPFYKYDKAGNRTQ QVKAVRVEQVQKTGVWVRNHNGIADNATMVRVDVFEKGDKYYLVPIYSWQV AKGILPDRAVVQGKDEEDWQLIDDSFNFKFSLHPNDLVEVITKKARMFGYF ASCHRGTGNINIRIHDLDHKIGKNGILEGIGVKTALSFQKYQIDELGKEIR PCRLKKRPPVRSGKRTADGSEFESPKKKRKVE	
G390 single guide RNA targeting the rat TTR gene	mG*mC*mC*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	269
trRNA	AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUUUUU	270
	Not Used	271
G534 single guide RNA targeting the rat TTR gene	mA*mC*mG*CAAAUAUCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	272
G000395 5′ truncated inactive sgRNA modified sequence	mG*mC*mA*AUGGUGUAGCGGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmG mCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	273
SV40 NLS	PKKKRKV	274
Alternate SV40 NLS	PKKKRRV	275
Nucleoplasmin NLS	KRPAATKKAGQAKKKK	276
Exemplary Kozak sequence	gccRccAUGG	277
Exemplary Kozak sequence	gccgccRccAUGG	278

* = PS linkage; 'm' = 2'-O-Me nucleotide

What is Claimed is:

1. A method of inducing a double-stranded break (DSB) within the *TTR* gene, comprising delivering a composition to a cell, wherein the composition comprises

a. a guide RNA comprising a guide sequence selected from SEQ ID NOs: 5-82;
b. a guide RNA comprising at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide RNA comprising a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

2. A method of modifying the *TTR* gene comprising delivering a composition to a cell, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

3. A method of treating amyloidosis associated with TTR (ATTR), comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82,

thereby treating ATTR.

4. A method of reducing TTR serum concentration, comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82,

thereby reducing TTR serum concentration.

5. A method for reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in a subject, comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82,

thereby reducing accumulation of amyloids or amyloid fibrils.

6. A composition comprising a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

7. A composition comprising a vector encoding a guide RNA, wherein the guide RNA comprises:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

8. The composition of claim 6 or 7, for use in inducing a double-stranded break (DSB) within the *TTR* gene in a cell or subject.

9. The composition of claim 6 or 7, for use in modifying the *TTR* gene in a cell or subject.

10. The composition of claim 6 or 7, for use in treating amyloidosis associated with TTR (ATTR) in a subject.

11. The composition of claim 6 or 7, for use in reducing TTR serum concentration in a subject.

12. The composition of claim 6 or 7, for use in reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

13. The method of any one of claims 1-5 or the composition for use of any one of claims 8-12, wherein the composition reduces serum TTR levels.

14. The method or composition for use of claim 13, wherein the serum TTR levels are reduced by at least 50% as compared to serum TTR levels before administration of the composition.

15. The method or composition for use of claim 13, wherein the serum TTR levels are reduced by 50-60%, 60-70%, 70-80%, 80-90%, 90-95%, 95-98%, 98-99%, or 99-100% as compared to serum TTR levels before administration of the composition.

16. The method or composition for use of any one of claims 1-5 or 8-15, wherein the composition results in editing of the TTR gene.

17. The method or composition for use of claim 16, wherein the editing is calculated as a percentage of the population that is edited (percent editing).

18. The method or composition for use of claim 17, wherein the percent editing is between 30 and 99% of the population.

19. The method or composition for use of claim 17, wherein the percent editing is between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the population.

20. The method of any one of claims 1-5 or the composition for use of any one of claims 8-19, wherein the composition reduces amyloid deposition in at least one tissue.

21. The method or composition for use of claim 20, wherein the at least one tissue comprises one or more of stomach, colon, sciatic nerve, or dorsal root ganglion.

22. The method or composition for use of claim 20 or 21, wherein amyloid deposition is measured 8 weeks after administration of the composition.

23. The method or composition for use of any one of claims 20-22, wherein amyloid deposition is compared to a negative control or a level measured before administration of the composition.

24. The method or composition for use of any one of claims 20-23, wherein amyloid deposition is measured in a biopsy sample and/or by immunostaining.

25. The method or composition for use of any one of claims 20-24, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen in a negative control.

26. The method or composition for use of any one of claims 20-25, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen before administration of the composition.

27. The method or composition for use of any one of claims 1-5 or 8-26, wherein the composition is administered or delivered at least two times.

28. The method or composition for use of claim 27, wherein the composition is administered or delivered at least three times.

29. The method or composition for use of claim 27, wherein the composition is administered or delivered at least four times.

30. The method or composition for use of claim 27, wherein the composition is administered or delivered up to five, six, seven, eight, nine, or ten times.

31. The method or composition for use of any one of claims 27-30, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days.

32. The method or composition for use of any one of claims 27-30, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks.

33. The method or composition for use of any one of claims 27-30, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 months.

34. The method or composition of any one of the preceding claims, wherein the guide sequence is selected from SEQ ID NOs: 5-82.

35. The method or composition of any one of the preceding claims, wherein the guide RNA is at least partially complementary to a target sequence present in the human *TTR* gene.

36. The method or composition of claim 35, wherein the target sequence is in exon 1, 2, 3, or 4 of the human *TTR* gene.

37. The method or composition of claim 35, wherein the target sequence is in exon 1 of the human *TTR* gene.

38. The method or composition of claim 35, wherein the target sequence is in exon 2 of the human *TTR* gene.

39. The method or composition of claim 35, wherein the target sequence is in exon 3 of the human *TTR* gene.

40. The method or composition of claim 35, wherein the target sequence is in exon 4 of the human *TTR* gene.

41. The method or composition of any one of claims 1-40, wherein the guide sequence is complementary to a target sequence in the positive strand of *TTR*.

42. The method or composition of any one of claims 1-40, wherein the guide sequence is complementary to a target sequence in the negative strand of *TTR*.

43. The method or composition of any one of claims 1-40, wherein the first guide sequence is complementary to a first target sequence in the positive strand of the *TTR* gene, and wherein the composition further comprises a second guide sequence that is complementary to a second target sequence in the negative strand of the *TTR* gene.

44. The method or composition of any one of the preceding claims, wherein the guide RNA comprises a crRNA that comprises the guide sequence and further comprises a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end.

45. The method or composition of any one of the preceding claims, wherein the guide RNA is a dual guide (dgRNA).

46. The method or composition of claim 45, wherein the dual guide RNA comprises a crRNA comprising a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end, and a trRNA.

47. The method or composition of any one of claims 1-43, wherein the guide RNA is a single guide (sgRNA).

48. The method or composition of claim 47, wherein the sgRNA comprises a guide sequence that has the pattern of SEQ ID NO: 3.

49. The method or composition of claim 47, wherein the sgRNA comprises the sequence of SEQ ID NO: 3.

50. The method or composition of claim 48 or 49, wherein each N in SEQ ID NO: 3 is any natural or non-natural nucleotide, wherein the N's form the guide sequence, and the guide sequence targets Cas9 to the *TTR* gene.

51. The method or composition of any one of claims 47-50, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-82 and the nucleotides of SEQ ID NO: 126.

52. The method or composition of any one of claims 47-51, wherein the sgRNA comprises a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID Nos: 87-124.

53. The method or composition of claim 47, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 87-124.

54. The method or composition of any one of the preceding claims, wherein the guide RNA comprises at least one modification.

55. The method or composition of claim 54, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

56. The method or composition of claim 54 or 55, wherein the at least one modification includes a phosphorothioate (PS) bond between nucleotides.

57. The method or composition of any one of claims 54-56, wherein the at least one modification includes a 2'-fluoro (2'-F) modified nucleotide.

58. The method or composition of any one of claims 54-57, wherein the at least one modification includes a modification at one or more of the first five nucleotides at the 5' end.

59. The method or composition of any one of claims 54-58, wherein the at least one modification includes a modification at one or more of the last five nucleotides at the 3' end.

60. The method or composition of any one of claims 54-59, wherein the at least one modification includes PS bonds between the first four nucleotides.

61. The method or composition of any one of claims 54-60, wherein the at least one modification includes PS bonds between the last four nucleotides.

62. The method or composition of any one of claims 54-61, wherein the at least one modification includes 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end.

63. The method or composition of any one of claims 54-62, wherein the at least one modification includes 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

64. The method or composition of any one of claims 54-63, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.

65. The method or composition of any one of claims 1-64, wherein the composition further comprises a pharmaceutically acceptable excipient.

66. The method or composition of any one of claims 1-65, wherein the guide RNA is associated with a lipid nanoparticle (LNP).

67. The method or composition of claim 66, wherein the LNP comprises a CCD lipid.

68. The method or composition of claim 67, wherein the CCD lipid is Lipid A or Lipid B.

69. The method or composition of claim 66-68, wherein the LNP comprises a neutral lipid.

70. The method or composition of claim 69, wherein the neutral lipid is DSPC

71. The method or composition of any one of claims 66-70, wherein the LNP comprises a helper lipid.

72. The method or composition of claim 71, wherein the helper lipid is cholesterol.

73. The method or composition of any one of claims 66-72, wherein the LNP comprises a stealth lipid.

74. The method or composition of claim 73, wherein the stealth lipid is PEG2k-DMG.

75. The method or composition of any one of the preceding claims, wherein the composition further comprises an RNA-guided DNA binding agent.

76. The method or composition of any one of the preceding claims, wherein the composition further comprises an mRNA that encodes an RNA-guided DNA binding agent.

77. The method or composition of claim 75 or 76, wherein the RNA-guided DNA binding agent is a Cas cleavase.

78. The method or composition of claim 77, wherein the RNA-guided DNA binding agent is Cas9.

79. The method or composition of any one of claims 75-78, wherein the RNA-guided DNA binding agent is modified.

80. The method or composition of any one of claims 75-79, wherein the RNA-guided DNA binding agent is a nickase.

81. The method or composition of claim 79 or 80, wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).

82. The method or composition of any one of claims 75-81, wherein the RNA-guided DNA binding agent is a Cas from a Type-II CRISPR/Cas system.

83. The method or composition of any one of the preceding claims, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.

84. The method or composition for use of any one of claims 1-5 or 8-83, wherein the composition reduces or prevents amyloids or amyloid fibrils comprising TTR.

85. The method or composition for use of claim 84, wherein the amyloids or amyloid fibrils are in the nerves, heart, or gastrointestinal track.

86. The method or composition for use of any one of claims 1-5 or 8-83, wherein non-homologous ending joining (NHEJ) leads to a mutation during repair of a DSB in the *TTR* gene.

87. The method or composition for use of claim 86, wherein NHEJ leads to a deletion or insertion of a nucleotide(s) during repair of a DSB in the *TTR* gene.

88. The method or composition for use of claim 87, wherein the deletion or insertion of a nucleotide(s) induces a frame shift or nonsense mutation in the *TTR* gene.

89. The method or composition for use of claim 87, wherein a frame shift or nonsense mutation is induced in the *TTR* gene of at least 50% of liver cells.

90. The method or composition for use of claim 89, wherein a frame shift or nonsense mutation is induced in the *TTR* gene of 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100% of liver cells.

91. The method or composition for use of any one of claims 87-90, wherein a deletion or insertion of a nucleotide(s) occurs in the *TTR* gene at least 50-fold or more than in off-target sites.

92. The method or composition for use of claim 91, wherein the deletion or insertion of a nucleotide(s) occurs in the *TTR* gene 50-fold to 150-fold, 150-fold to 500-fold, 500-fold to 1500-fold, 1500-fold to 5000-fold, 5000-fold to 15000-fold, 15000-fold to 30000-fold, or 30000-fold to 60000-fold more than in off-target sites.

93. The method or composition for use of any one of claims 87-92, wherein the deletion or insertion of a nucleotide(s) occurs at less than or equal to 3, 2, 1, or 0 off-target site(s) in primary human hepatocytes, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

94. The method or composition for use of claim 93, wherein the deletion or insertion of a nucleotide(s) occurs at a number of off-target sites in primary human hepatocytes that is less

than the number of off-target sites at which a deletion or insertion of a nucleotide(s) occurs in Cas9-overexpressing cells, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

95. The method or composition for use of claim 94, wherein the Cas9-overexpressing cells are HEK293 cells stably expressing Cas9.

96. The method or composition for use of any one of claims 93-95, wherein the number of off-target sites in primary human hepatocytes is determined by analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA and the guide RNA, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

97. The method or composition for use of any one of claims 93-95, wherein the number of off-target sites in primary human hepatocytes is determined by an oligonucleotide insertion assay comprising analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA, the guide RNA, and a donor oligonucleotide, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

98. The method or composition of any one of claims 1-43 or 47-97, wherein the sequence of the guide RNA is:

a) SEQ ID NO: 92 or 104;

b) SEQ ID NO: 87, 89, 96, or 113;

c) SEQ ID NO: 100, 102, 106, 111, or 112; or

d) SEQ ID NO: 88, 90, 91, 93, 94, 95, 97, 101, 103, 108, or 109,

optionally wherein the guide RNA does not produce indels at off-target site(s) that occur in a protein coding region in the genome of primary human hepatocytes.

99. The method or composition for use of any one of claims 1-5 or 8-98, wherein administering the composition reduces levels of TTR in the subject.

100. The method or composition for use of claim 99, wherein the levels of TTR are reduced by at least 50%.

101. The method or composition for use of claim 100, wherein the levels of TTR are reduced by 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100%.

102. The method or composition for use of claim 100 or 101, wherein the levels of TTR are measured in serum, plasma, blood, cerebral spinal fluid, or sputum.

103. The method or composition for use of claim 100 or 101, wherein the levels of TTR are measured in liver, choroid plexus, and/or retina.

104. The method or composition for use of any one of claims 99-103, wherein the levels of TTR are measured via enzyme-linked immunosorbent assay (ELISA).

105. The method or composition for use of any one of claims 1-5 or 8-104, wherein the subject has ATTR.

106. The method or composition for use of any one of claims 1-5 or 8-105, wherein the subject is human.

107. The method or composition for use of claim 105 or 106, wherein the subject has ATTRwt.

108. The method or composition for use of claim 105 or 106, wherein the subject has hereditary ATTR.

109. The method or composition for use of any one of claims 1-5, 8-106, or 108, wherein the subject has a family history of ATTR.

110. The method or composition for use of any one of claims 1-5, 8-106, or 108-109, wherein the subject has familial amyloid polyneuropathy.

111. The method or composition for use of any one of claims 1-5 or 8-110, wherein the subject has only or predominantly nerve symptoms of ATTR.

112. The method or composition for use of any one of claims 1-5 or 8-110, wherein the subject has familial amyloid cardiomyopathy.

113. The method or composition for use of any one of claims 1-5, 8-109, or 112, wherein the subject has only or predominantly cardiac symptoms of ATTR.

114. The method or composition for use of any one of claims 1-5 or 8-113, wherein the subject expresses TTR having a V30 mutation.

115. The method or composition for use of claim 114, wherein the V30 mutation is V30A, V30G, V30L, or V30M.

116. The method or composition for use of claim any one of claims 1-5 or 8-113, wherein the subject expresses TTR having a T60 mutation.

117. The method or composition for use of claim 116, wherein the T60 mutation is T60A.

118. The method or composition for use of claim any one of claims 1-5 or 8-113, wherein the subject expresses TTR having a V122 mutation.

119. The method or composition for use of claim 118, wherein the V122 mutation is V122A, V122I, or V122(-).

120. The method or composition for use of any one of claims 1-5 or 8-119, wherein the subject expresses wild-type TTR.

121. The method or composition for use of any one of claims 1-5, 8-107, or 120, wherein the subject does not express TTR having a V30, T60, or V122 mutation.

122. The method or composition for use of any one of claims 1-5, 8-107, or 120-121, wherein the subject does not express TTR having a pathological mutation.

123. The method or composition for use of claim 121, wherein the subject is homozygous for wild-type *TTR*.

124. The method or composition for use of any one of claims 1-5 or 8-123, wherein after administration the subject has an improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy.

125. The method or composition for use of claim 124, wherein the improvement, stabilization, or slowing of change in sensory neuropathy is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

126. The method or composition for use of any one of claims 1-5 or 8-125, wherein the subject has an improvement, stabilization, or slowing of change in symptoms of congestive heart failure.

127. The method or composition for use of claim 126, wherein the improvement, stabilization, or slowing of change in congestive heart failure is measured using cardiac biomarker tests, lung function tests, chest x-rays, or electrocardiography.

128. The method or composition for use of any one of claims 1-5 or 8-127, wherein the composition or pharmaceutical formulation is administered via a viral vector.

129. The method or composition for use of any one of claims 1-5 or 8-127, wherein the composition or pharmaceutical formulation is administered via lipid nanoparticles.

130. The method or composition for use of any one of claims 1-5 or 8-129, wherein the subject is tested for specific mutations in the *TTR* gene before administering the composition or formulation.

131. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 5.

132. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 6.

133. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 7.

134. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 8.

135. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 9.

136. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 10.

137. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 11.

138. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 12.

139. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 13.

140. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 14.

141. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 15.

142. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 16.

143. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 17.

144. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 18.

145. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 19.

146. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 20.

147. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 21.

148. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 22.

149. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 23.

150. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 24.

151. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 25.

152. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 26.

153. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 27.

154. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 28.

155. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 29.

156. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 30.

157. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 31.

158. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 32.

159. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 33.

160. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 34.

161. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 35.

162. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 36.

163. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 37.

164. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 38.

165. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 39.

166. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 40.

167. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 41.

168. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 42.

169. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 43.

170. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 44.

171. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 45.

172. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 46.

173. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 47.

174. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 48.

175. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 49.

176. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 50.

177. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 51.

178. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 52.

179. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 53.

180. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 54.

181. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 55.

182. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 56.

183. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 57.

184. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 58.

185. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 59.

186. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 60.

187. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 61.

188. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 62.

189. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 63.

190. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 64.

191. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 65.

192. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 66.

193. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 67.

194. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 68.

195. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 69.

196. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 70.

197. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 71.

198. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 72.

199. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 73.

200. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 74.

201. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 75.

202. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 76.

203. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 77.

204. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 78.

205. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 79.

206. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 80.

207. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 81.

208. The method or composition of any one of claims 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 82.

209. Use of a composition or formulation of any of claims 6-208 for the preparation of a medicament for treating a human subject having ATTR.

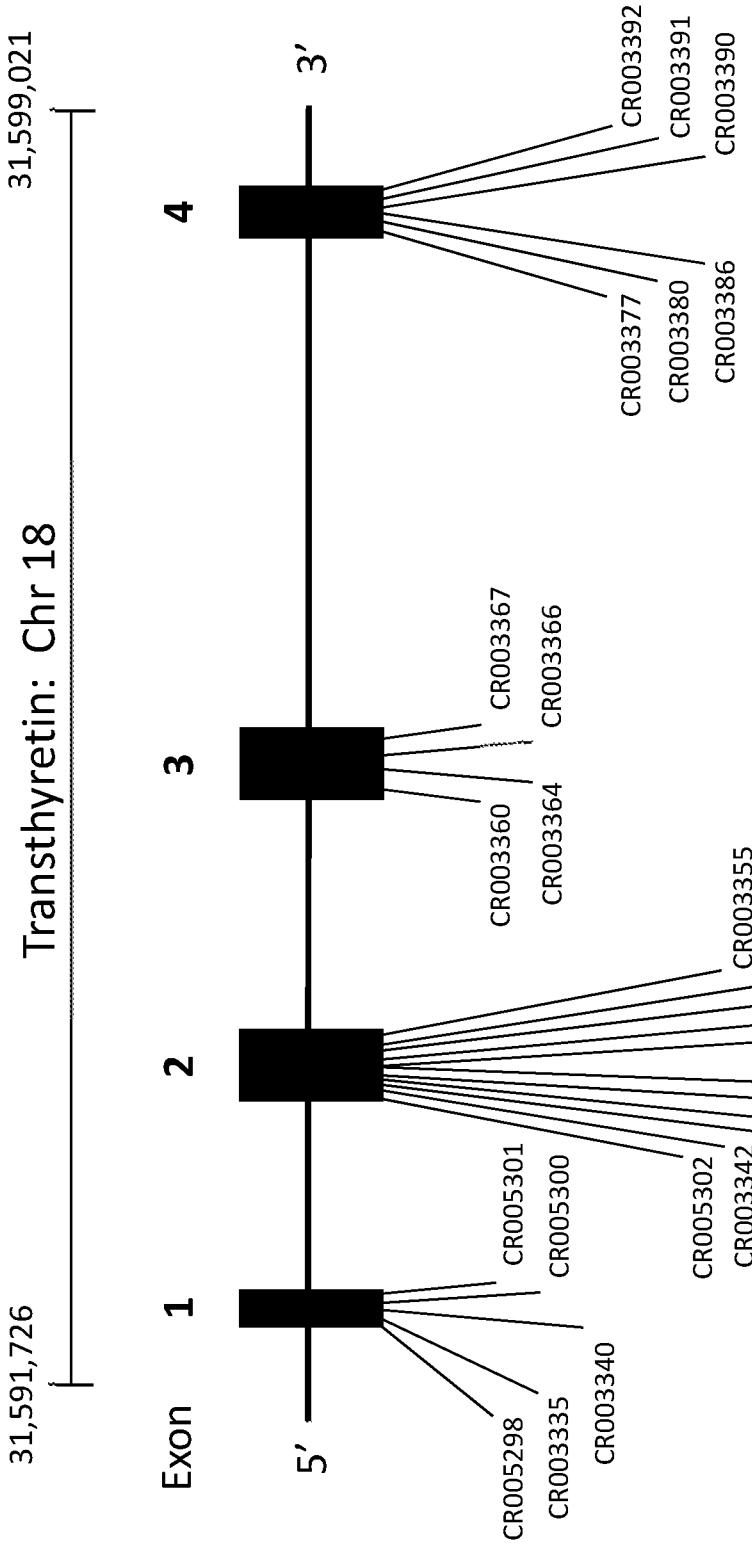

Transthyretin: Chr 18
31,591,726
31,599,021
Exon
1
2
3
4
5'
3'
CR005298
CR003335
CR003340
CR005301
CR005300
CR005302
CR003342
CR005303
CR003344
CR003345
CR003346
CR003347
CR003350
CR003351
CR005304
CR003355
CR003360
CR003364
CR003367
CR003366
CR003377
CR003380
CR003386
CR003392
CR003391
CR003390

