

COMPOSITIONS AND METHODS FOR ANGIOPOIETIN LIKE 3 (ANGPTL3) EDITING

REFERENCE TO CROSS-RELATED APPLICATIONS

[001] This application claims the priority to and the benefit of U.S. Provisional Patent Application Serial No. 63/559,459 filed February 29, 2024, the entire contents of which is incorporated herein by this reference.

REFERENCE TO SEQUENCE LISTING

[002] The application contains a Sequence Listing which has been submitted electronically in .XML format and is hereby incorporated by reference in its entirety. Said .XML copy, created on February 27, 2025, is named "ILH-03360.xml" and is 936,887 bytes in size. The sequence listing contained in this .XML file is part of the specification and is hereby incorporated by reference herein in its entirety.

INTRODUCTION

[003] Angiopoietin like 3 (ANGPTL3) encodes a member of a family of secreted proteins that function in angiogenesis, expressed predominantly in the liver. It is further processed into an N-terminal coiled-coil domain containing chain and a C-terminal fibrinogen chain. The N-terminal chain is involved in regulation of lipid and glucose metabolism, and the C-terminal chain may be involved in angiogenesis. Mutations in the *ANGPTL3* gene have been demonstrated to cause familial hypobetalipoproteinemia type 2 and can increase susceptibility to atherosclerosis.

SUMMARY

[004] The present disclosure provides compositions, systems, kits, and methods for modifying an *ANGPTL3* gene. The present disclosure provides a guide RNA, e.g., a modified guide RNA, compositions thereof, and pharmaceutical compositions comprising a guide RNA or a composition as described herein for modifying an *ANGPTL3* gene. In some aspects, the present disclosure provides systems and kits for modifying an *ANGPTL3* gene comprising a guide RNA and an *S. pyogenes* ("Spy") Cas9 cleavase. In some aspects, the present disclosure also provides uses and methods of using a guide RNA in combination with a *SpyCas9* cleavase, a composition thereof, or a pharmaceutical composition as described herein, for inducing a double-stranded break in an *ANGPTL3* gene, for reducing expression

of an *ANGPTL3* gene in a cell or subject, and for treating a patient having or at risk of having an *ANGPTL3*-related disease or condition.

[005] In some embodiments, the guide RNA comprises a guide region and a conserved region, wherein the guide RNA comprises a nucleotide sequence targeting a locus of an *ANGPTL3* gene. In some embodiments, the guide RNA is a modified guide RNA.

[006] In some aspects, the present disclosure provides a composition, system, or kit comprising a guide RNA and a SpyCas9 cleavase, *i.e.*, a polypeptide SpyCas9 cleavase, or a nucleic acid encoding a SpyCas9 cleavase, each as described herein. In some embodiments, the nucleic acid encoding a SpyCas9 cleavase comprises an mRNA comprising an open reading frame (ORF) encoding a SpyCas9 cleavase.

[007] In some embodiments, a composition, system, or kit as described herein further comprises a pharmaceutical excipient. In some embodiments, the guide RNA comprised in the composition is associated with a lipid nanoparticle (LNP). In some embodiments, a nucleic acid encoding a SpyCas9 cleavase is associated with the same LNP as the guide RNA. In some embodiments, a nucleic acid encoding a SpyCas9 cleavase is associated with a different LNP from the guide RNA. In some embodiments, the LNP comprises a cationic lipid. In some embodiments, the LNP comprises a helper lipid. In some embodiments, the helper lipid is cholesterol. In some embodiments, the LNP comprises a neutral lipid. In some embodiments, the neutral lipid is 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). In some embodiments, the LNP comprises a stealth lipid. In some embodiments, the stealth lipid is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2k-DMG).

[008] In some aspects, the present disclosure provides a pharmaceutical composition, system, or kit. In some embodiments, the pharmaceutical composition comprises a guide RNA as described herein, or a composition as described herein.