FIG. 1

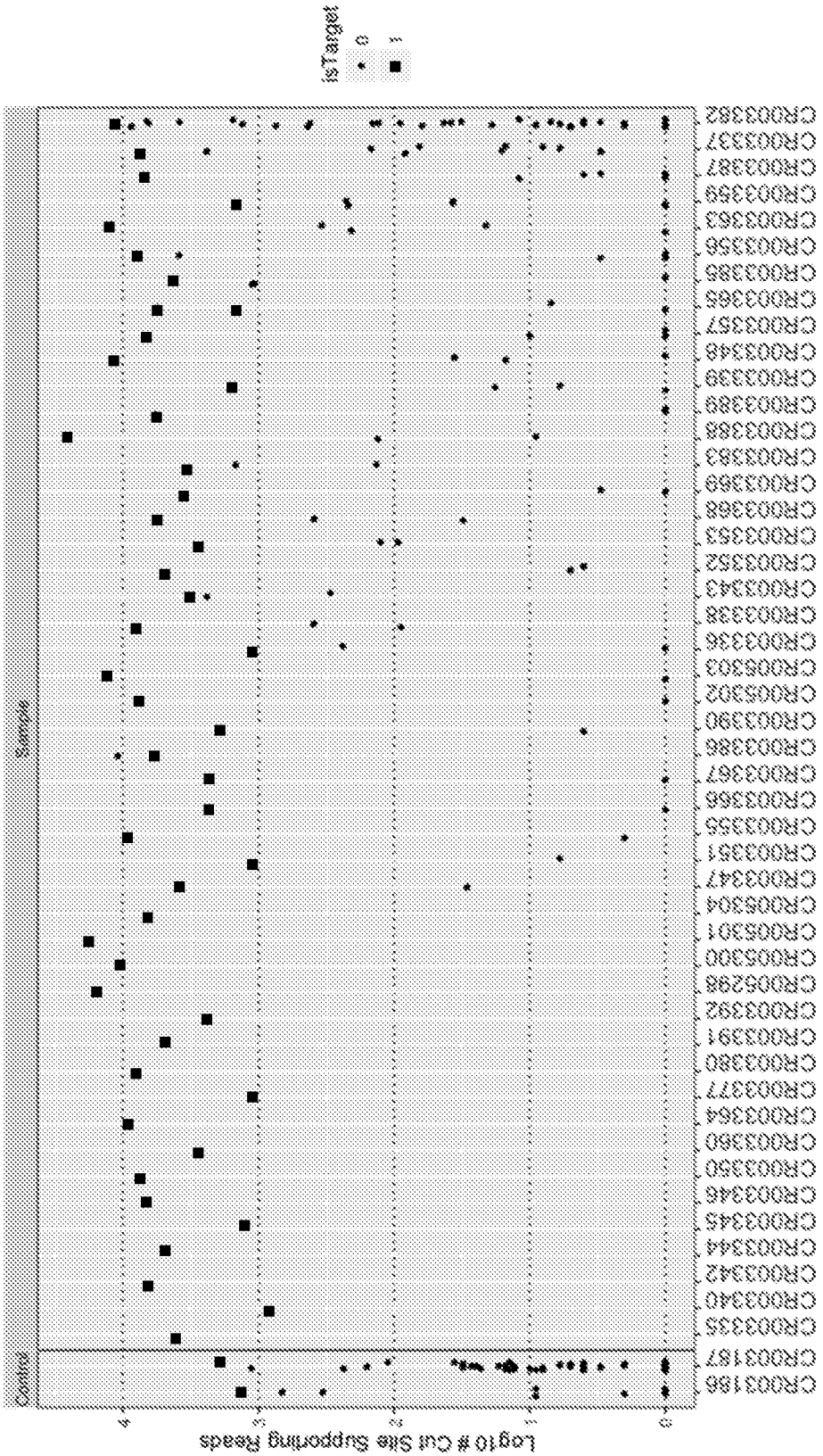

isTarget
0
1
Control
Sample
Guide Name
CR003156
CR003187
CR003335
CR003340
CR003342
CR003344
CR003345
CR003346
CR003350
CR003360
CR003364
CR003377
CR003380
CR003391
CR003392
CR005298
CR005300
CR005301
CR005304
CR003347
CR003351
CR003355
CR003366
CR003367
CR003386
CR003390
CR005302
CR005303
CR003336
CR003338
CR003343
CR003352
CR003353
CR003368
CR003369
CR003383
CR003388
CR003389
CR003339
CR003348
CR003357
CR003365
CR003385
CR003356
CR003363
CR003359
CR003387
CR003337
CR003382
4
3
2
1
0
Log10 # Cut Site Supporting Reads

FIG. 2

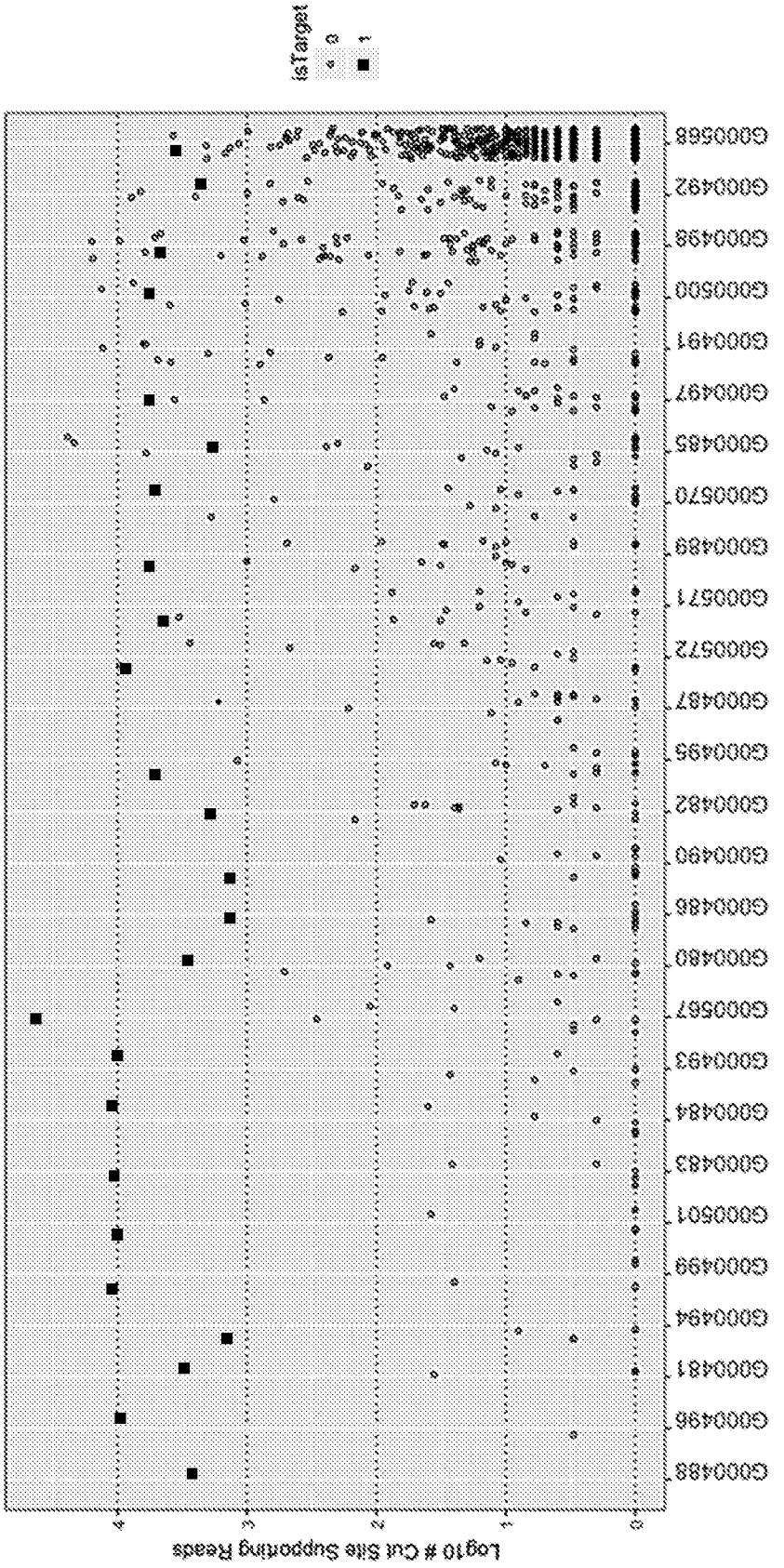

isTarget
0
1
log10 # Cut Site Supporting Reads
4
3
2
1
0
G000488
G000496
G000481
G000494
G000499
G000501
G000483
G000484
G000493
G000567
G000480
G000486
G000490
G000482
G000495
G000487
G000572
G000571
G000489
G000570
G000485
G000497
G000491
G000500
G000498
G000492
G000588

FIG. 3

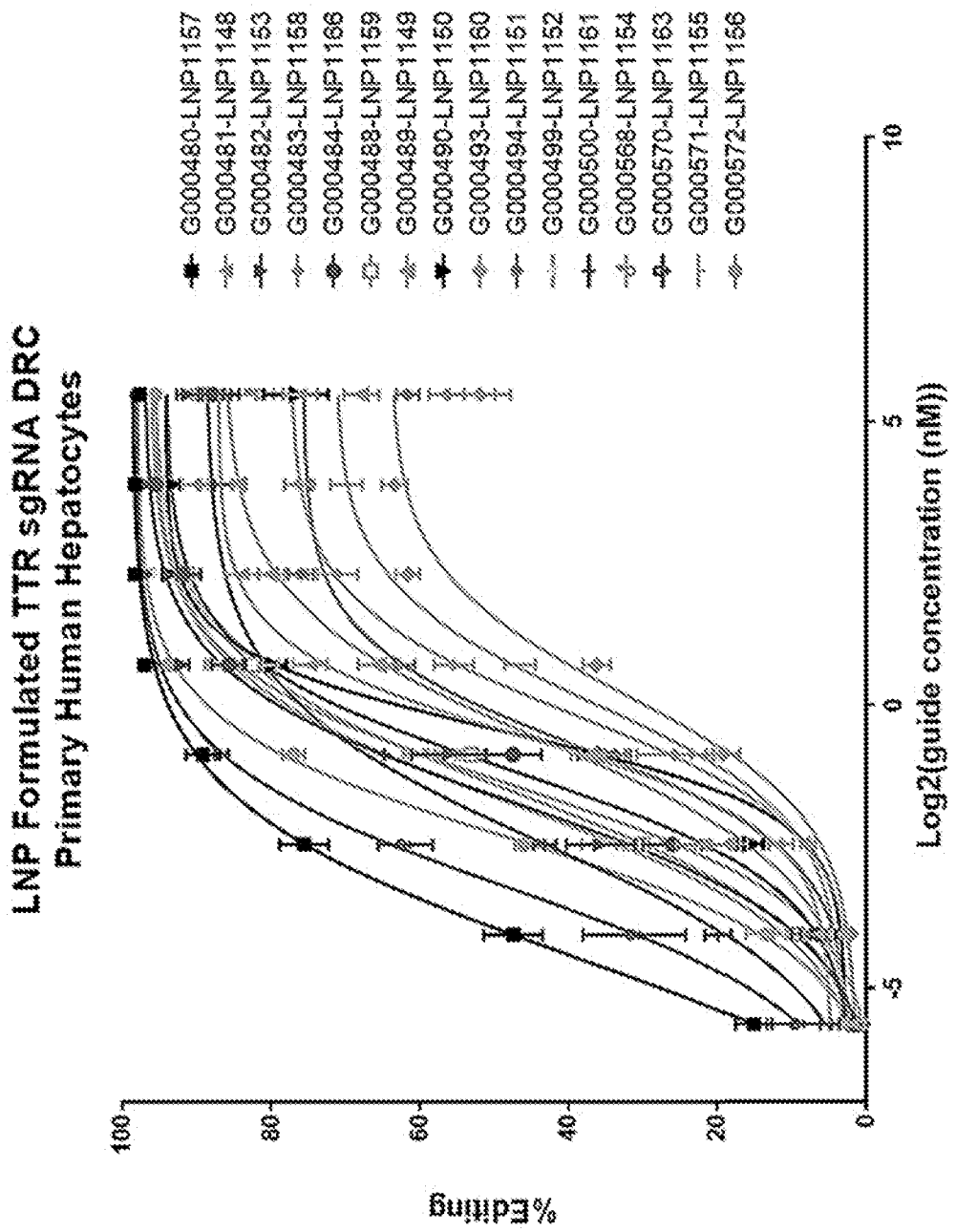

LNP Formulated TTR sgRNA DRC
Primary Human Hepatocytes
G000480-LNP1157
G000481-LNP1148
G000482-LNP1153
G000483-LNP1158
G000484-LNP1166
G000488-LNP1159
G000489-LNP1149
G000490-LNP1150
G000493-LNP1160
G000494-LNP1151
G000499-LNP1152
G000500-LNP1161
G000568-LNP1154
G000570-LNP1163
G000571-LNP1155
G000572-LNP1156
%Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 4

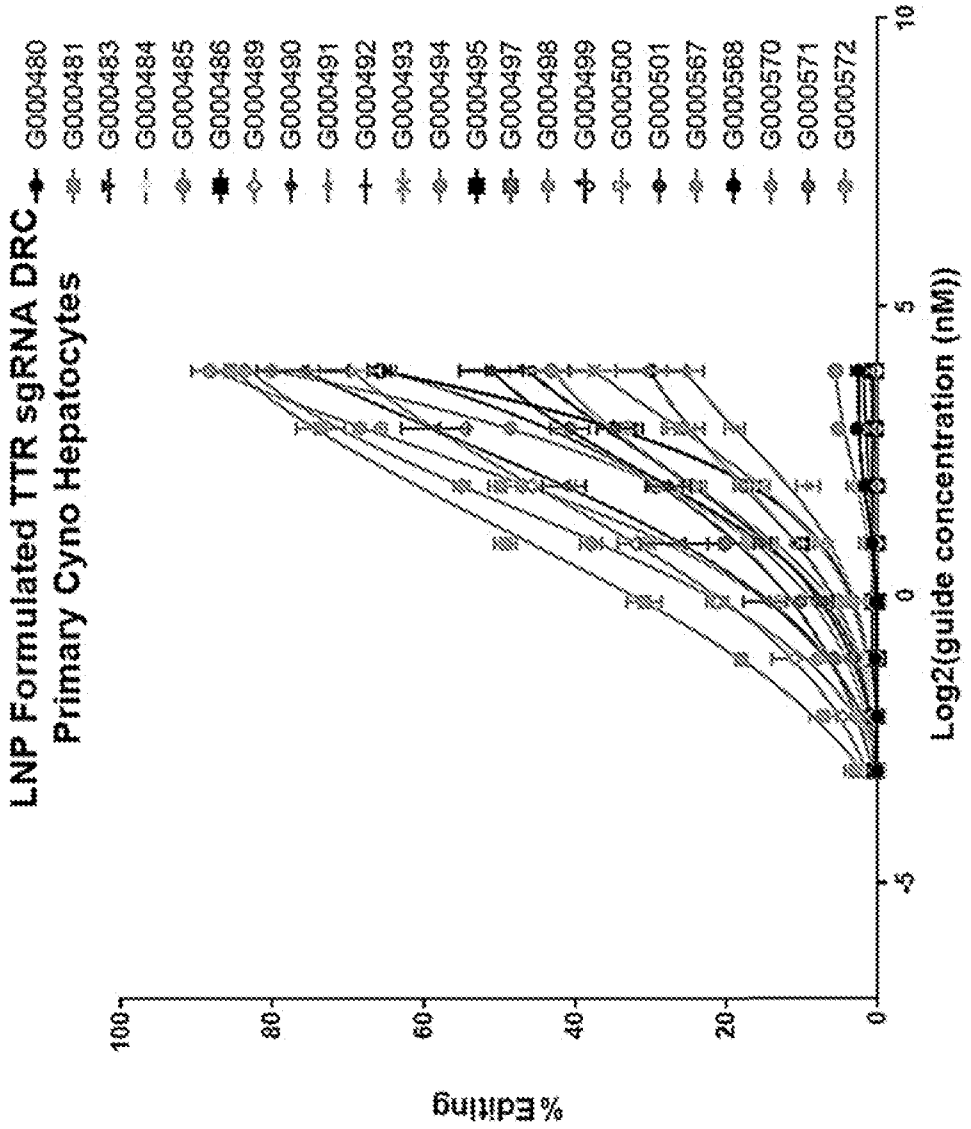

LNP Formulated TTR sgRNA DRC
Primary Cyno Hepatocytes
G000480
G000481
G000483
G000484
G000485
G000486
G000489
G000490
G000491
G000492
G000493
G000494
G000495
G000497
G000498
G000499
G000500
G000501
G000567
G000568
G000570
G000571
G000572
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 5

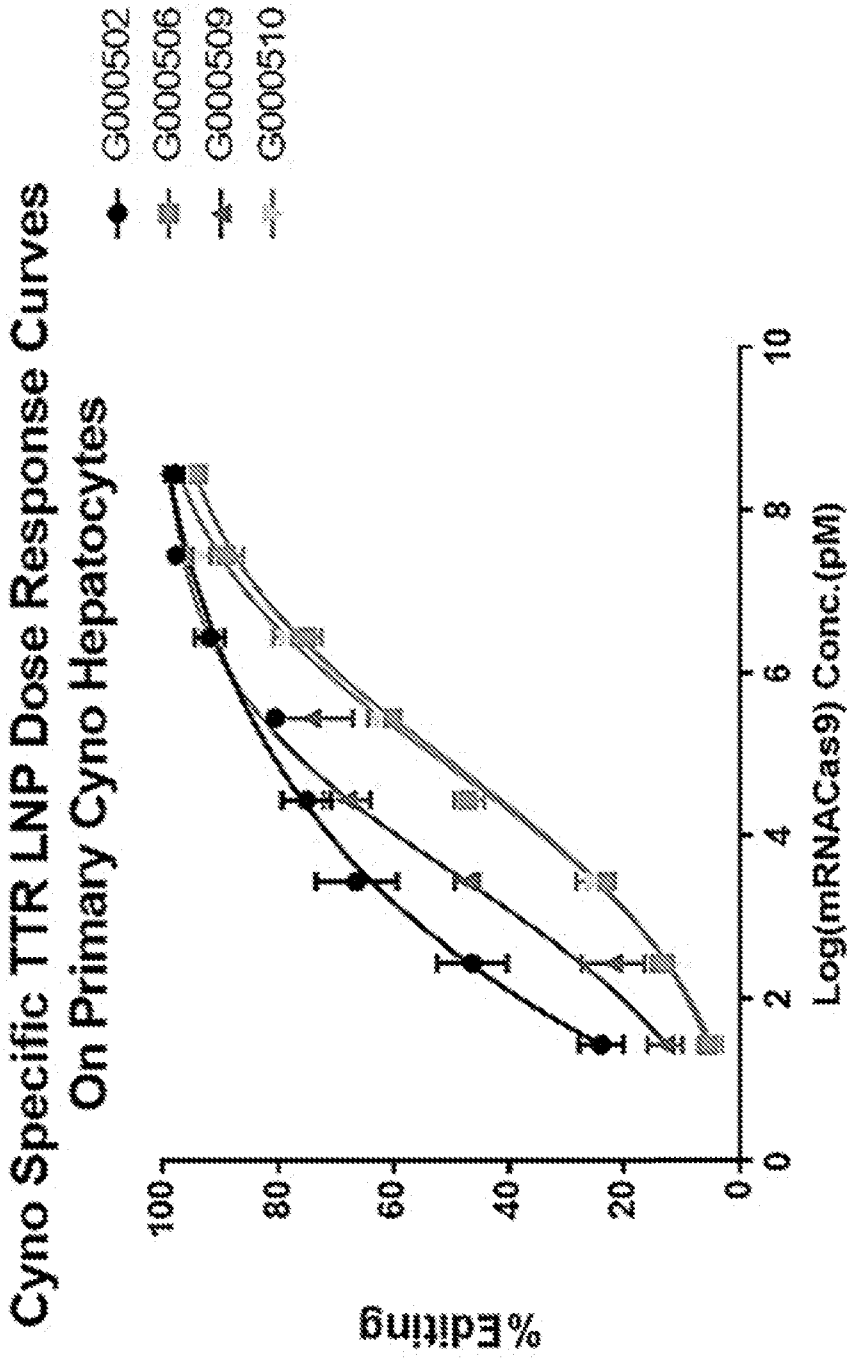

Cyno Specific TTR LNP Dose Response Curves
On Primary Cyno Hepatocytes
G000502
G000506
G000509
G000510
%Editing
100
80
60
40
20
0
0
2
4
6
8
10
Log(mRNACas9) Conc.(pM)

FIG. 6

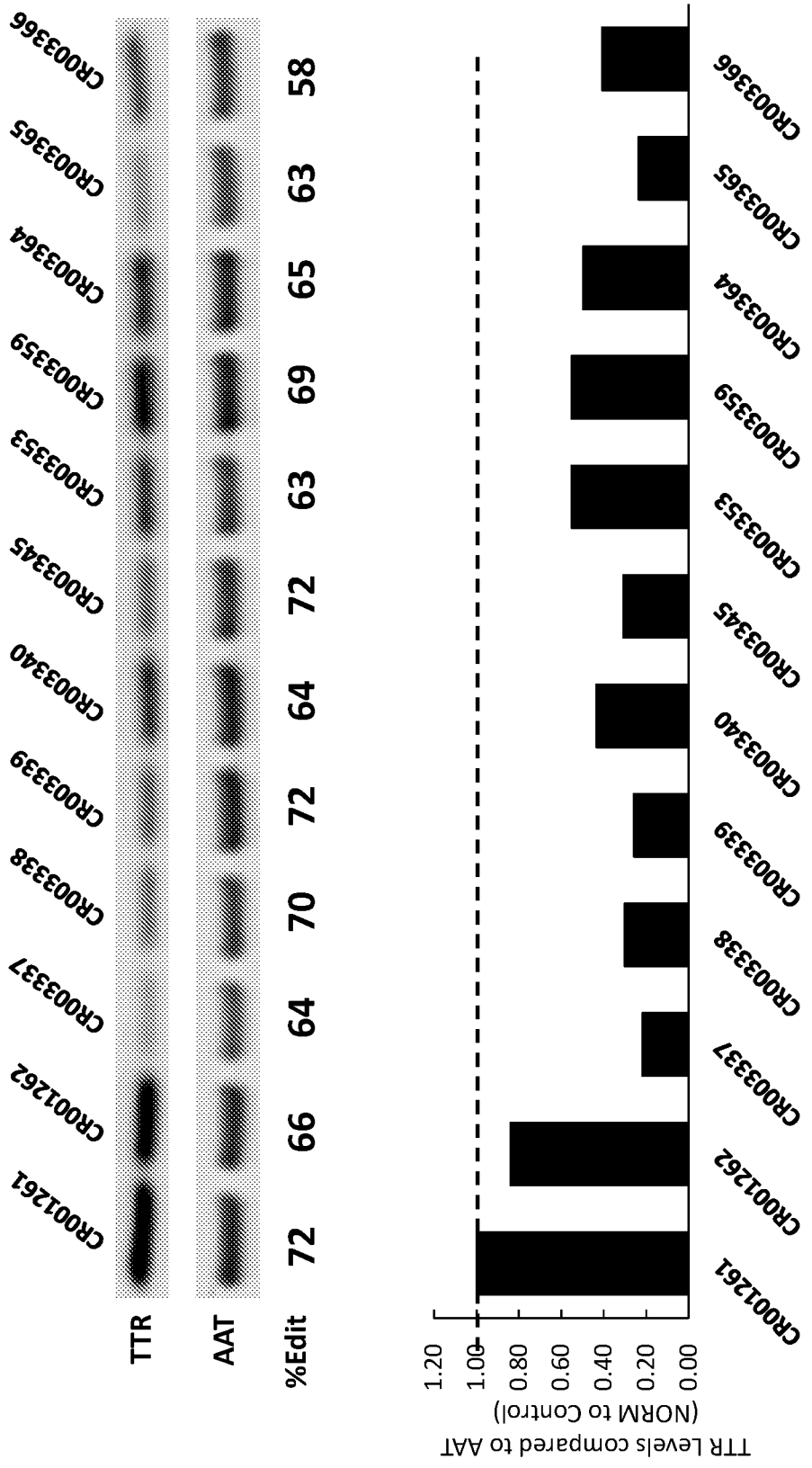

TTR
AAT
%Edit
72 66 64 64 70 72 64 72 63 69 65 63 58
CR001261
CR001262
CR003337
CR003338
CR003339
CR003340
CR003345
CR003353
CR003359
CR003364
CR003365
CR003366
TTR Levels compared to AAT
(NORM to Control)
1.20
1.00
0.80
0.60
0.40
0.20
0.00

FIG. 7

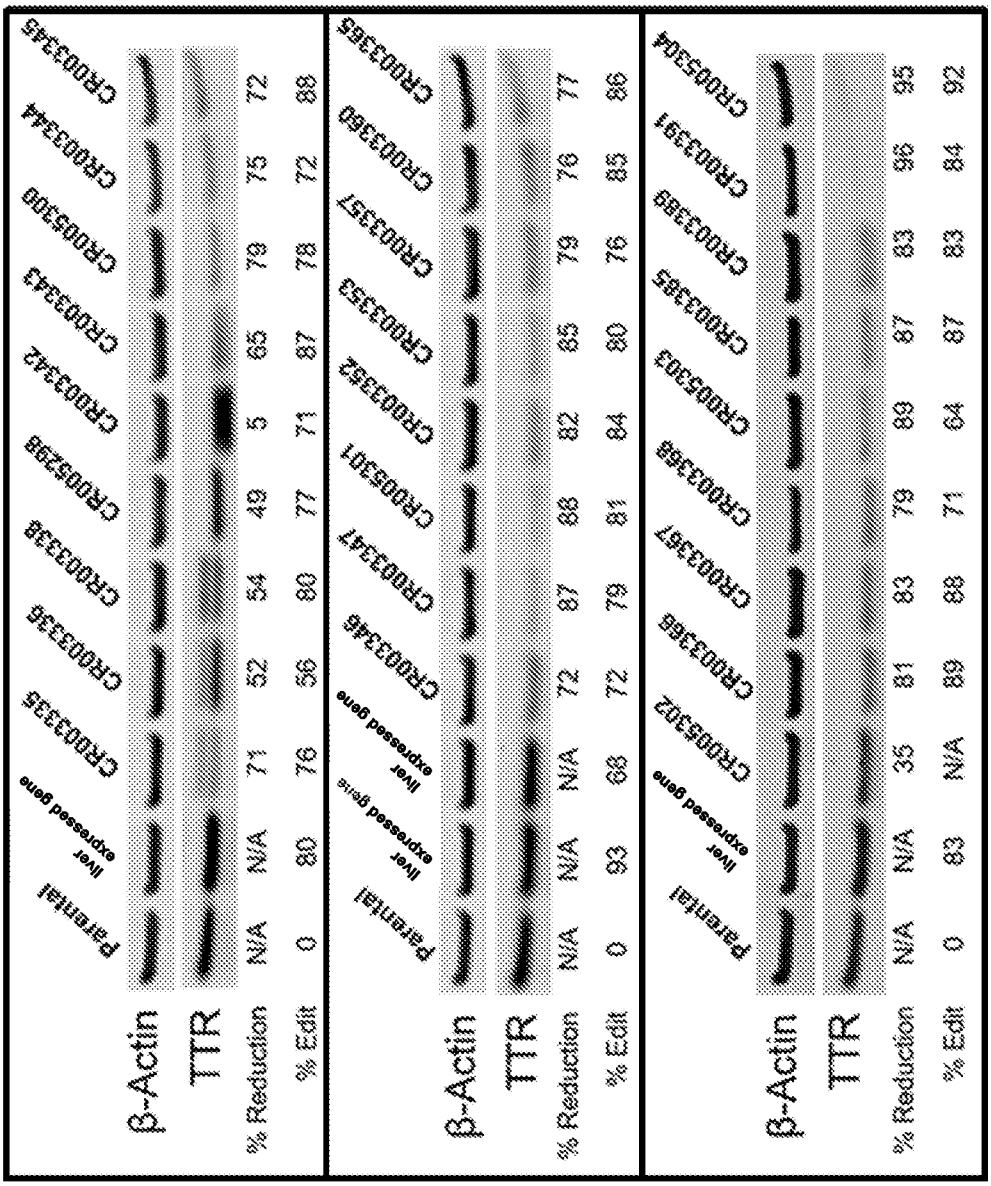

β-Actin
TTR
% Reduction
% Edit
Parental
liver expressed gene
CR003335
CR003336
CR003338
CR005298
CR003342
CR003343
CR005300
CR003344
CR003345
N/A 71 52 54 49 5 65 79 75 72
0 80 76 80 77 71 87 78 72 88
liver expressed gene
CR003346
CR003347
CR005301
CR003352
CR003353
CR003357
CR003360
CR003365
N/A N/A 72 87 88 82 85 79 76 77
0 93 68 72 79 81 84 80 76 85 86
liver expressed gene
CR005302
CR003366
CR003367
CR003368
CR005303
CR003385
CR003389
CR003391
CR005304
N/A 35 81 83 79 89 87 83 96 95
0 83 N/A 89 88 71 64 87 83 84 92

FIG. 8

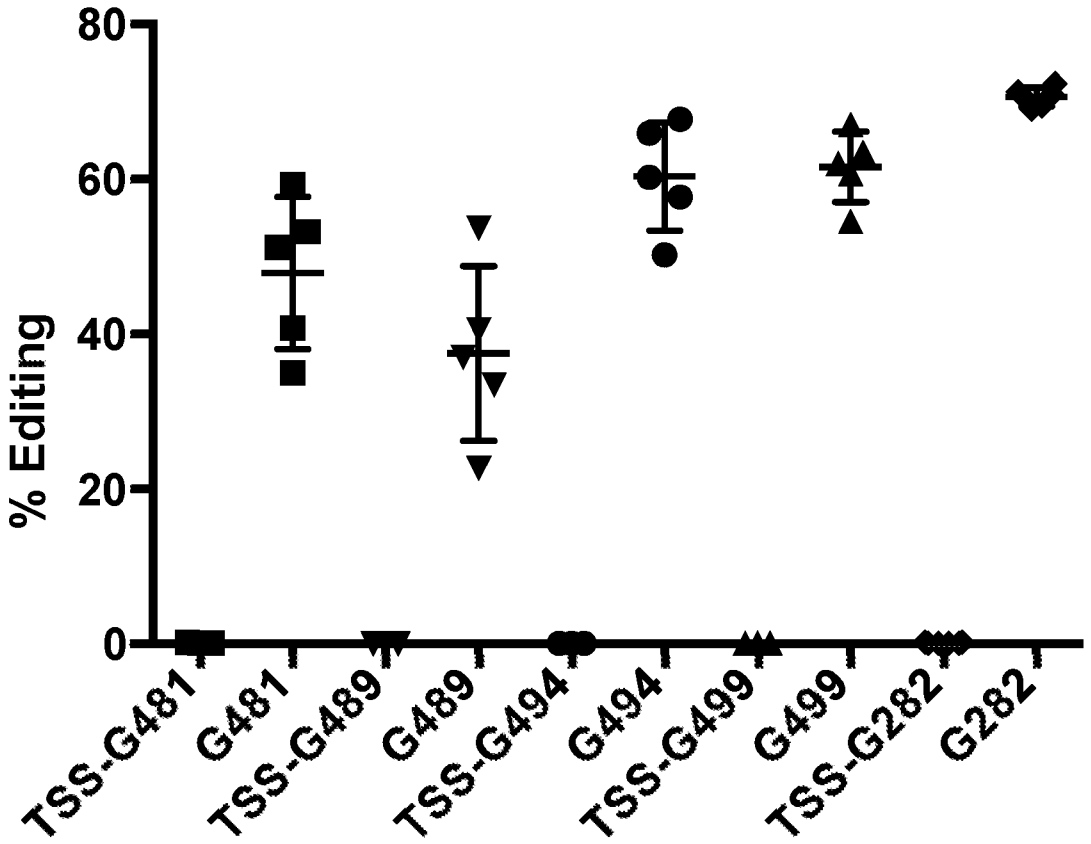

80
60
40
20
0
% Editing
TSS-G481
G481
TSS-G489
G489
TSS-G494
G494
TSS-G499
G499
TSS-G282
G282

FIG. 9

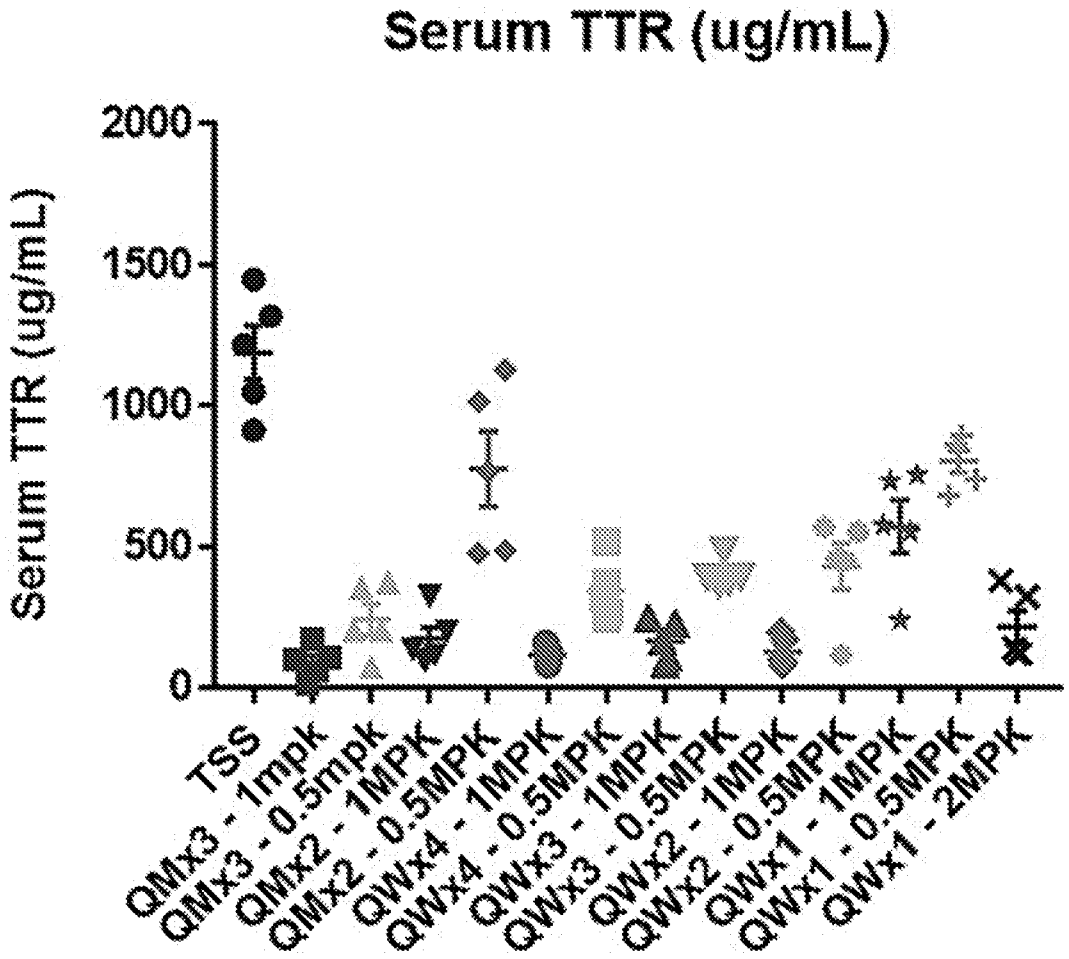

Serum TTR (ug/mL)
Serum TTR (ug/mL)
2000
1500
1000
500
0
TSS
QMx3 - 1mpk
QMx3 - 0.5mpk
QMx2 - 1MPK
QMx2 - 0.5MPK
QWx4 - 1MPK
QWx4 - 0.5MPK
QWx3 - 1MPK
QWx3 - 0.5MPK
QWx2 - 1MPK
QWx2 - 0.5MPK
QWx1 - 1MPK
QWx1 - 0.5MPK
QWx1 - 2MPK

FIG. 10A

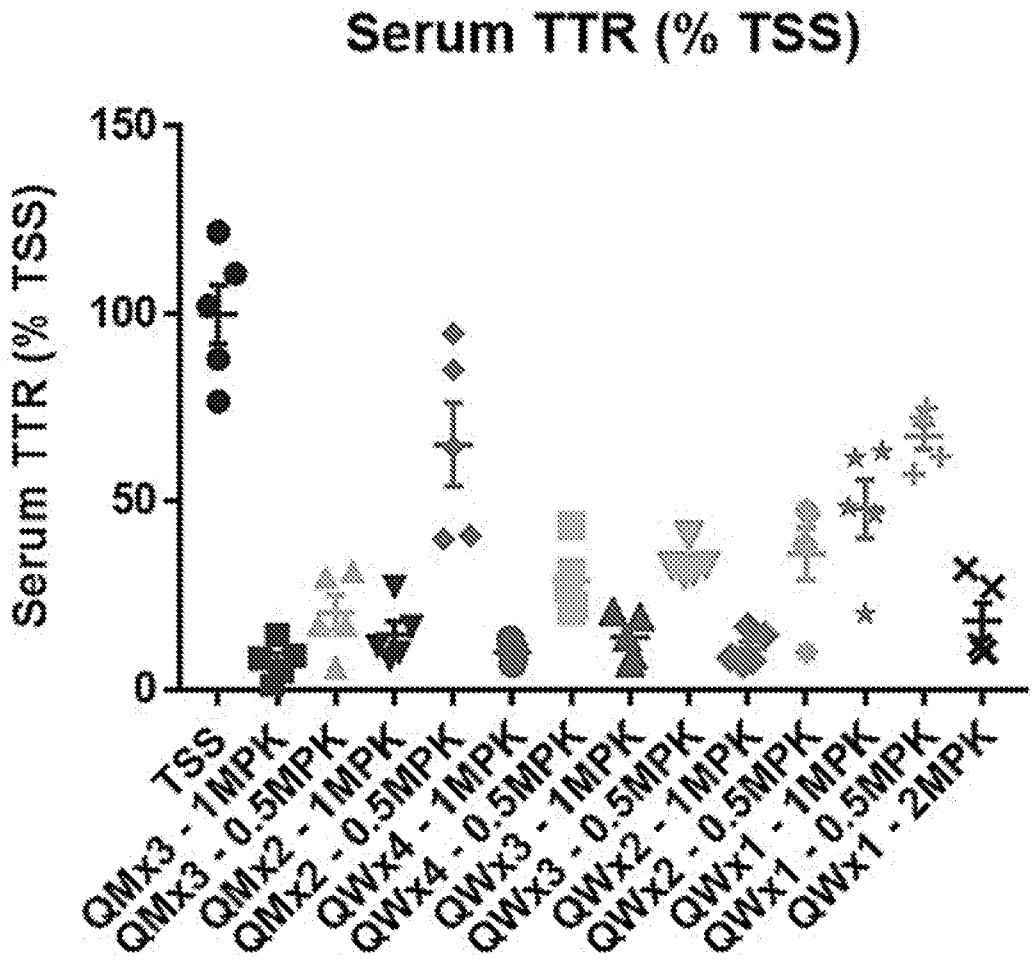

Serum TTR (% TSS)
Serum TTR (% TSS)
150
100
50
0
TSS
QMx3 - 1MPK
QMx3 - 0.5MPK
QMx2 - 1MPK
QMx2 - 0.5MPK
QWx4 - 1MPK
QWx4 - 0.5MPK
QWx3 - 1MPK
QWx3 - 0.5MPK
QWx2 - 1MPK
QWx2 - 0.5MPK
QWx1 - 1MPK
QWx1 - 0.5MPK
QWx1 - 2MPK

FIG. 10B

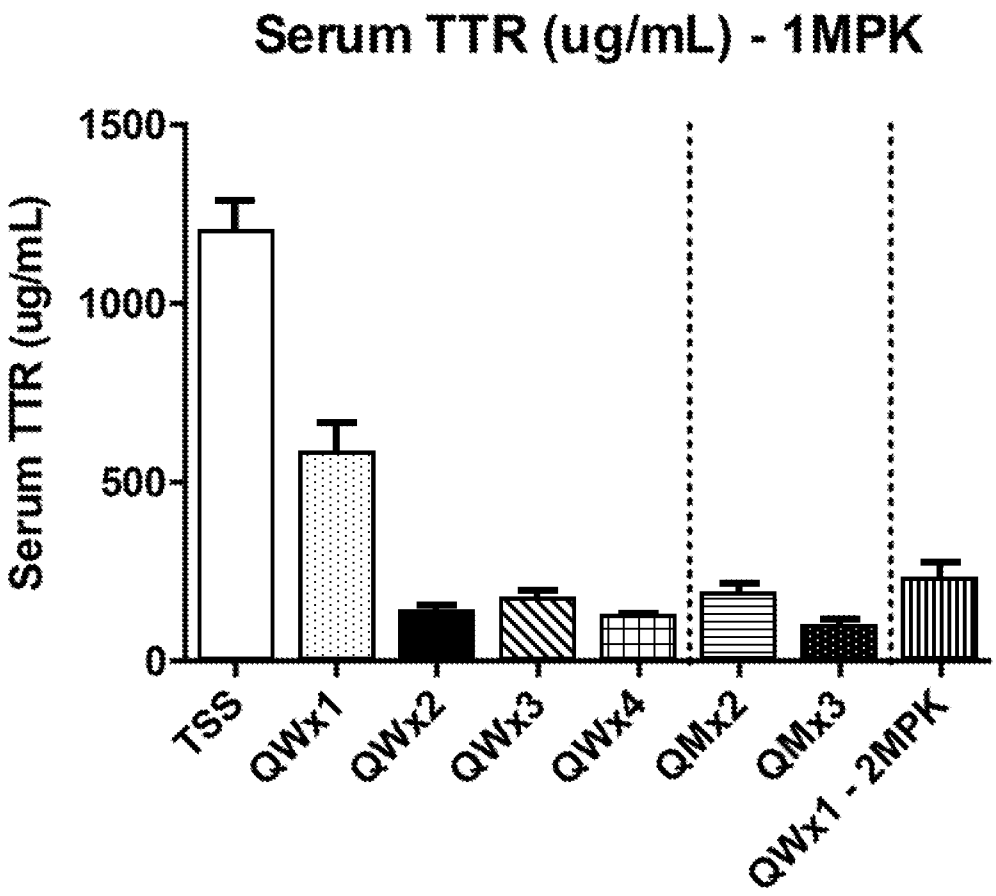

Serum TTR (ug/mL) - 1MPK
Serum TTR (ug/mL)
1500
1000
500
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 11A

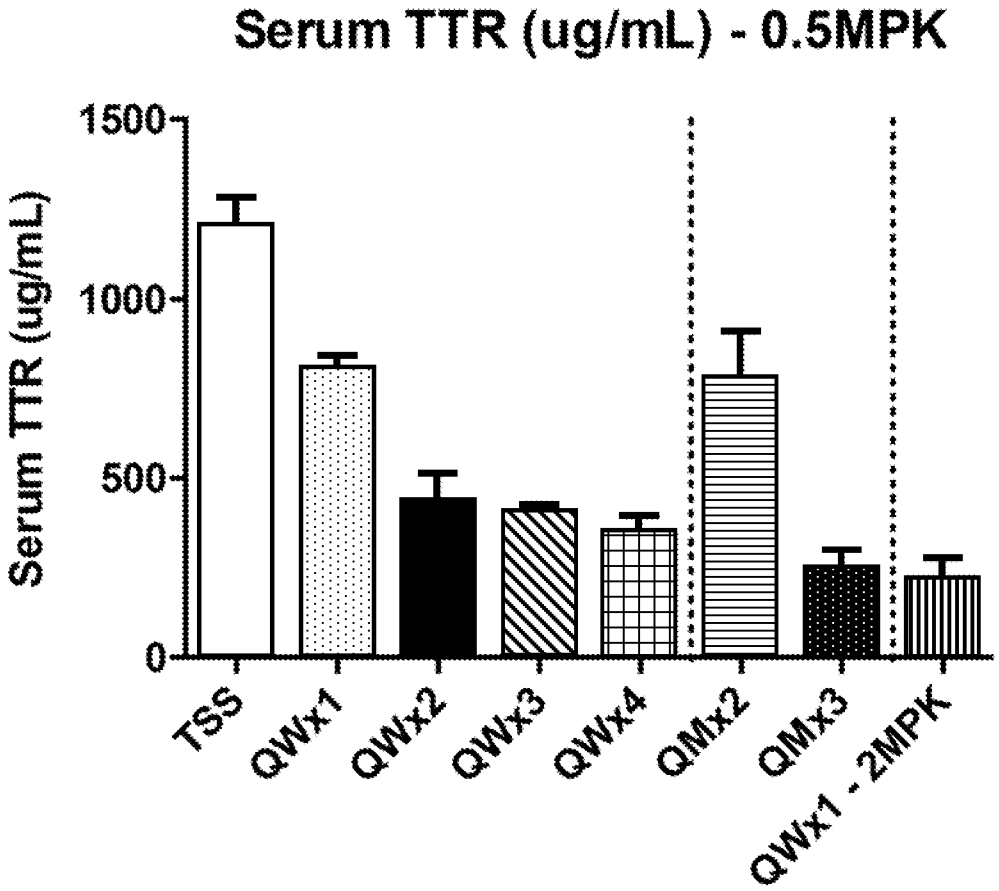

Serum TTR (ug/mL) - 0.5MPK
Serum TTR (ug/mL)
1500
1000
500
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 11B

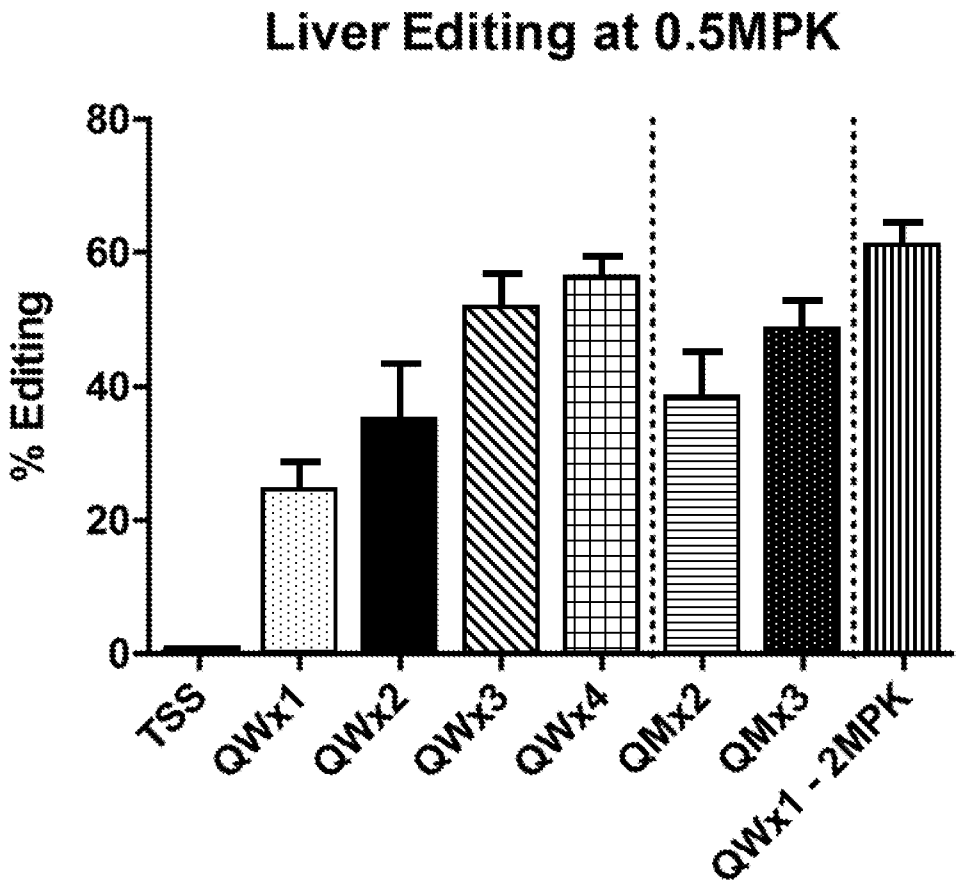

Liver Editing at 0.5MPK
% Editing
80
60
40
20
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 12A

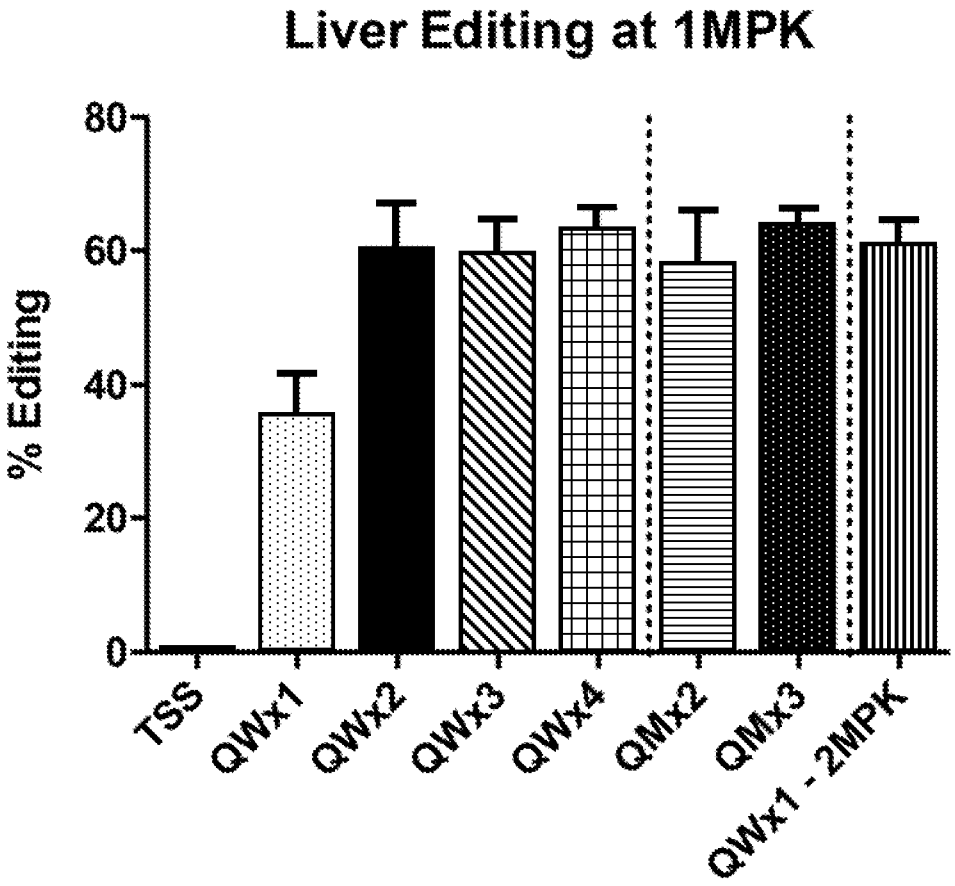

Liver Editing at 1MPK
% Editing
80
60
40
20
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 12B