[009] In some aspects, the present disclosure provides use of a guide RNA and a SpyCas9 cleavase as described herein, or a composition, system, or kit as described herein, for inducing a double-stranded break within an *ANGPTL3* gene in a cell. In some aspects, the present disclosure provides a pharmaceutical composition comprising a guide RNA and a pharmaceutical composition comprising a SpyCas9 cleavase as described herein, or a composition as described herein, for inducing a double-stranded break within an *ANGPTL3* gene in a cell. In some embodiments, the cell is in a subject. In some aspects, the present disclosure provides use of a guide RNA and a SpyCas9 cleavase as described herein, or a composition, system, or kit as described herein, for reducing expression of an *ANGPTL3* gene in a cell or subject. In some aspects, the present disclosure provides a pharmaceutical

composition comprising a guide RNA and a pharmaceutical composition comprising a SpyCas9 cleavase as described herein, or a composition as described herein, for reducing expression of a *ANGPTL3* gene in a cell or subject. In some embodiments, the cell is in a subject. In some embodiments, the guide RNA and the SpyCas9 cleavase are present in the same pharmaceutical composition.

[0010] In some aspects, the present disclosure provides use of a guide RNA and a SpyCas9 cleavase as described herein, or a composition as described herein, for treating a subject having an *ANGPTL3*-related disease or condition. In some aspects, the present disclosure provides a pharmaceutical composition comprising a guide RNA and a pharmaceutical composition comprising a SpyCas9 cleavase as described herein, or a composition, system, or kit as described herein, for treating a subject having an *ANGPTL3*-related disease or condition. In some embodiments, the guide RNA and the SpyCas9 cleavase are present in the same composition.

[0011] In some aspects, the present disclosure provides a method of inducing a double-stranded break within an *ANGPTL3* gene in a cell, or reducing expression of an *ANGPTL3* protein in a cell, comprising contacting a cell with a guide RNA and a SpyCas9 cleavase as described herein, or a composition as described herein. In some embodiments, the guide RNA and the SpyCas9 cleavase are present in the same composition. In some embodiments, the cell is in a subject. In some embodiments, the level of *ANGPTL3* protein is measured in a subject sample selected from blood or serum.

[0012] In some aspects, the present disclosure provides use of a guide RNA as described herein, or a composition as described herein, in the preparation of a medicament for practicing any of the methods as described herein.

[0013] In some aspects, provided herein is a system comprising: A. a modified *S. pyogenes* Cas9 (SpyCas9) guide RNA comprising: 1. a targeting sequence comprising a sequence at least 95%, or 90%, identical to the nucleotide sequence of SEQ ID NOs: 1, 2, 3, 4, or 5; 2. a targeting sequence comprising a sequence identical to at least 18, 19, or 20 contiguous nucleotides of the nucleotide sequence of SEQ ID NOs: 1, 2, 3, 4, or 5; or 3. a targeting sequence comprising a sequence identical to the nucleotide sequence of SEQ ID NOs: 1, 2, 3, 4, or 5; and B. an *S. pyogenes* Cas9 (SpyCas9) cleavase.

[0014] In some embodiments, the SpyCas9 guide RNA comprises a targeting sequence identical to the nucleotide sequence of SEQ ID NOs: 1, 2, 3, 4, or 5.

[0015] In some embodiments, the SpyCas9 guide RNA further comprises one or more of: A. a shortened hairpin 1 region, or a substituted and optionally shortened hairpin 1 region

relative to SEQ ID NO: 303, wherein 1. at least one of the following pairs of nucleotides are substituted in the substituted and optionally shortened hairpin 1 region with Watson-Crick pairing nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, or H1-4 and H1-9, and the hairpin 1 region optionally lacks a. any one or two of H1-5 through H1-8, b. one, two, or three of the following pairs of nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, and H1-4 and H1-9, or c. 1-8 nucleotides of the hairpin 1 region; or 2. the shortened hairpin 1 region lacks 4-8 nucleotides, preferably 4-6 nucleotides; and a. one or more of positions H1-1, H1-2, or H1-3 is deleted or substituted relative to SEQ ID NO: 303; or b. one or more of positions H1-6 through H1-10 is substituted relative to SEQ ID NO: 303; or 3. the shortened hairpin 1 region lacks 5-10 nucleotides, preferably 5-6 nucleotides, and one or more of positions N18, H1-12, or N is substituted relative to SEQ ID NO: 303; or B. a shortened upper stem region, wherein the shortened upper stem region lacks 1-6 nucleotides and wherein the 6, 7, 8, 9, 10, or 11 nucleotides of the shortened upper stem region include less than or equal to 4 substitutions relative to SEQ ID NO: 303; or C. a substitution relative to SEQ ID NO: 303 at any one or more of LS6, LS7, US3, US10, B3, N7, N15, N17, H2-2 and H2-14, wherein the substituent nucleotide is neither a pyrimidine that is followed by an adenine, nor an adenine that is preceded by a pyrimidine; or D. SEQ ID NO: 303 with an upper stem region, wherein the upper stem modification comprises a modification to any one or more of US1-US12 in the upper stem region.