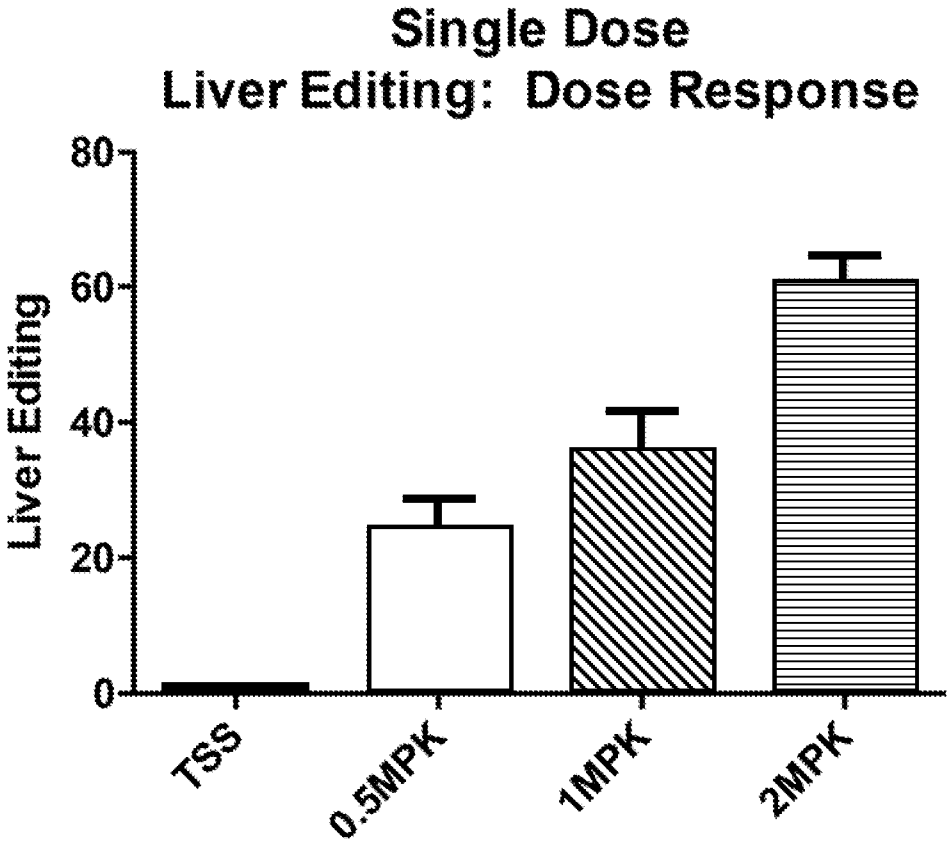

Single Dose
Liver Editing: Dose Response
Liver Editing
80
60
40
20
0
TSS
0.5MPK
1MPK
2MPK

FIG. 12C

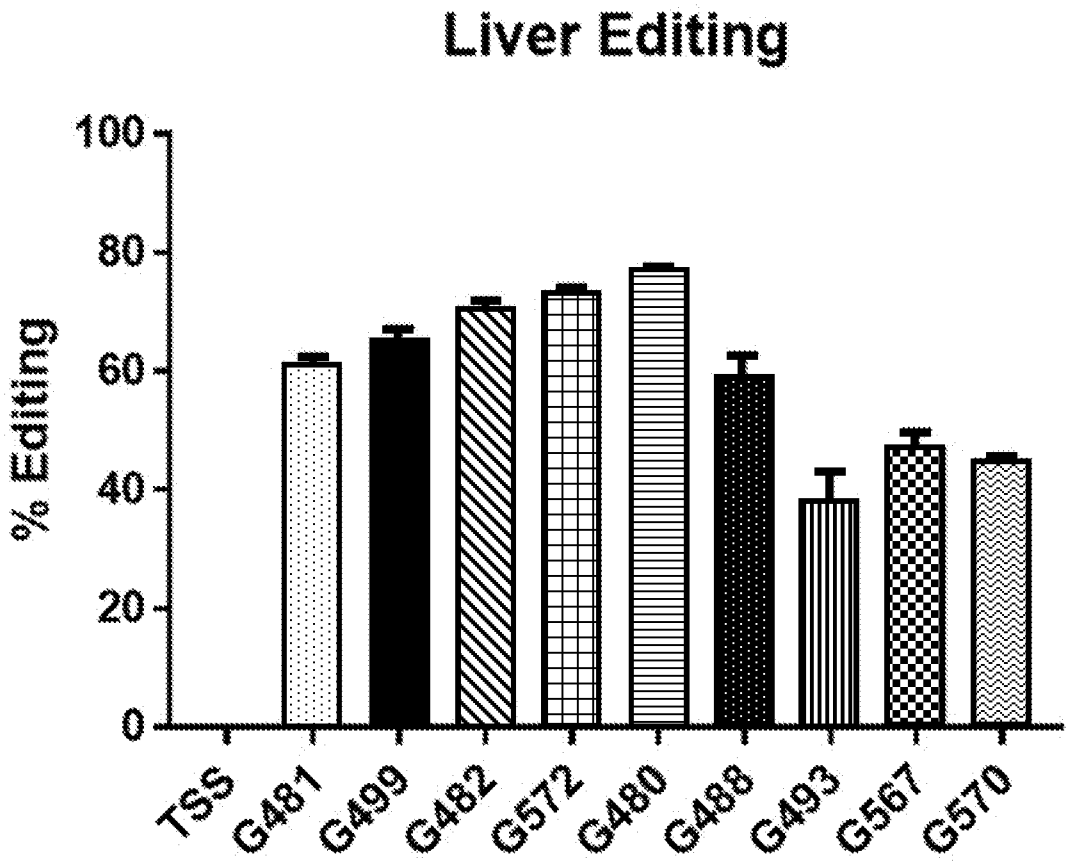

Liver Editing
% Editing
100
80
60
40
20
0
TSS
G481
G499
G482
G572
G480
G488
G493
G567
G570

FIG. 13

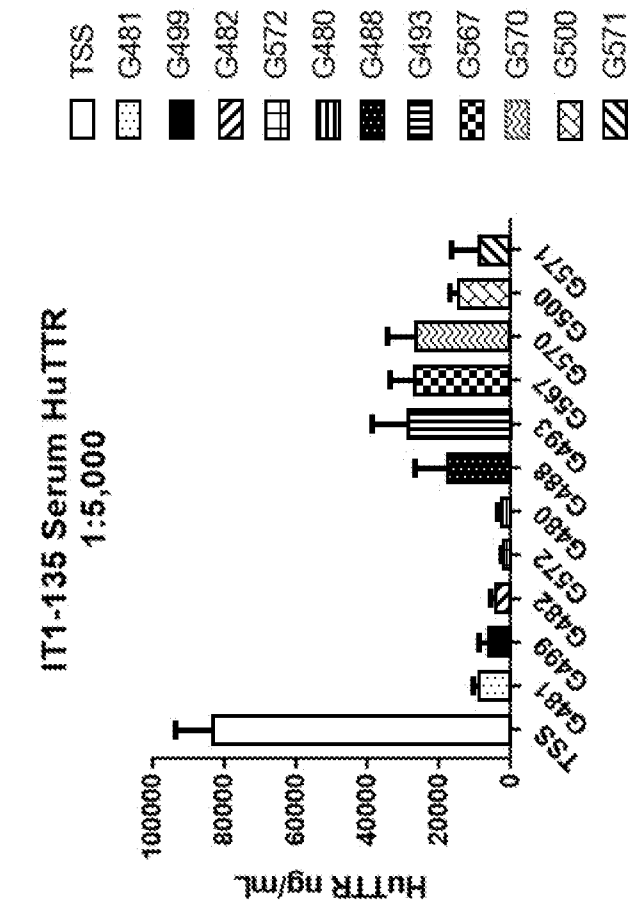


FIG. 14B

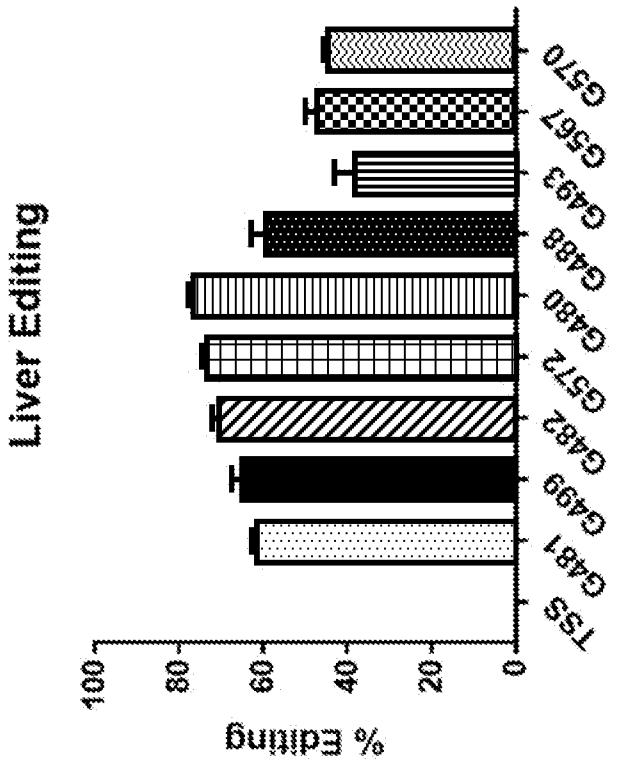


FIG. 14A

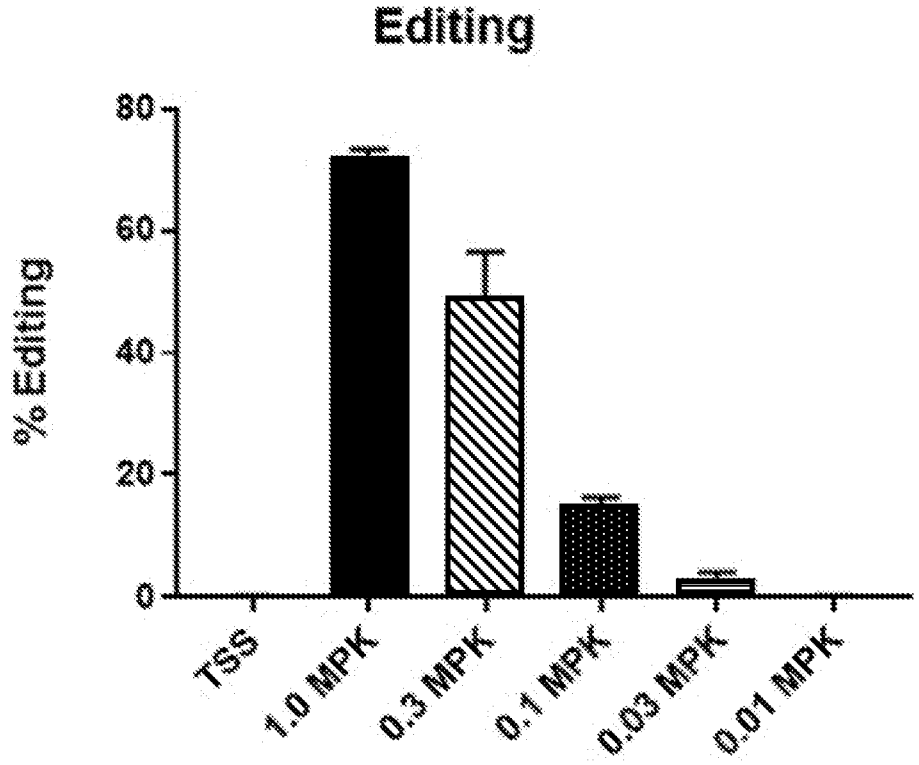

Editing
% Editing
80
60
40
20
0
TSS
1.0 MPK
0.3 MPK
0.1 MPK
0.03 MPK
0.01 MPK

FIG. 15A

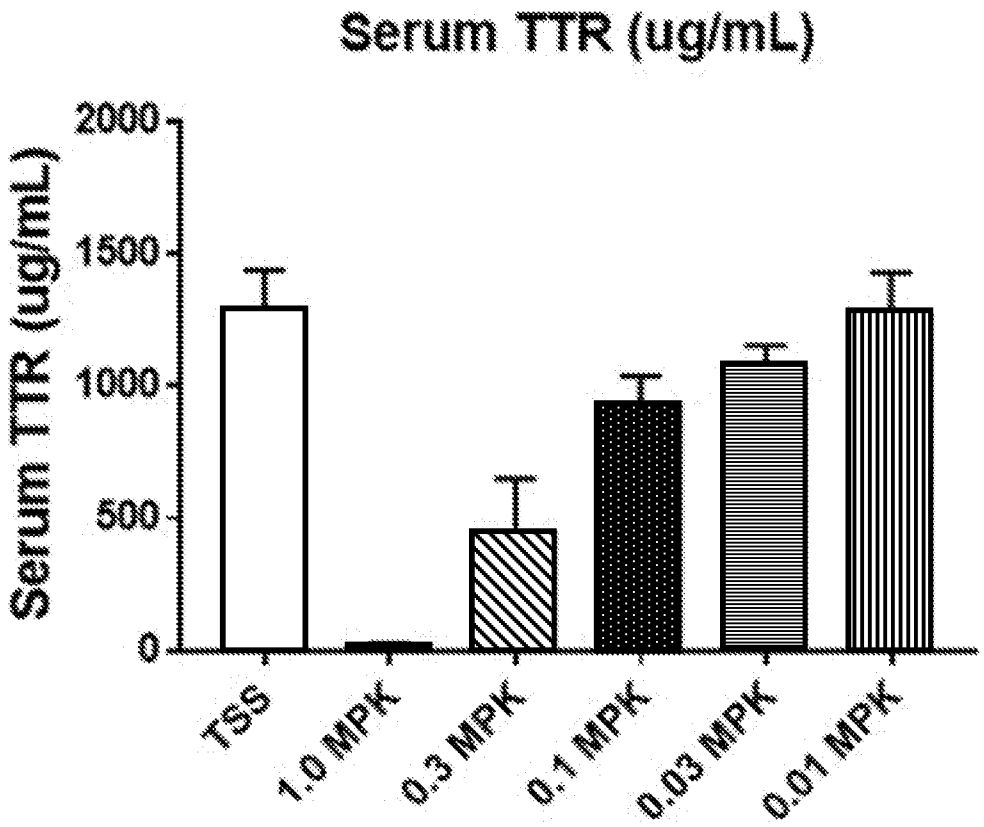

Serum TTR (ug/mL)
Serum TTR (ug/mL)
2000
1500
1000
500
0
TSS
1.0 MPK
0.3 MPK
0.1 MPK
0.03 MPK
0.01 MPK

FIG. 15B

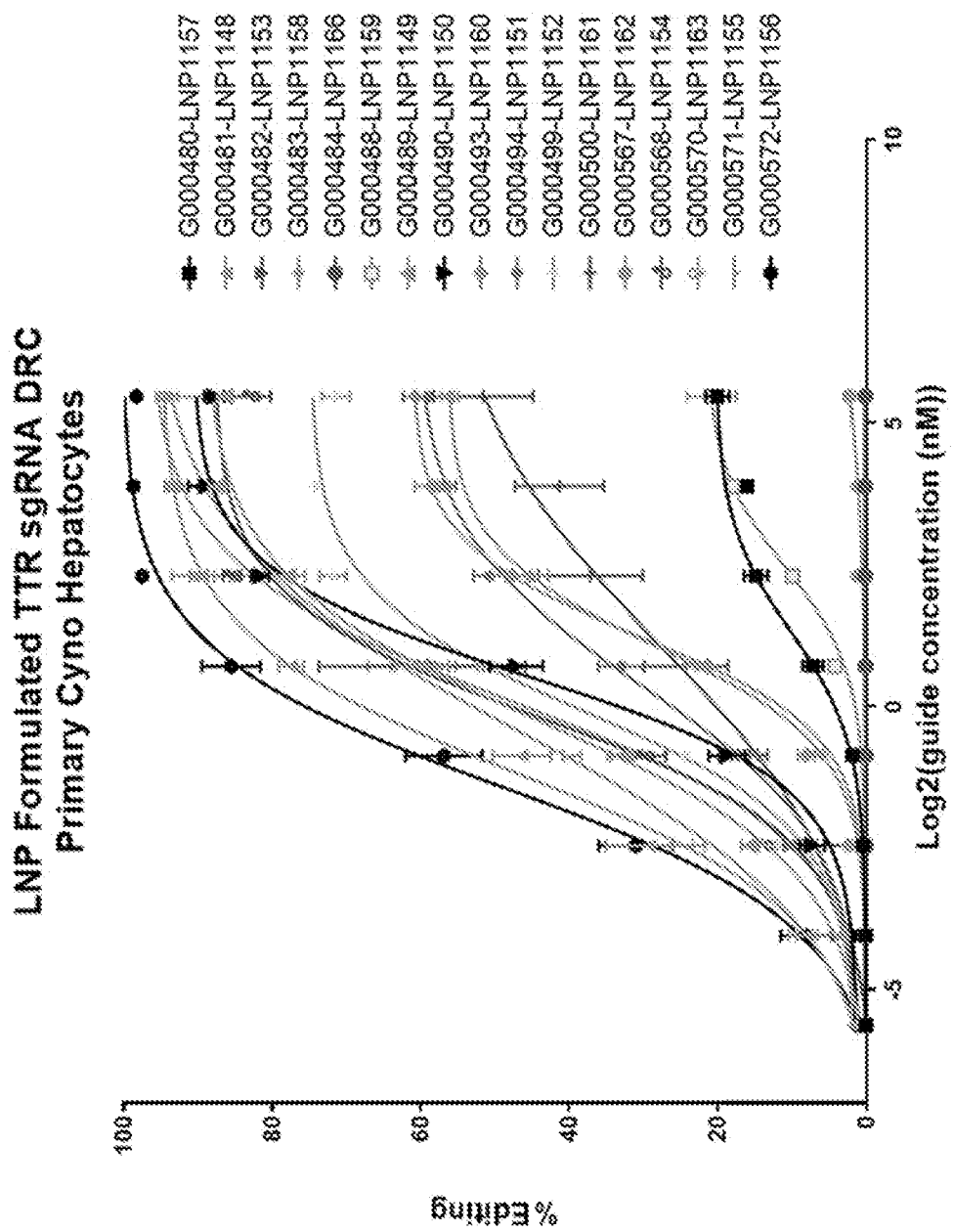

LNP Formulated TTR sgRNA DRC
Primary Cyno Hepatocytes
G000480-LNP1157
G000481-LNP1148
G000482-LNP1153
G000483-LNP1158
G000484-LNP1166
G000488-LNP1159
G000489-LNP1149
G000490-LNP1150
G000493-LNP1160
G000494-LNP1151
G000499-LNP1152
G000500-LNP1161
G000567-LNP1162
G000568-LNP1154
G000570-LNP1163
G000571-LNP1155
G000572-LNP1156
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 16

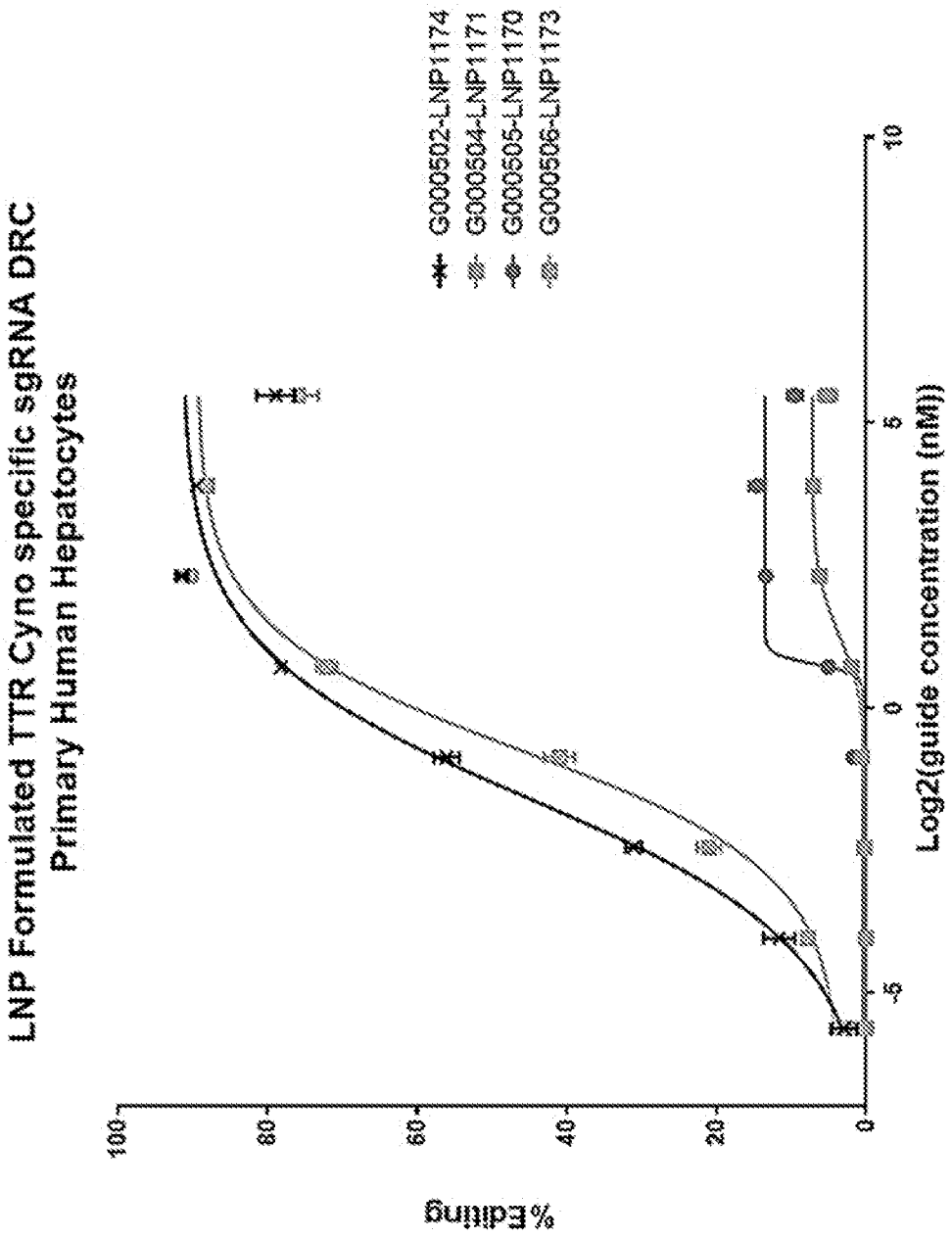

LNP Formulated TTR Cyno specific sgRNA DRC
Primary Human Hepatocytes
G000502-LNP1174
G000504-LNP1171
G000505-LNP1170
G000506-LNP1173
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 17

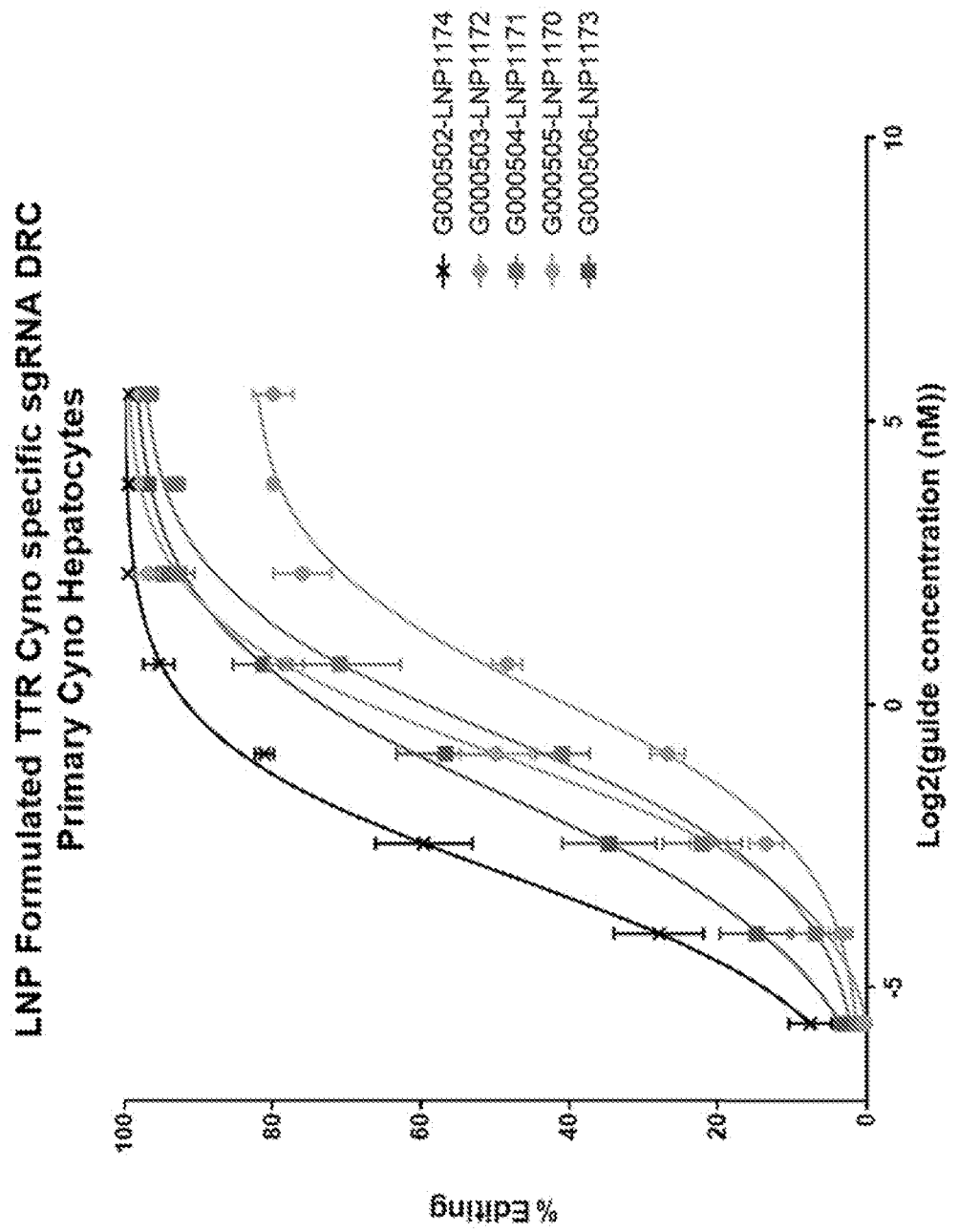

LNP Formulated TTR Cyno specific sgRNA DRC
Primary Cyno Hepatocytes
G000502-LNP1174
G000503-LNP1172
G000504-LNP1171
G000505-LNP1170
G000506-LNP1173
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 18

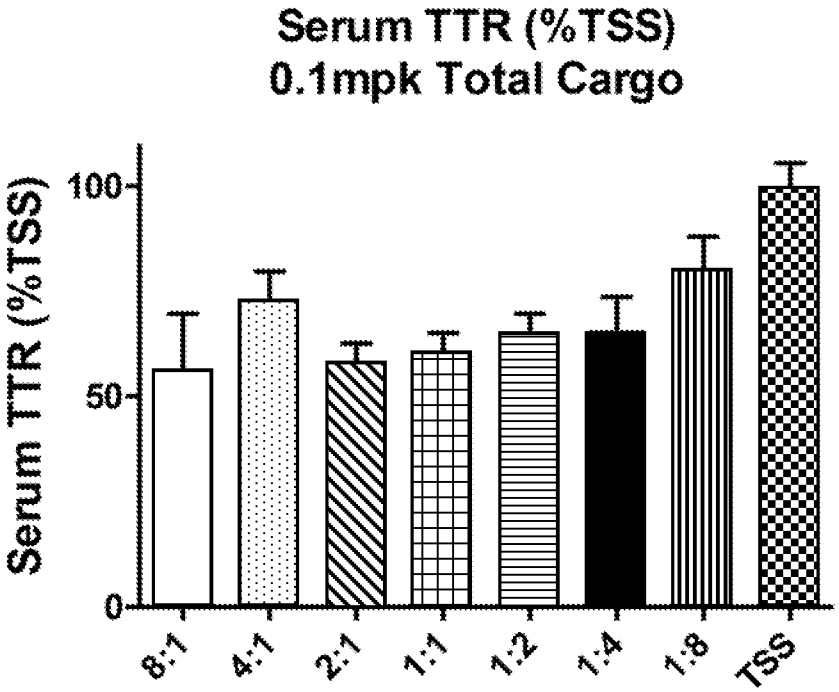


FIG. 19A

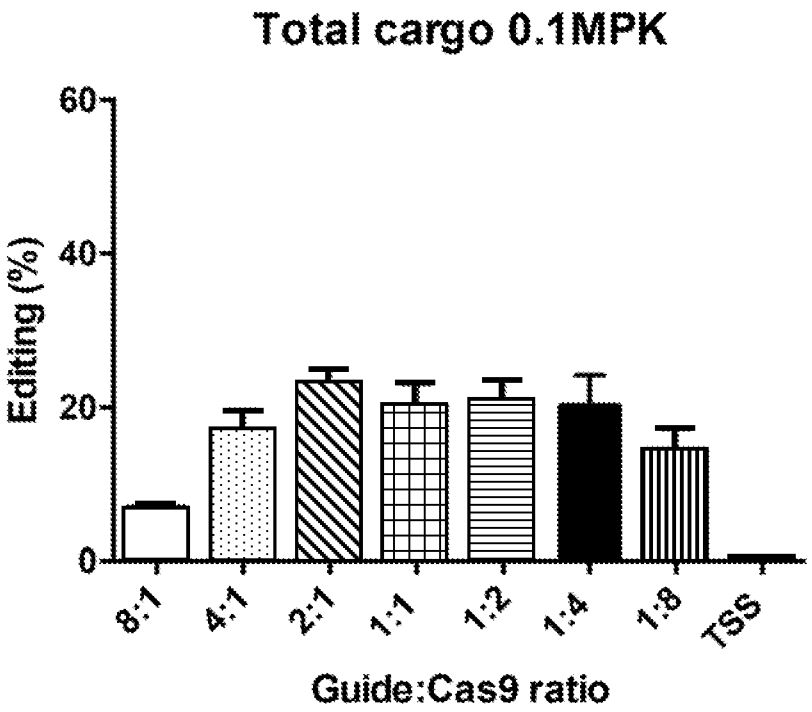


FIG. 19B

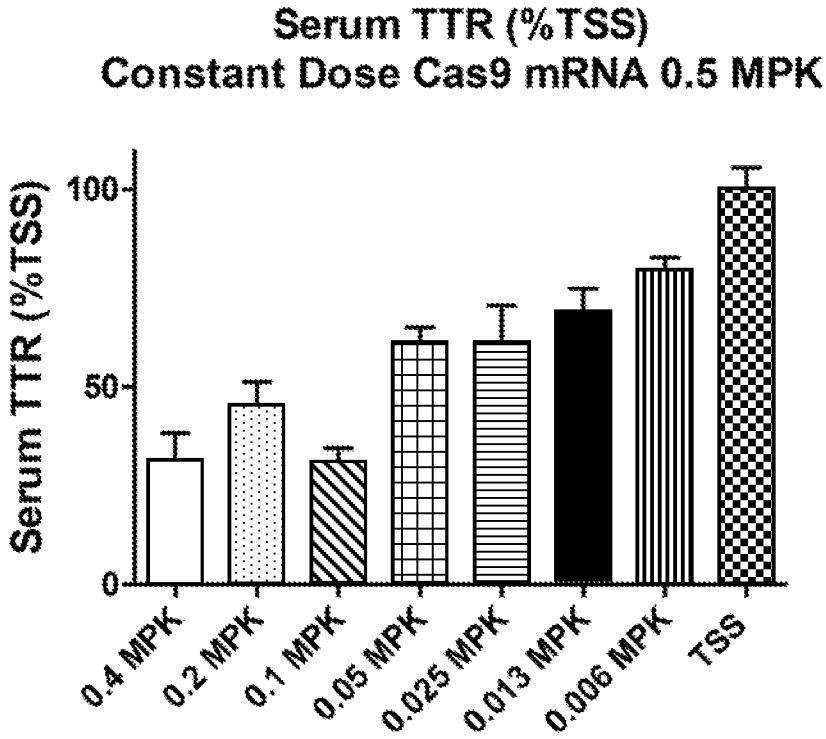


FIG. 19C

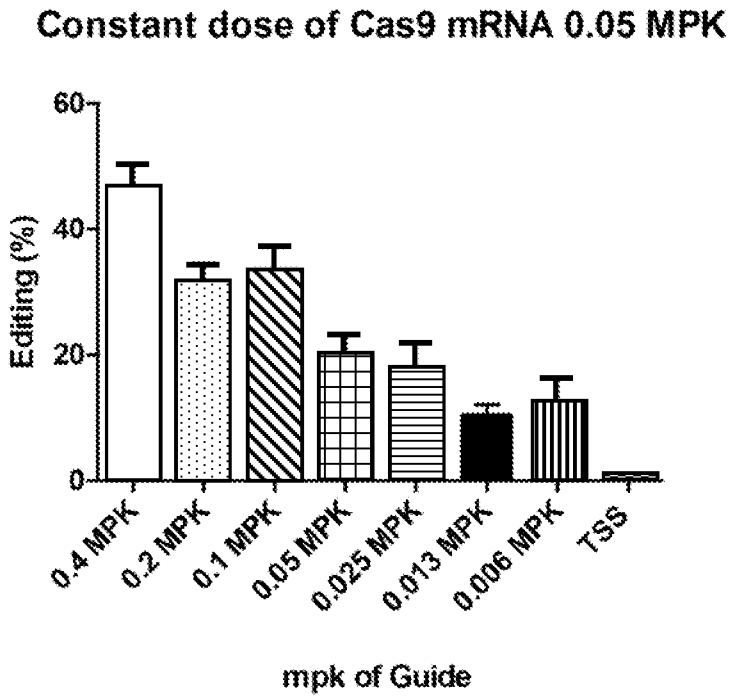


FIG. 19D

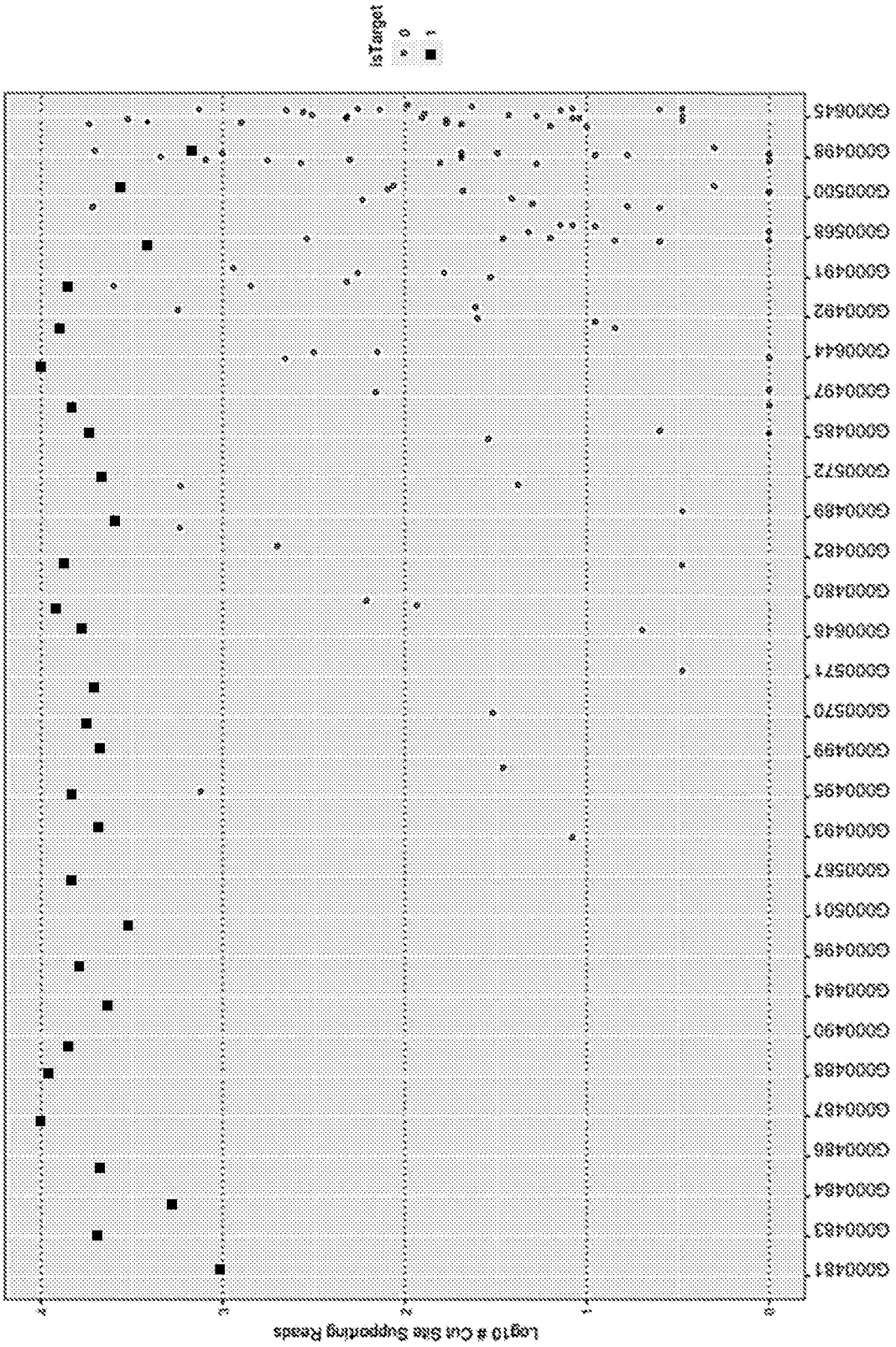

isTarget
0
1
log10 # Cut Site Supporting Reads
0
1
2
3
4
G0006451
G0004981
G0008501
G0005681
G0004911
G0004921
G0006441
G0004971
G0004851
G0005721
G0004891
G0004821
G0004801
G0006461
G0005711
G0005701
G0004991
G0004951
G0004931
G0005671
G0005011
G0004961
G0004941
G0004901
G0004681
G0004871
G0004861
G0004641
G0004631

FIG. 20

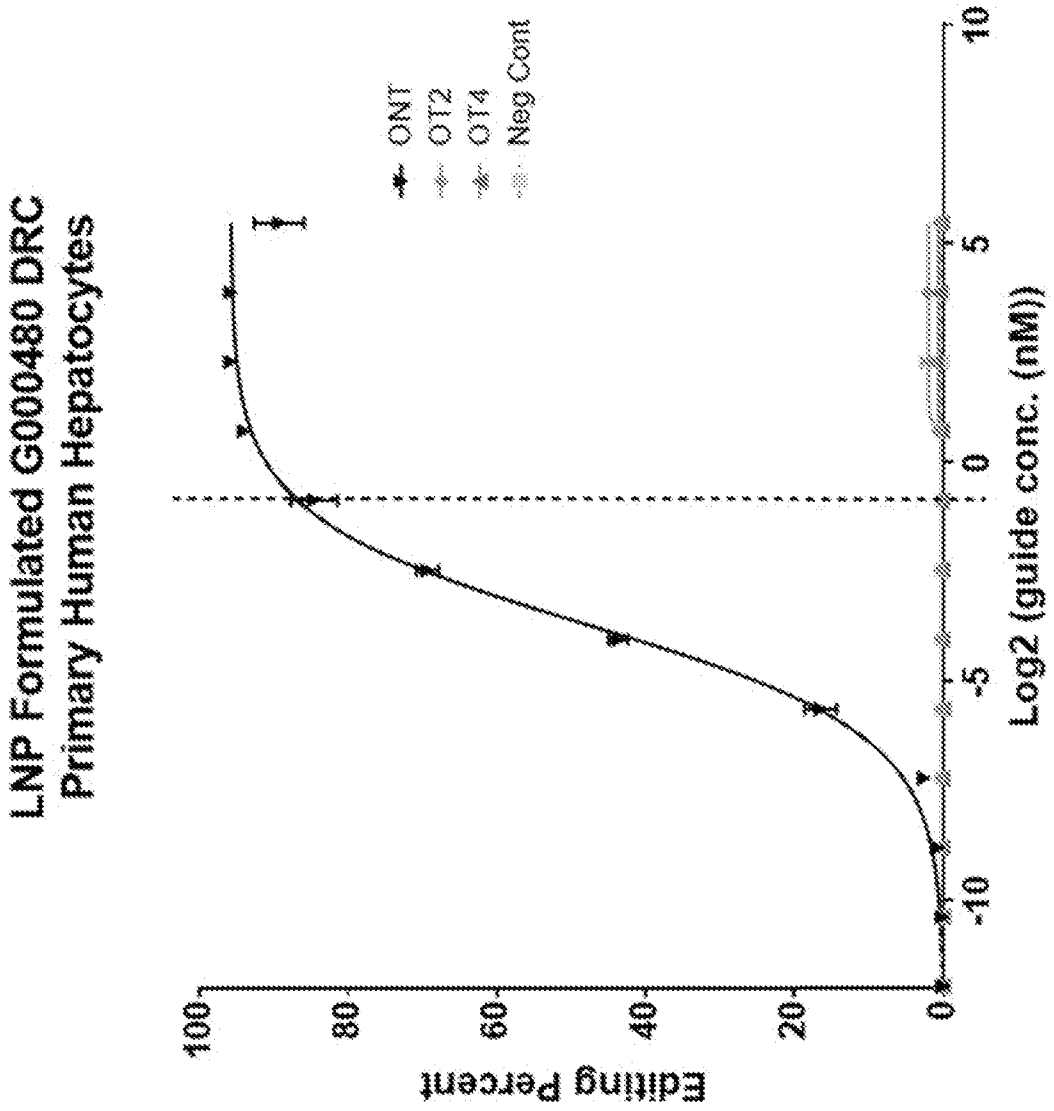

LNP Formulated G000480 DRC
Primary Human Hepatocytes
ONT
OT2
OT4
Neg Cont
Editing Percent
100
80
60
40
20
0
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 21A

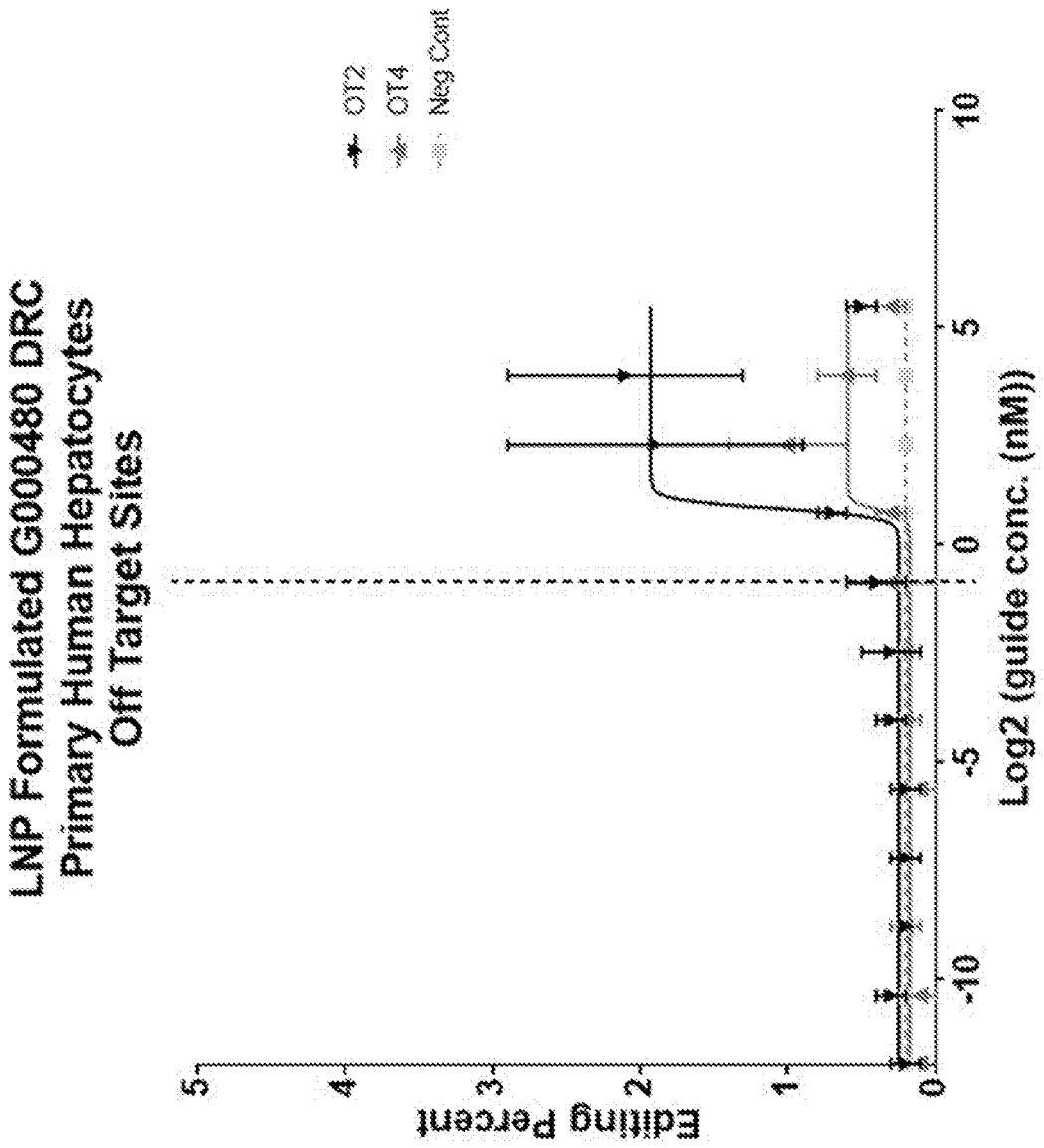

LNP Formulated G000480 DRC
Primary Human Hepatocytes
Off Target Sites
OT2
OT4
Neg Cont
Editing Percent
0
1
2
3
4
5
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 21B

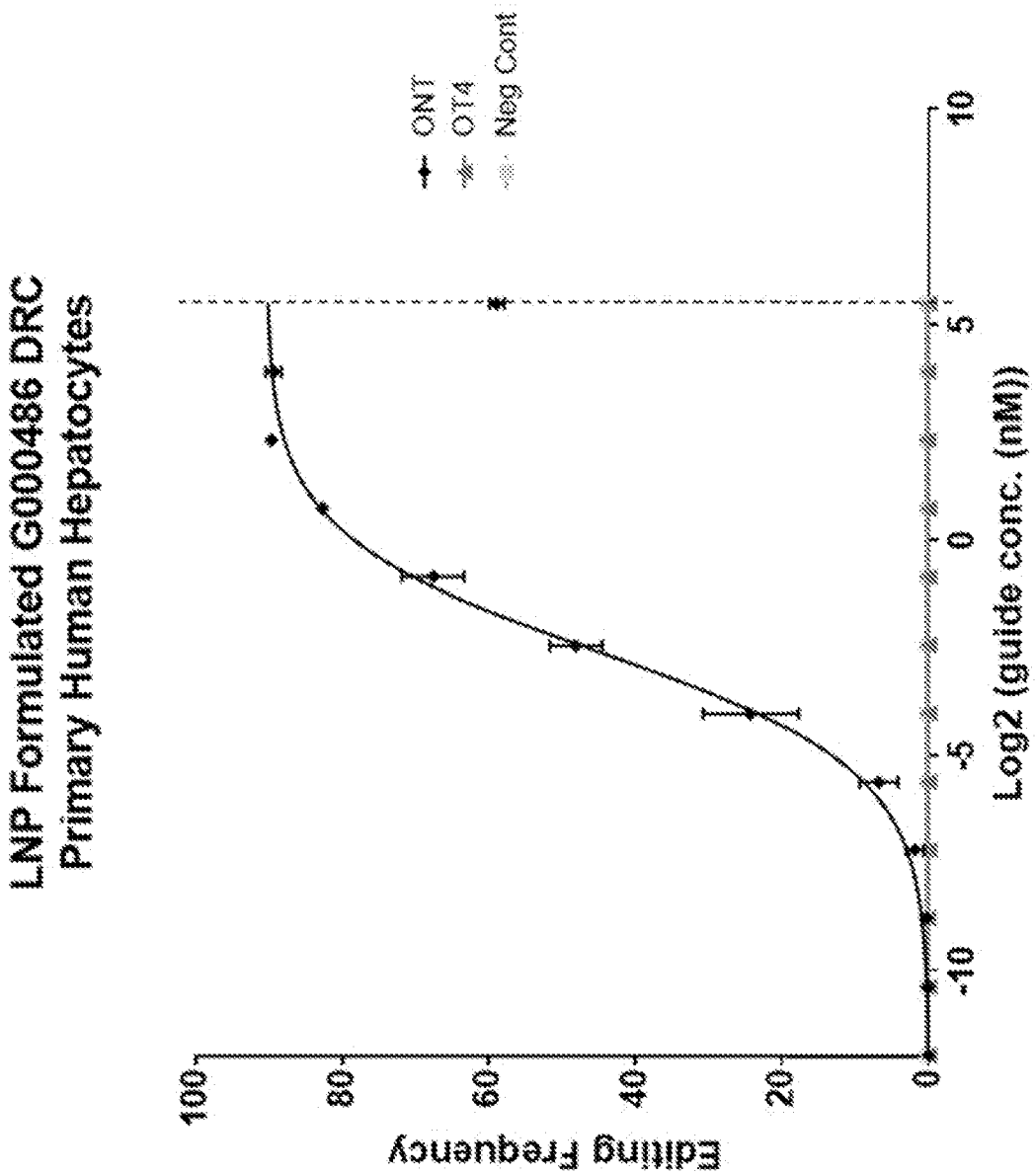

LNP Formulated G000486 DRC
Primary Human Hepatocytes
ONT
OT4
Neg Cont
Editing Frequency
0
20
40
60
80
100
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 22A