[0016] In some embodiments, the SpyCas9 guide RNA lacks 6 nucleotides in the shortened hairpin 1. In some embodiments, the SpyCas9 guide RNA lacks 8 nucleotides in the shortened hairpin 1. In some embodiments, H-1 and H-3 are deleted. In some embodiments, the SpyCas9 guide RNA further comprises a 3' tail. In some embodiments, the 3' tail is 1-4 nucleotides in length, optionally 1 nucleotide in length. In some embodiments, the SpyCas9 guide RNA comprises an upper stem region comprising a modification to any one or more of US1-US12 in the upper stem region.

[0017] In some embodiments, the targeting sequence comprises a modified nucleotide sequence according to the pattern (mN*)³(N)¹³⁻¹⁷, wherein "m" is indicative of a 2'-O-methyl modification, * is indicative of a phosphorothioate bond, and N is indicative of a 2'-OH and a phosphodiester bond. In some embodiments, the SpyCas9 guide RNA comprises a modified nucleotide sequence selected from the sequence of SEQ ID NO: 501-512, wherein the modified nucleotide sequence is at 3' of the guide sequence. In some embodiments, the SpyCas9 guide RNA is modified according to the pattern of a nucleotide sequence selected from SEQ ID NO: 601-612, wherein the (mN*)³N¹⁷ refers to the targeting sequence

described herein. In some embodiments, the SpyCas9 guide RNA comprises the nucleotide sequence selected from SEQ ID NOs: 102, 101, 104, and 103. In some embodiments, each nucleotide is a natural or non-natural nucleotide. In some embodiments, the SpyCas9 guide RNA comprises the modified nucleotide sequence selected from SEQ ID NOs: 202, 201, 204, and 203.

[0018] In some embodiments, the SpyCas9 cleavase comprises a SpyCas9 cleavase polypeptide or a nucleic acid encoding a SpyCas9 cleavase polypeptide. In some embodiments, the nucleic acid encoding the SpyCas9 cleavase comprises an mRNA comprising an open reading frame (ORF) encoding the SpyCas9 cleavase. In some embodiments, the SpyCas9 cleavase comprises an amino acid sequence having at least 95% identity to SEQ ID NO: 1001; or wherein the ORF encoding the SpyCas9 cleavase comprises a nucleotide sequence having at least 95% identity to a sequence selected from SEQ ID NOs: 1003 and 1006. In some embodiments, the ORF is a modified ORF.

[0019] In some aspects, provided herein is a composition comprising the system described herein. In some embodiments, the composition further comprises a pharmaceutical excipient. In some embodiments, the SpyCas9 guide RNA is associated with a lipid nanoparticle (LNP). In some embodiments, the LNP comprises a cationic lipid. In some embodiments, the cationic lipid 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. In some embodiments, the LNP comprises a helper lipid. In some embodiments, the helper lipid is cholesterol. In some embodiments, the LNP comprises a neutral lipid. In some embodiments, the neutral lipid is 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). In some embodiments, the LNP comprises a stealth lipid. In some embodiments, the stealth lipid is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol 2000 (PEG2k-DMG). In some embodiments, the LNP comprises (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, DSPC, cholesterol, and PEG2k-DMG.

[0020] In some aspects, provided herein is a pharmaceutical composition comprising the composition described herein. In some embodiments, the pharmaceutical composition described herein, or use of a pharmaceutical composition described herein is for inducing a double-stranded break within an *ANGPTL3* gene in a cell. In some embodiments, the

pharmaceutical composition or use described herein is for reducing expression of an *ANGPTL3* gene in a cell. In some embodiments, the cell is a liver cell. In some embodiments, the cell is in a subject. In some embodiments, the pharmaceutical composition or use described herein is for treating a subject having an *ANGPTL3*-related disease.