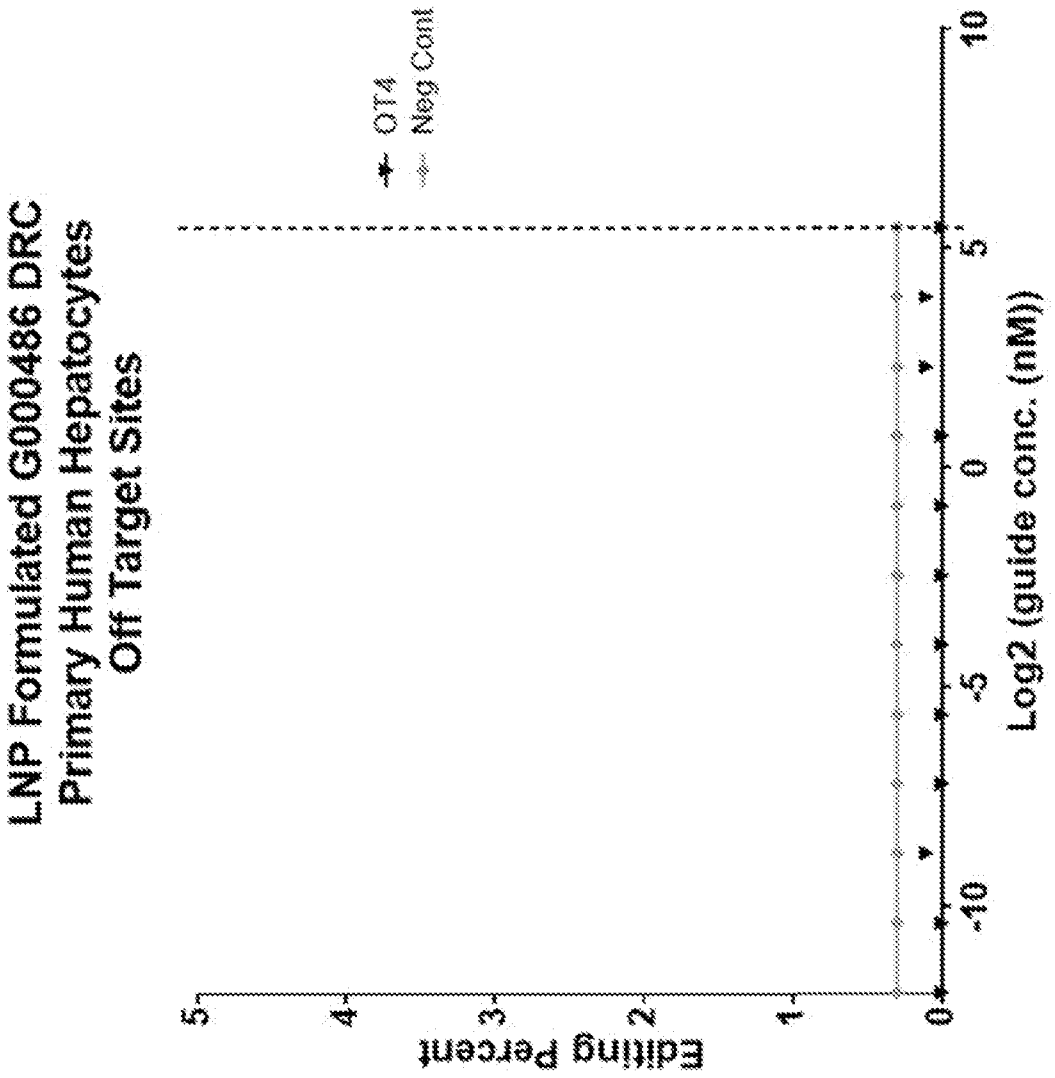

LNP Formulated G000486 DRC
Primary Human Hepatocytes
Off Target Sites
OT4
Neg Cont
Editing Percent
0
1
2
3
4
5
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 22B

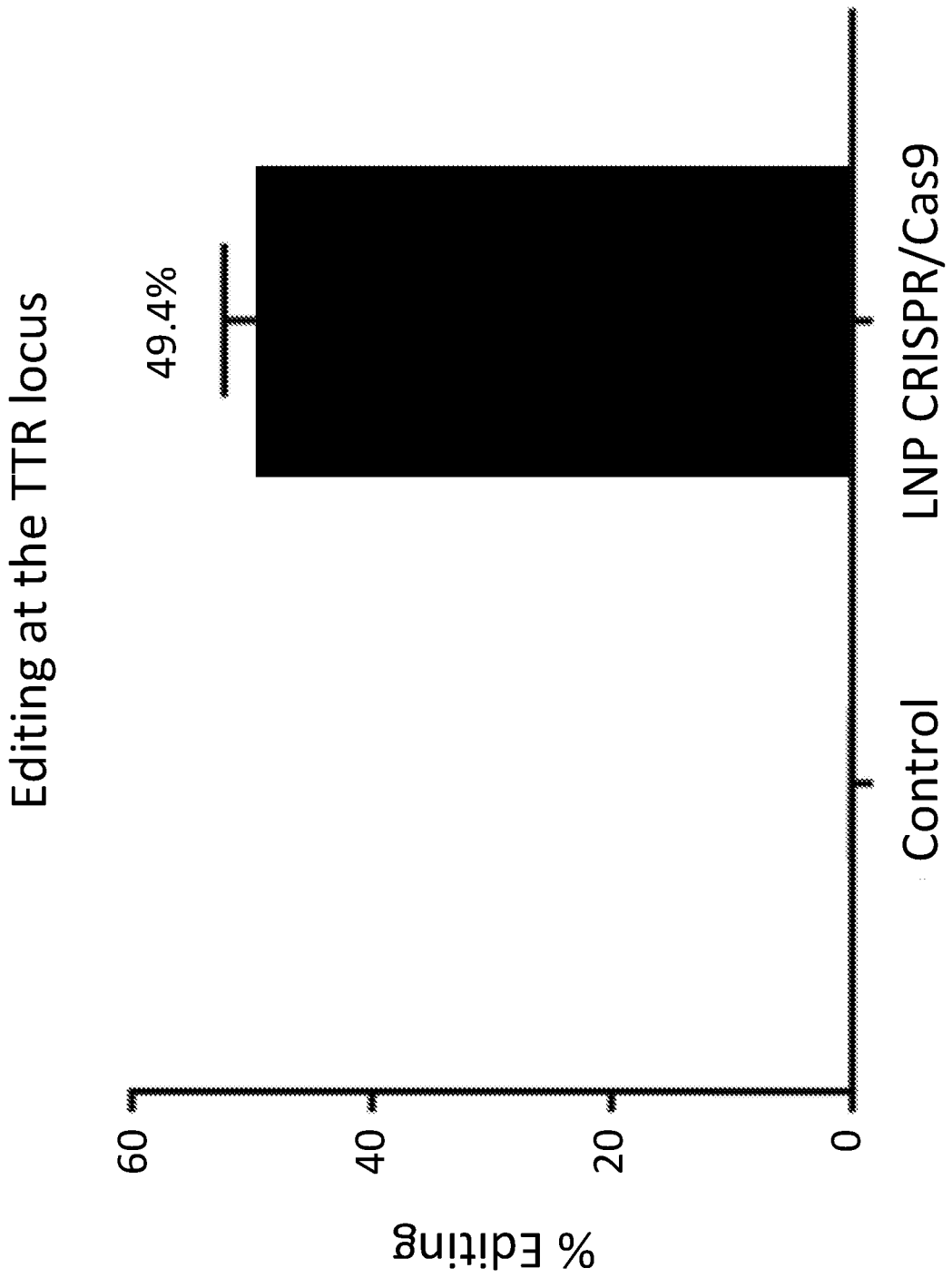

Editing at the TTR locus
49.4%
% Editing
60
40
20
0
Control
LNP CRISPR/Cas9

FIG. 23A

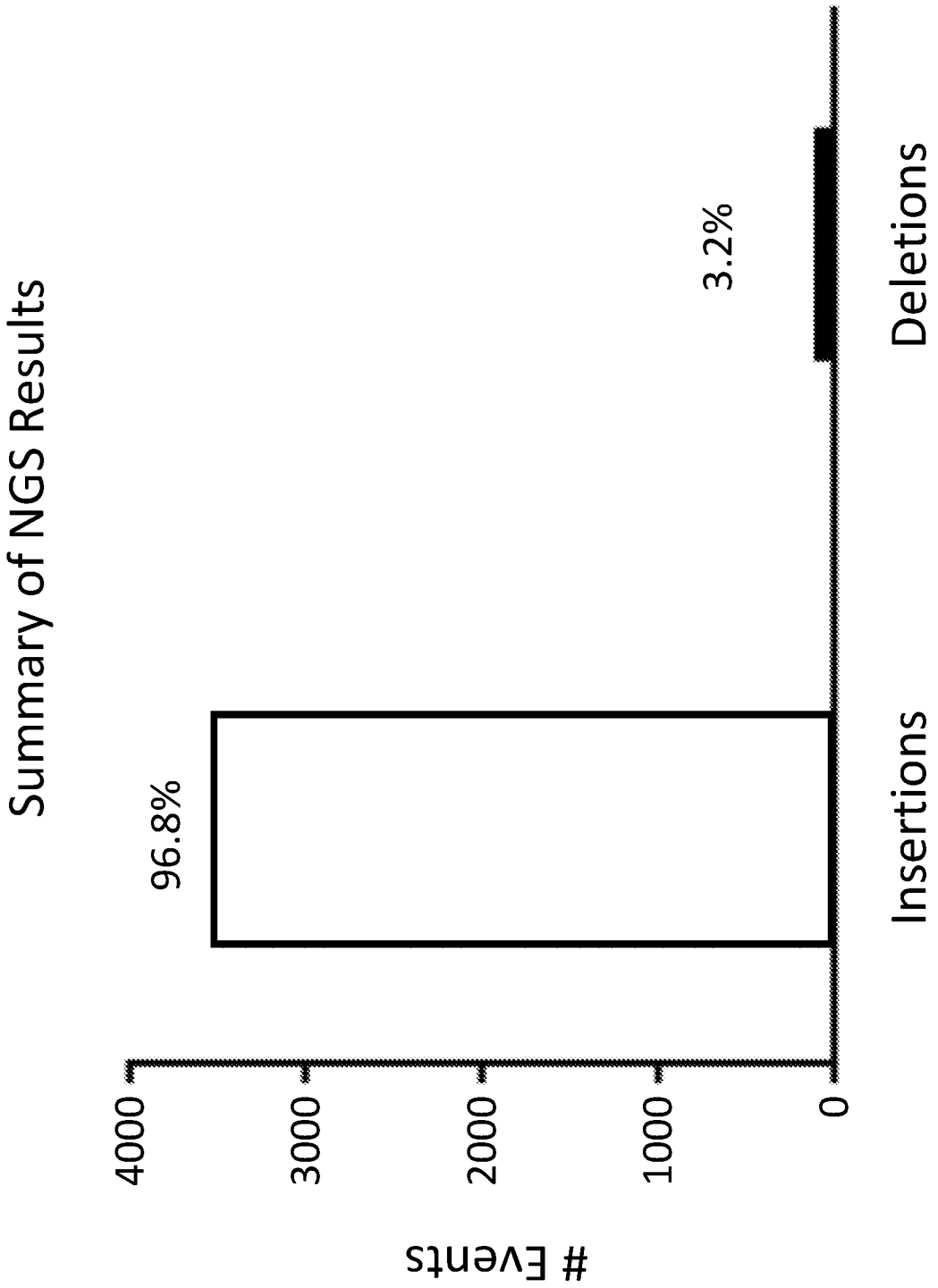

Summary of NGS Results
96.8%
3.2%
Insertions
Deletions
Events
4000
3000
2000
1000
0

FIG. 23B

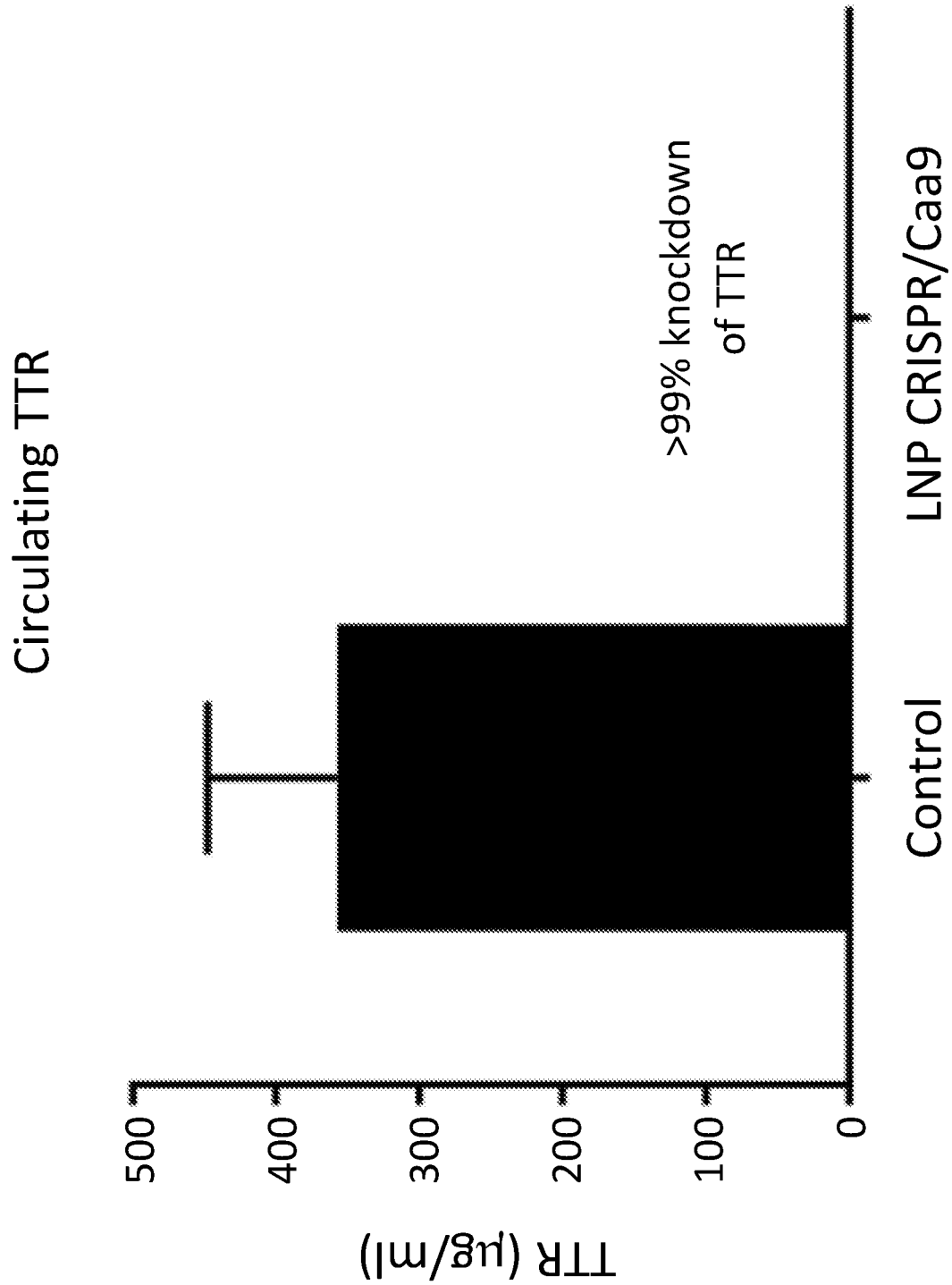

Circulating TTR
TTR (μg/ml)
500
400
300
200
100
0
>99% knockdown
of TTR
Control
LNP CRISPR/Caa9

FIG. 24A

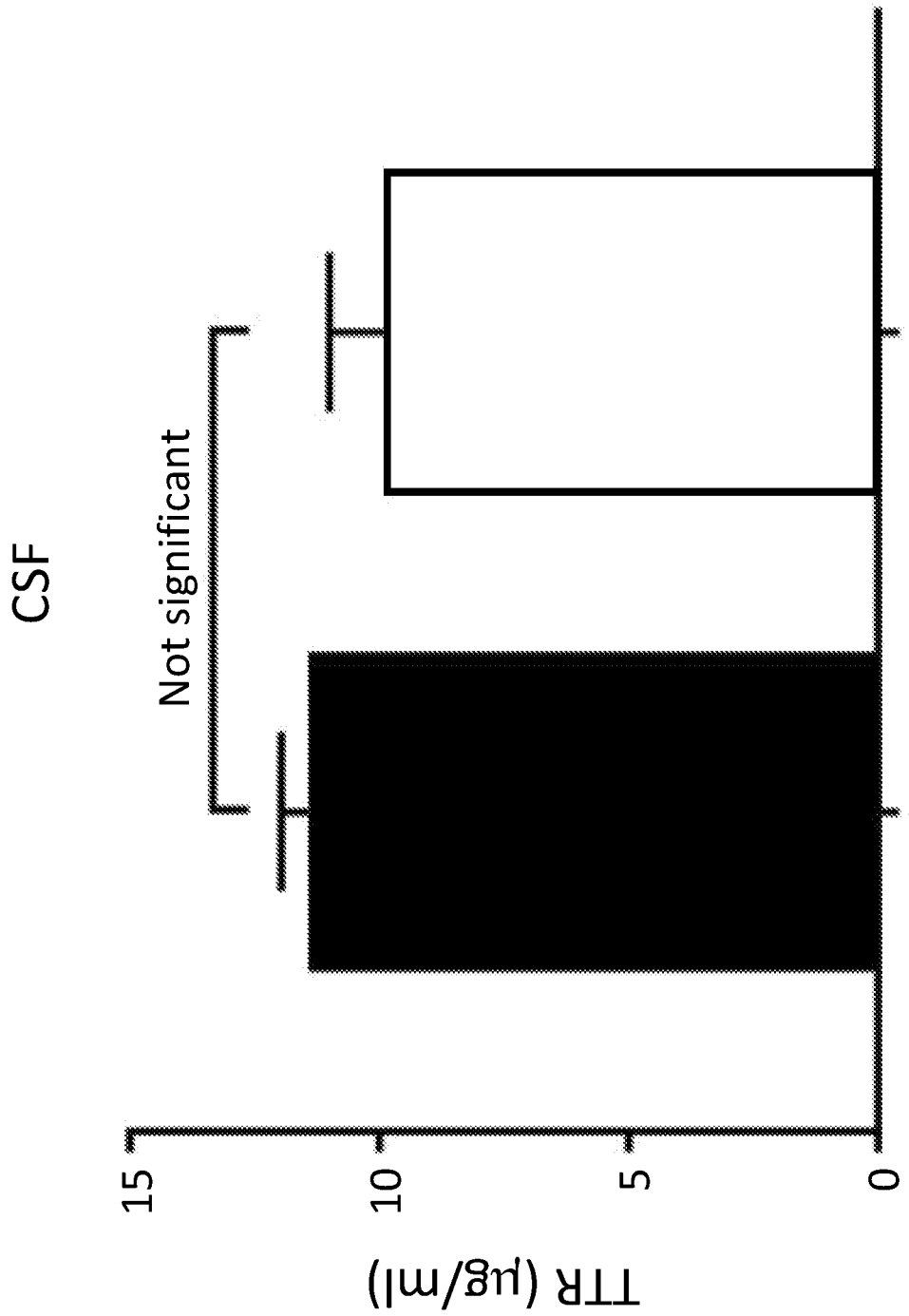

CSF
Not significant
TTR (µg/ml)
15
10
5
0
Control
LNP CRISPR/Caa9

FIG. 24B

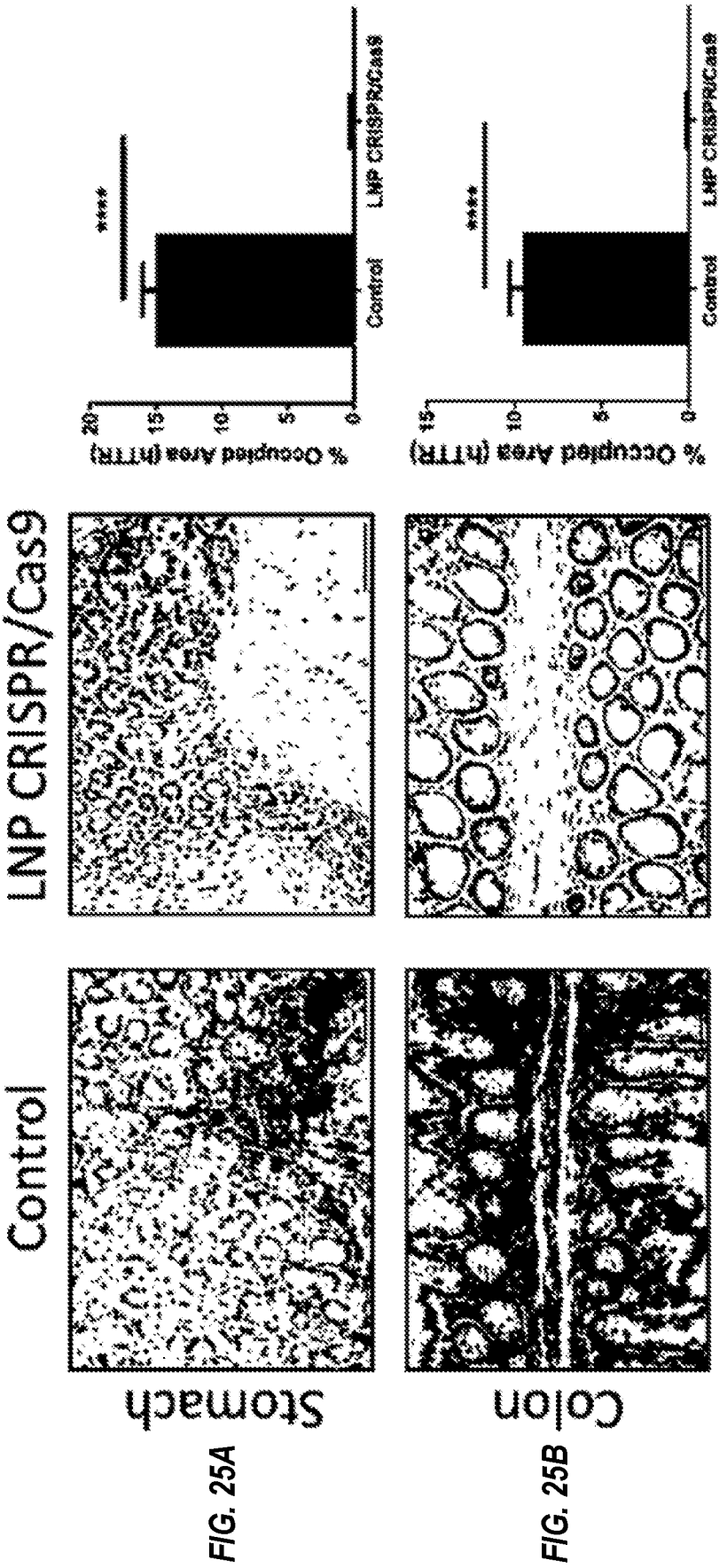
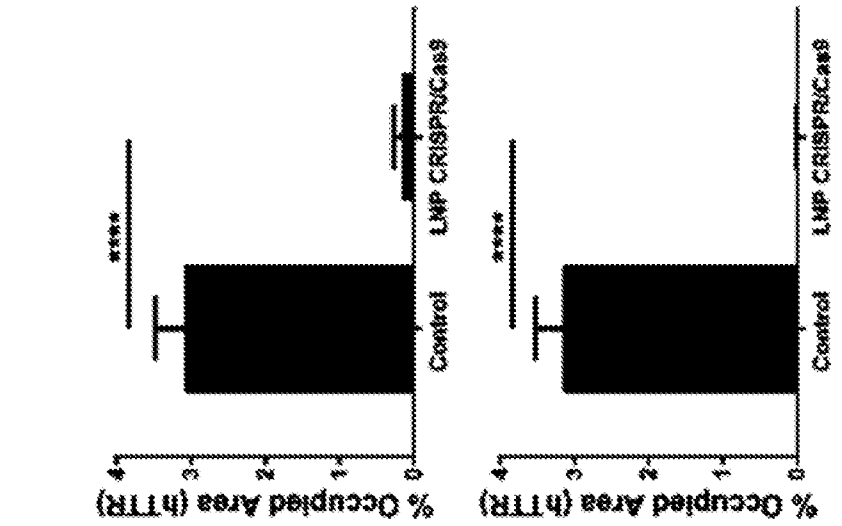


FIG. 25A

FIG. 25B



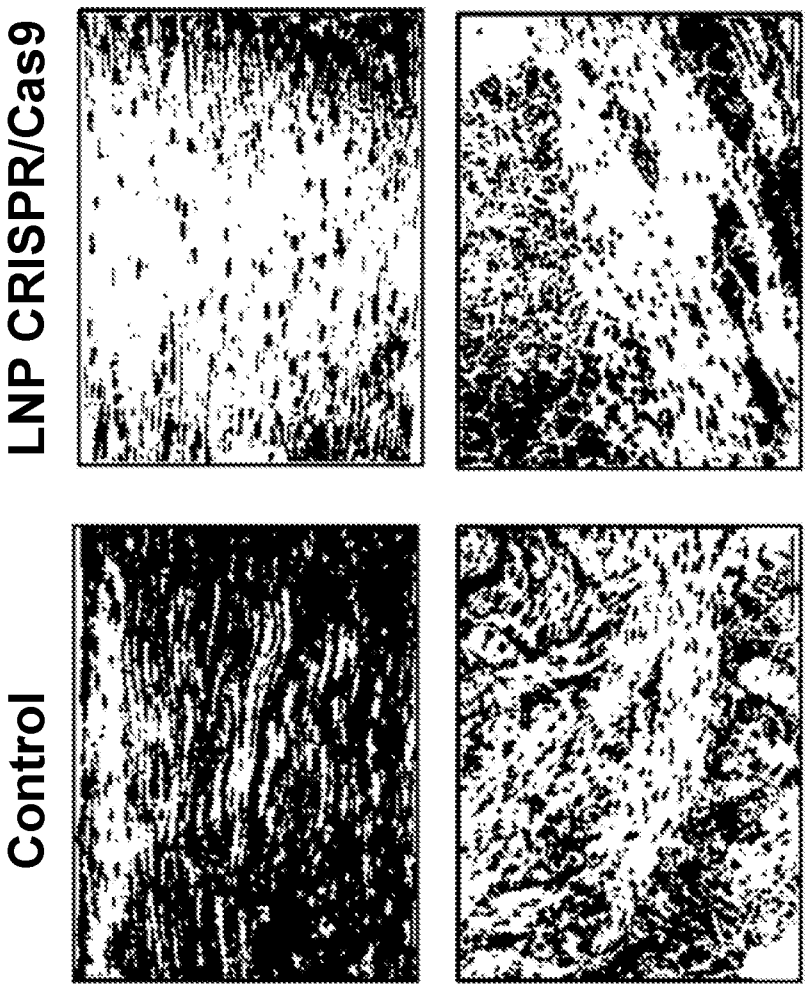


FIG. 25C

FIG. 25D

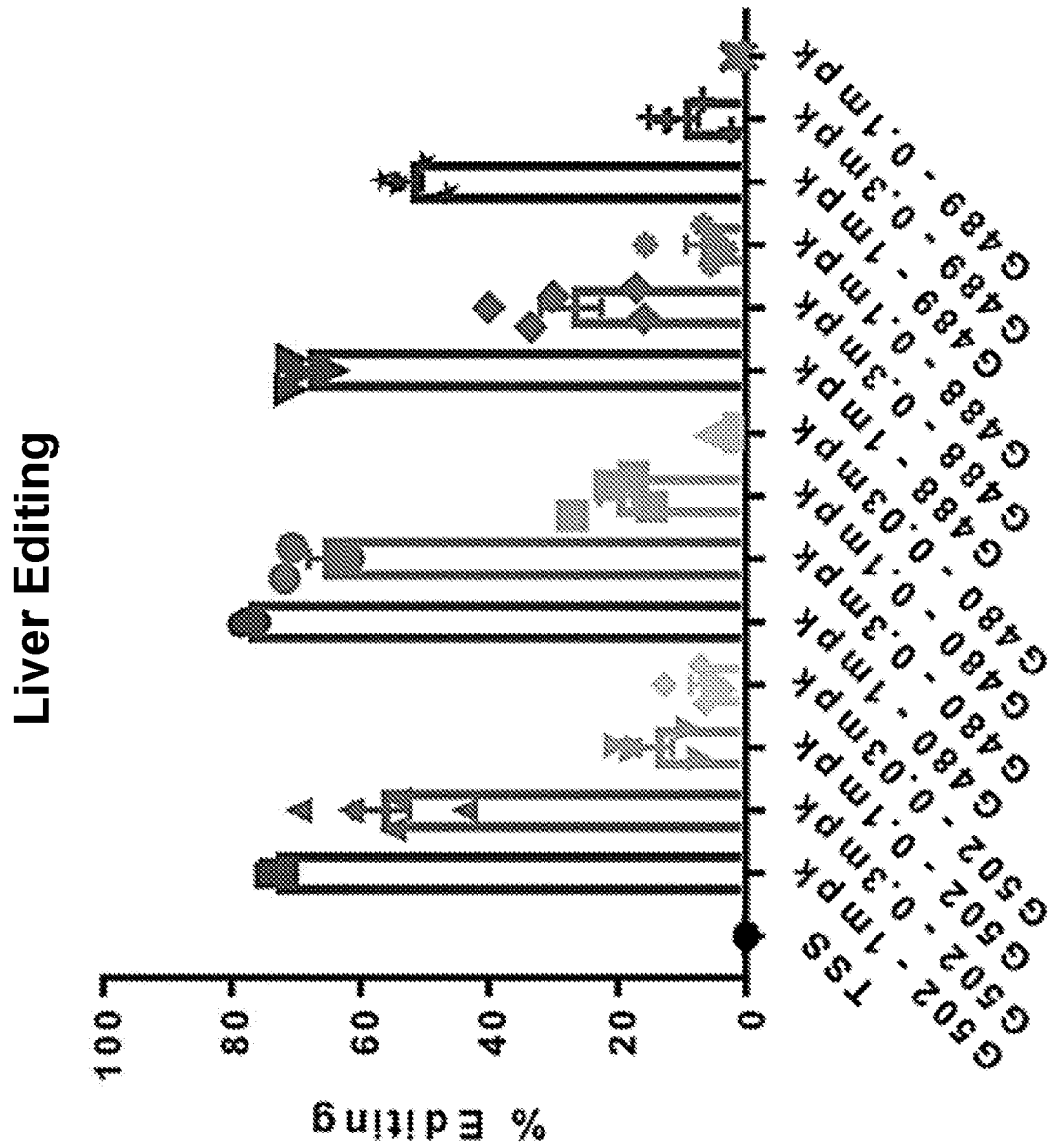

Liver Editing
% Editing
100
80
60
40
20
0
TSS - 1m pk
G502 - 0.3m pk
G502 - 0.1m pk
G502 - 0.03m pk
G502 - 1m pk
G480 - 0.3m pk
G480 - 0.1m pk
G480 - 0.03m pk
G480 - 1m pk
G488 - 0.3m pk
G488 - 0.1m pk
G488 - 0.03m pk
G488 - 1m pk
G489 - 0.3m pk
G489 - 0.1m pk
G489 - 1m pk

FIG. 26A

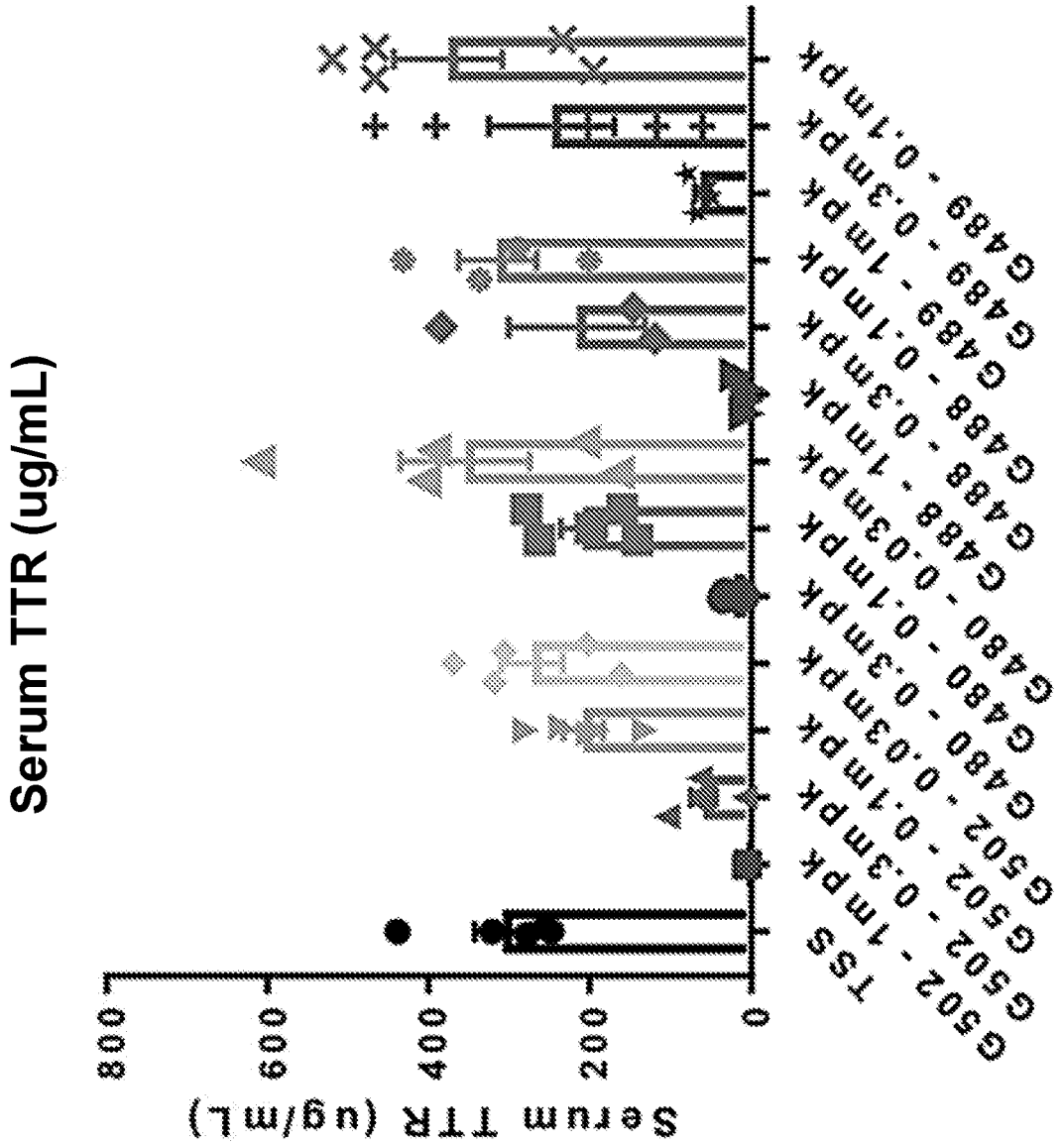

Serum TTR (ug/mL)
800
600
400
200
0
TSS - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk
G502 - 0.03mpk
G480 - 0.3mpk
G480 - 0.1mpk
G480 - 0.03mpk
G488 - 1mpk
G488 - 0.3mpk
G488 - 0.1mpk
G489 - 1mpk
G489 - 0.3mpk
G489 - 0.1mpk

FIG. 26B

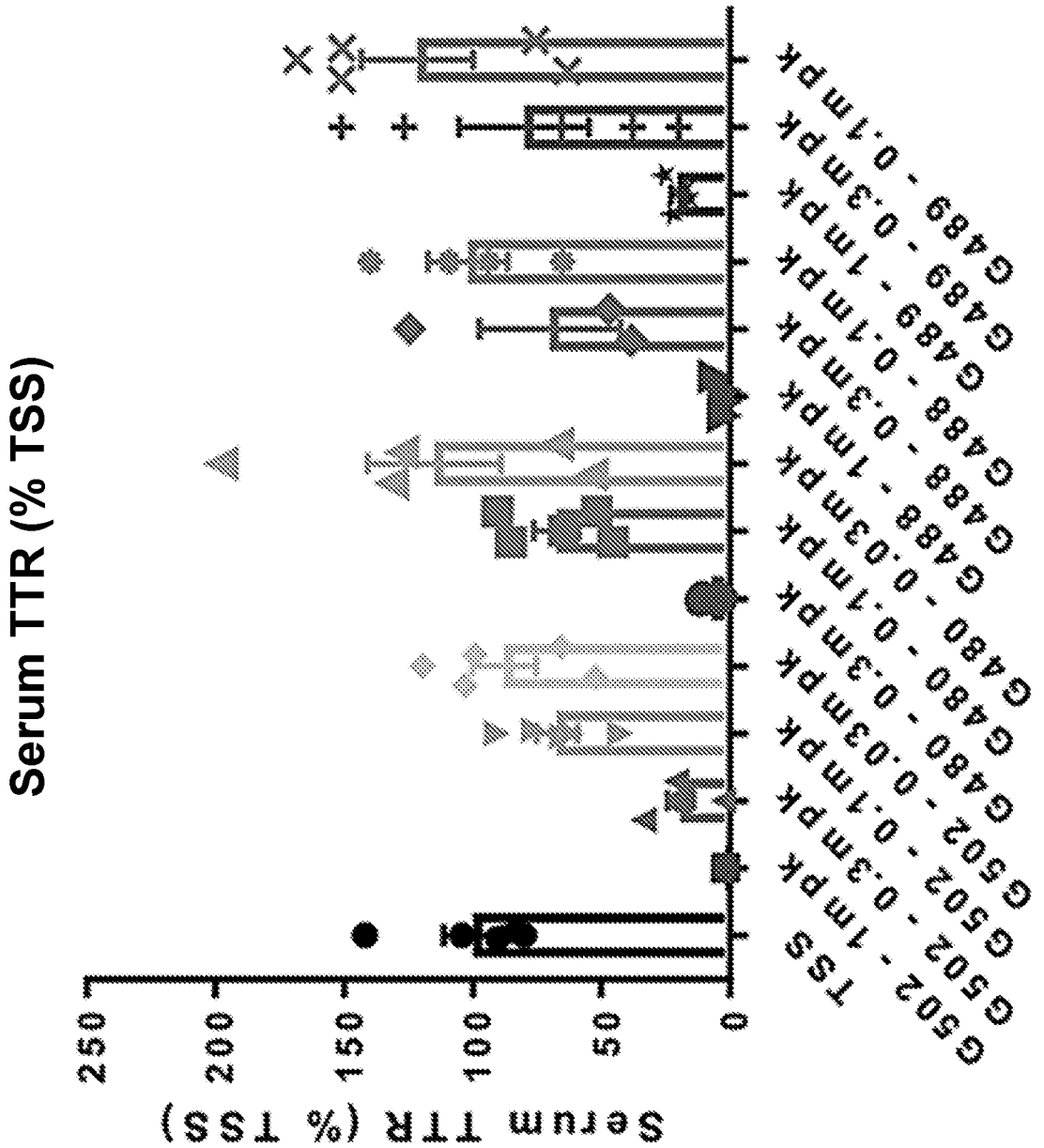

Serum TTR (% TSS)
250
200
150
100
50
0
TSS - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk
G502 - 0.03mpk
G480 - 0.3mpk
G480 - 0.1mpk
G480 - 0.03mpk
G488 - 1mpk
G488 - 0.3mpk
G488 - 0.1mpk
G489 - 1mpk
G489 - 0.3mpk
G489 - 0.1mpk

FIG. 26C

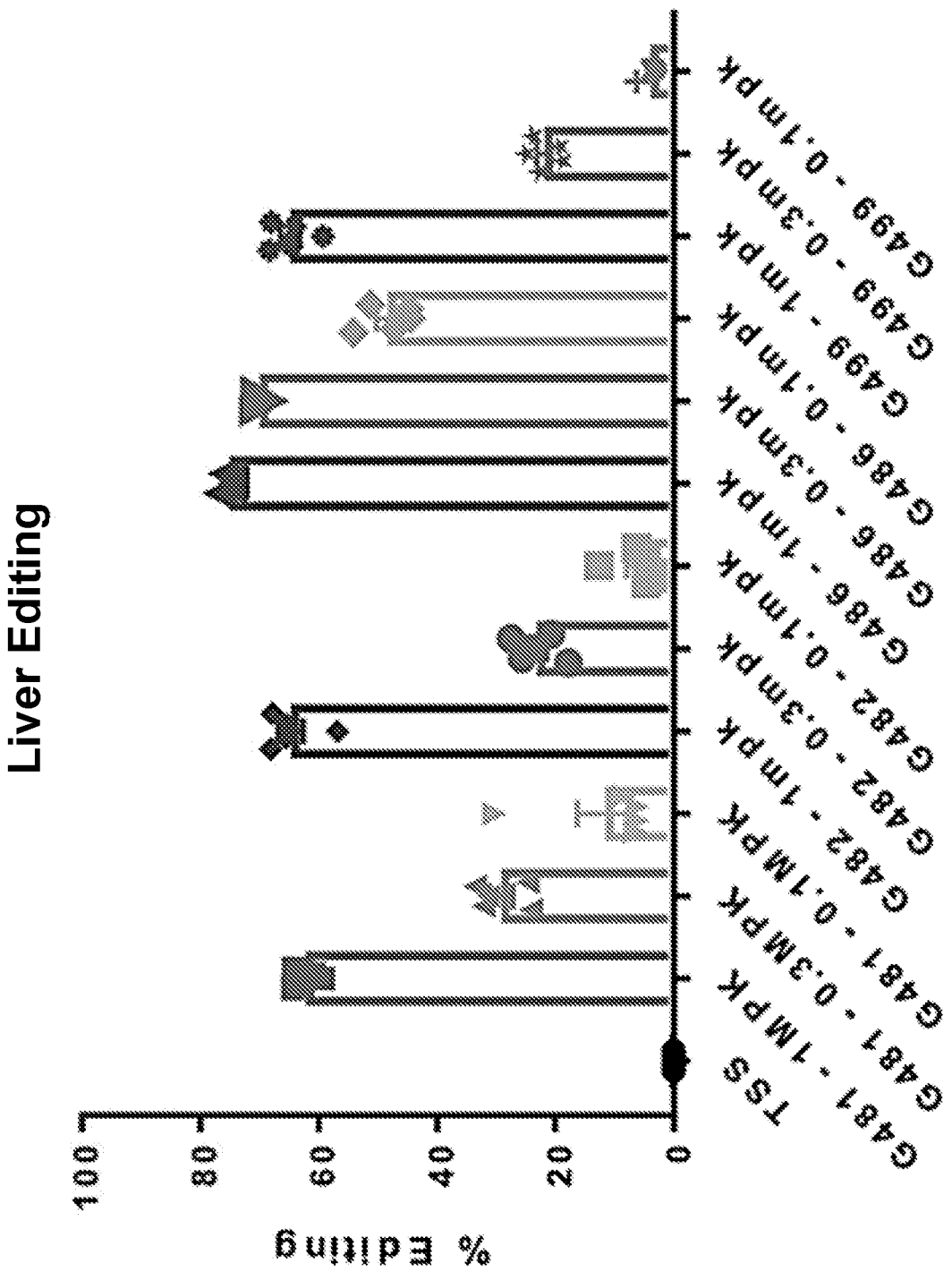

Liver Editing
% Editing
100
80
60
40
20
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G482 - 1MPK
G482 - 0.3MPK
G482 - 0.1MPK
G486 - 1MPK
G486 - 0.3MPK
G486 - 0.1MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK

FIG. 27A

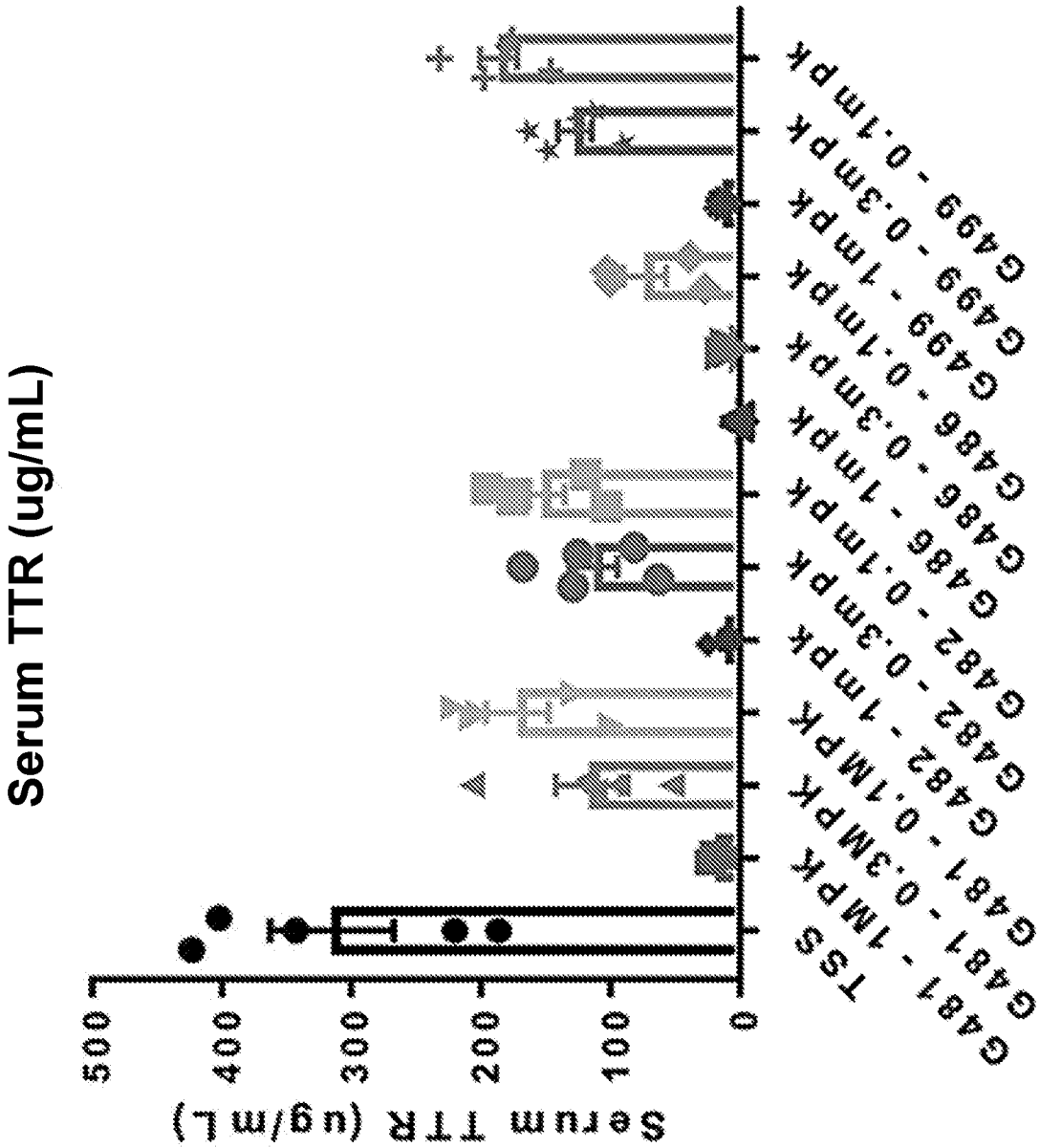

Serum TTR (ug/mL)
Serum TTR (ug/mL)
500
400
300
200
100
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G482 - 1MPK
G482 - 0.3MPK
G482 - 0.1MPK
G486 - 1MPK
G486 - 0.3MPK
G486 - 0.1MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK

FIG. 27B

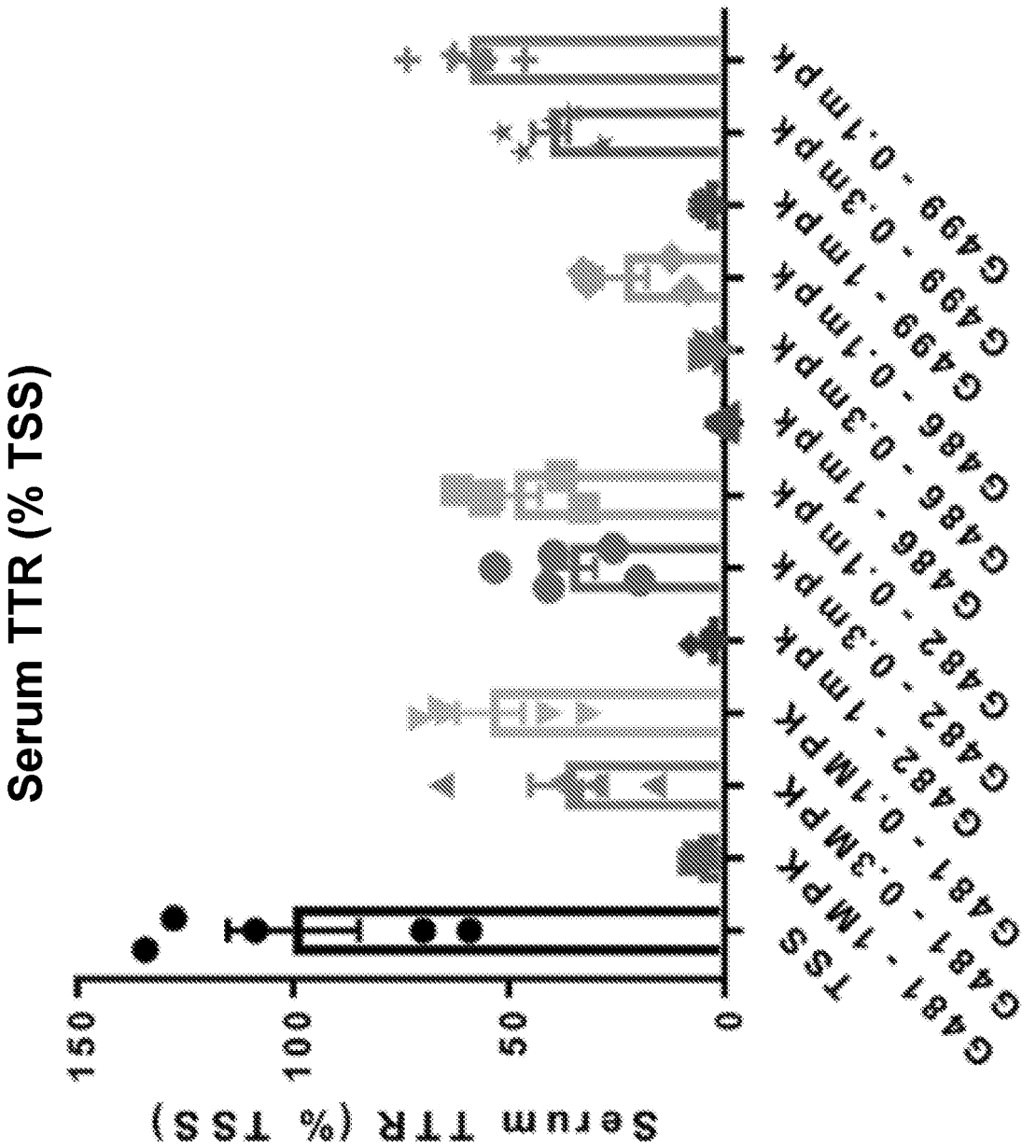

Serum TTR (% TSS)
150
100
50
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G482 - 1MPK
G482 - 0.3MPK
G482 - 0.1MPK
G486 - 1MPK
G486 - 0.3MPK
G486 - 0.1MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK

FIG. 27C

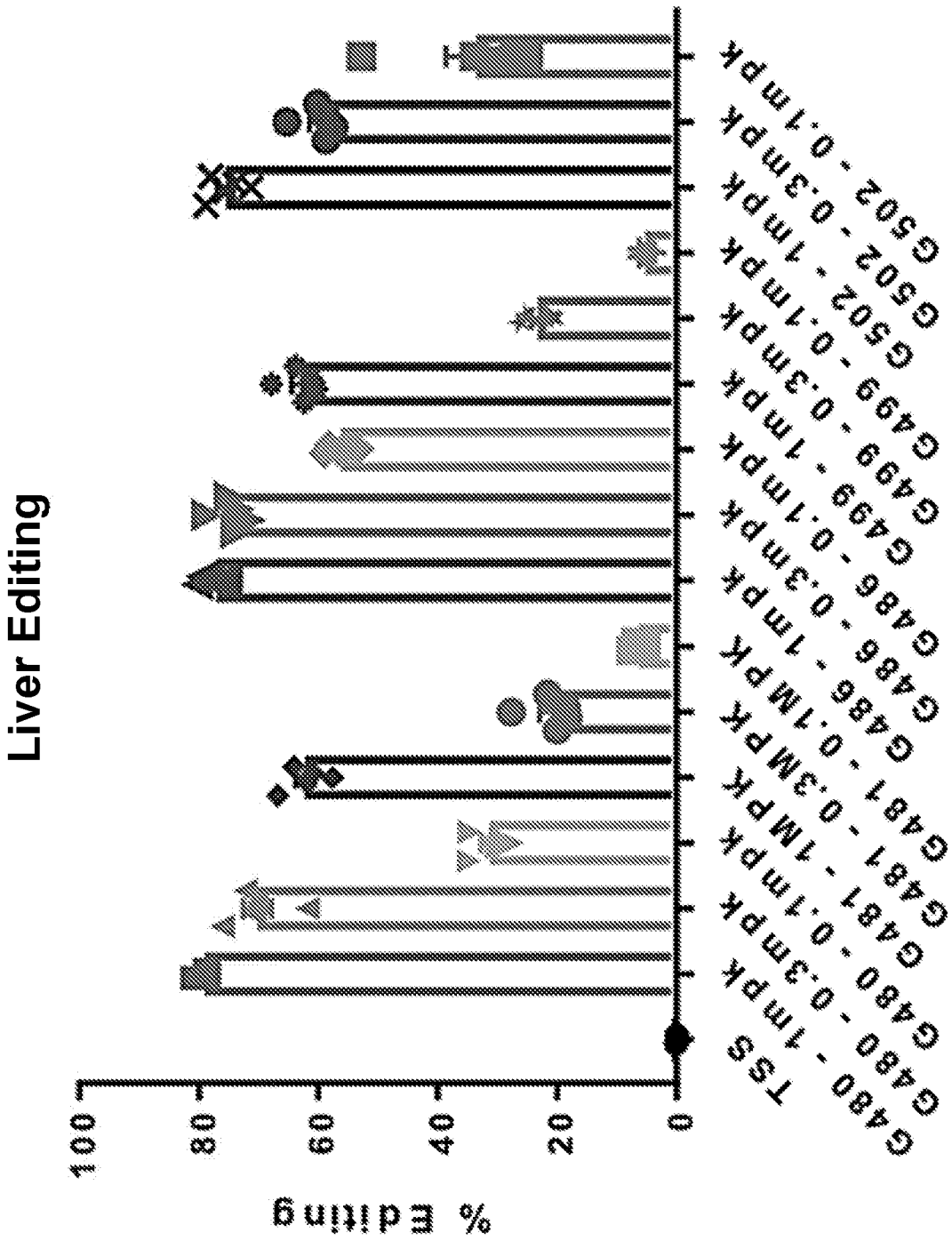

Liver Editing
% Editing
100
80
60
40
20
0
TSS
G480 - 1mpk
G480 - 0.3mpk
G480 - 0.1mpk
G481 - 1mpk
G481 - 0.3mpk
G481 - 0.1mpk
G486 - 1mpk
G486 - 0.3mpk
G486 - 0.1mpk
G499 - 1mpk
G499 - 0.3mpk
G499 - 0.1mpk
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

FIG. 28A

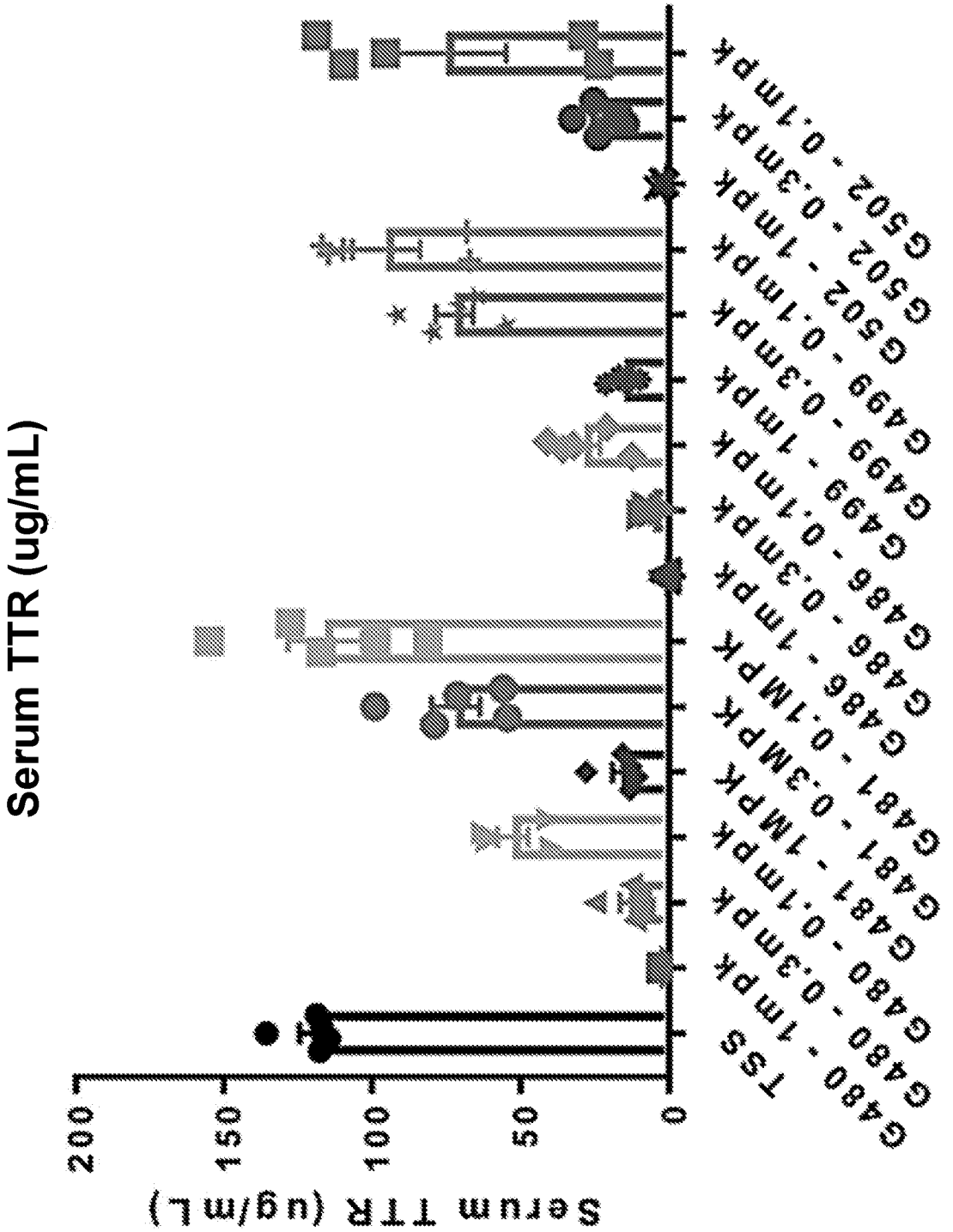

Serum TTR (ug/mL)
200
150
100
50
0
TSS - 1mpk
G480 - 0.3mpk
G480 - 1mpk
G481 - 0.1mpk
G481 - 1MPK
G481 - 0.3MPK
G486 - 1MPK
G486 - 0.3mpk
G486 - 1mpk
G499 - 0.1mpk
G499 - 1mpk
G499 - 0.3mpk
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

FIG. 28B

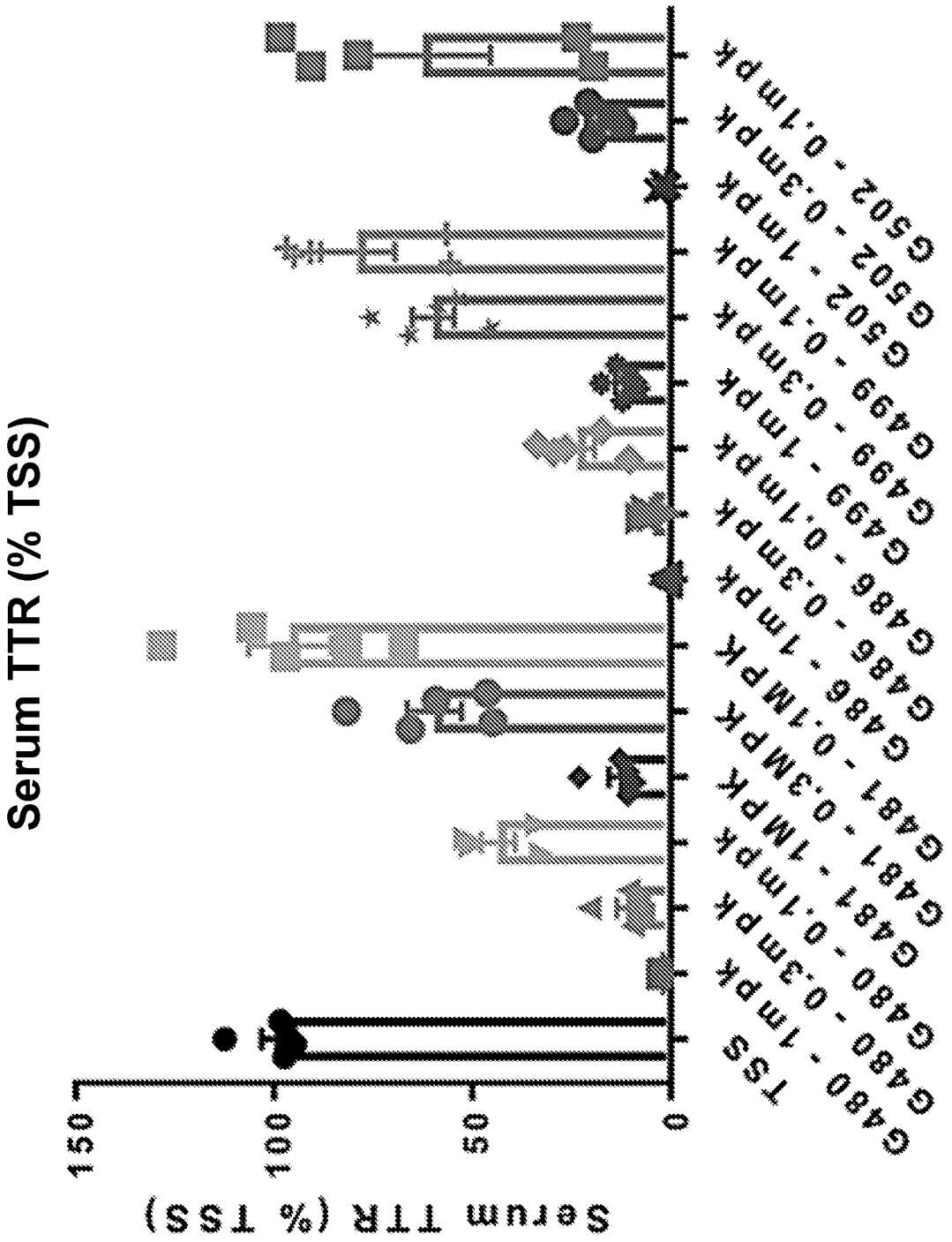

Serum TTR (% TSS)
150
100
50
0
TSS - 1mpk
G480 - 0.3mpk
G480 - 1mpk
G481 - 0.3MPK
G481 - 1MPK
G486 - 0.3mpk
G486 - 1mpk
G499 - 0.3mpk
G499 - 1mpk
G502 - 0.3mpk
G502 - 1mpk
G499 - 0.3mpk
G499 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

FIG. 28C

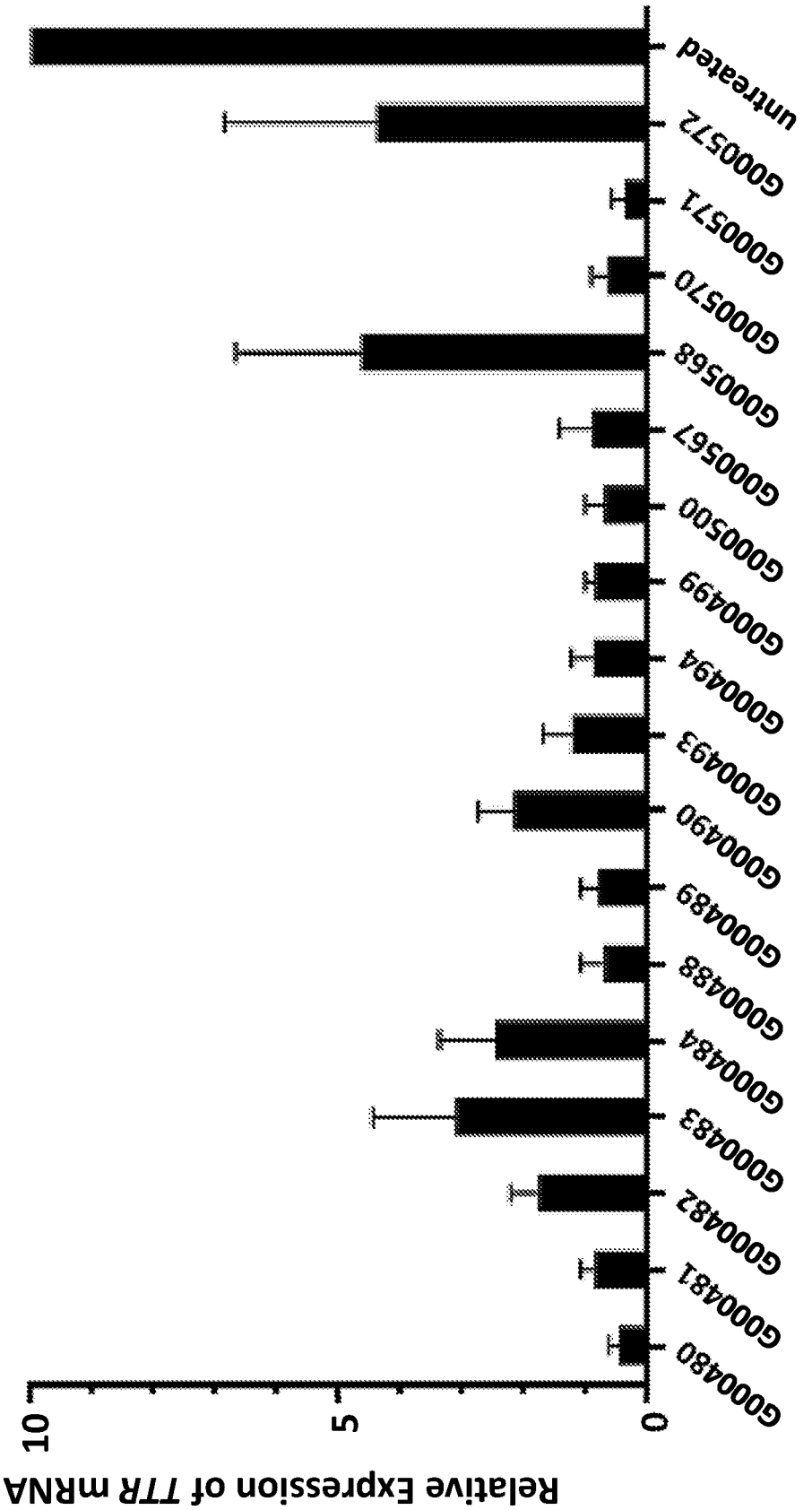

Relative Expression of TTR mRNA
10
5
0
G000480
G000481
G000482
G000483
G000484
G000488
G000489
G000490
G000493
G000494
G000499
G000500
G000567
G000568
G000570
G000571
G000572
untreated

FIG. 29

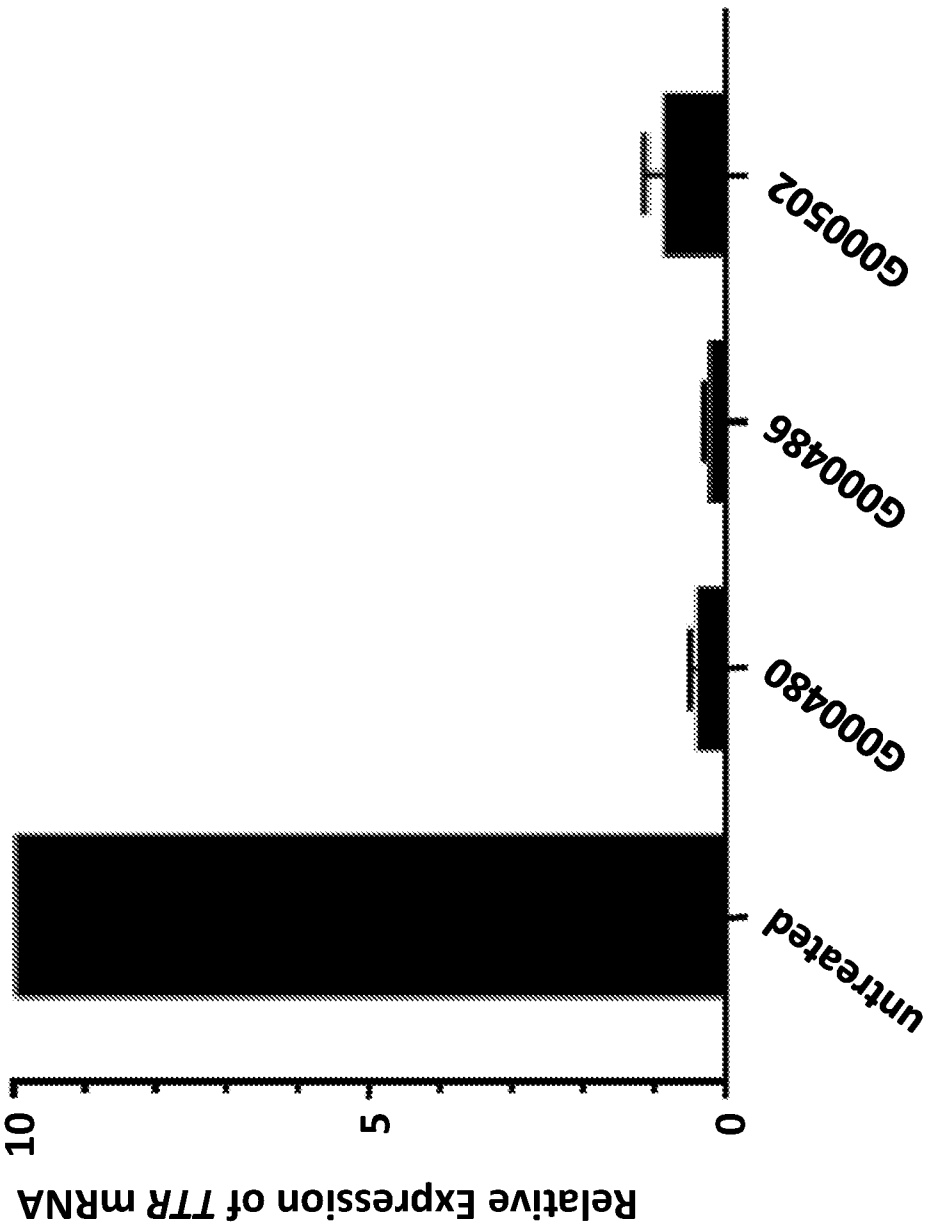

Relative Expression of TTR mRNA
10
5
0
untreated
G000480
G000486
G000502

FIG. 30