[0021] In some aspects, provided herein is a kit comprising the system described herein, the composition described herein, or the pharmaceutical composition described herein.

[0022] In some aspects, provided herein is a method of inducing a double-stranded break within an *ANGPTL3* gene in a cell comprising contacting the cell with the system described herein, the composition described herein, or the pharmaceutical composition described herein.

[0023] In some aspects, provided herein is a use of the system described herein, the composition described herein, or the pharmaceutical composition described herein in the preparation of a medicament for practicing the method described herein.

[0024] In some aspects, provided herein is a method of modifying a genomic locus in a human liver cell, comprising contacting a human liver cell with the system described herein, the composition described herein, or the pharmaceutical composition described herein.

[0025] In some embodiments, the cell or the liver cell is a hepatocyte. In some embodiments, the cell or the liver cell is in a subject with an *ANGPTL3*-related disease.

[0026] In some aspects, provided herein is a method of treating an *ANGPTL3*-related disease in a subject, comprising administering to the subject the system described herein, the composition described herein, or the pharmaceutical composition described herein.

[0027] In some embodiments, the pharmaceutical composition, use, method, or kit described herein further comprises determining the *ANGPTL3* protein level in a blood or serum sample from the subject.

[0028] In some aspects, provided herein is a use of the system described herein, the composition described herein, or the pharmaceutical composition described herein in the preparation of a medicament for practicing the method described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **Fig. 1A** shows the percent editing of five *ANGPTL3* guides in PHH donor 1.

[0030] **Fig. 1B** shows the levels of secreted *ANGPTL3* after treatment with five *ANGPTL3* guides in culture medium (hu*ANGPTL3* pg/mL) in a 7-point dilution series.

[0031] **Figs. 2A-C** show the percent editing of two *ANGPTL3* guides in an 8-point dilution series. **Fig. 2A** shows the percent editing in PHH donor 1. **Fig. 2B** shows the percent editing in PHH donor 2. **Fig. 2C** shows the percent editing in PHH donor 3. The data point for each of the highest gRNA doses is not shown in the graph. The values are provided in the data tables in the corresponding Example below.

[0032] **Figs. 3A-C** show the levels of secreted *ANGPTL3* after treatment with two *ANGPTL3* guides in culture medium (hu*ANGPTL3* pg/mL) in an 8-point dose dilution series. **Fig. 3A** shows the *ANGPTL3* serum levels in PHH donor 1. **Fig. 3B** shows the *ANGPTL3* serum levels in PHH donor 2. **Fig. 3C** shows the *ANGPTL3* serum levels in PHH donor 3. The data point for each of the highest gRNA doses is not shown in the graph. The values are provided in the data tables in the corresponding Example below.

[0033] **Figs. 4A-C** show the percent editing of an *ANGPTL3* guide in either 100-mer or 91-mer format in a 12-point dilution series. An sgRNA to a target other than *ANGPTL3* was used as a control to allow comparison across the cell lines. **Fig. 4A** shows the percent editing in PHH donor 1. **Fig. 4B** shows the percent editing in PHH donor 2. **Fig. 4C** shows the percent editing in PHH donor 3. The data point for each of the lowest and highest gRNA dose is not shown in the graph. The values are provided in the data tables in the corresponding Example below.

BRIEF DESCRIPTION OF DISCLOSED SEQUENCES

SEQ ID NO	Description
1-5	Exemplary <i>ANGPTL3</i> guide sequences and chromosomal coordinates
101-110	Exemplary unmodified sgRNA sequences targeting <i>ANGPTL3</i>
201-210	Exemplary modified sgRNA sequences targeting <i>ANGPTL3</i>
301-309	Exemplary unmodified SpyCas9 scaffold sequences
401-408	Exemplary unmodified SpyCas9 guide RNA sequences
501-512	Exemplary modified SpyCas9 guide scaffold sequences
601-612	Exemplary modified SpyCas9 guide sequences
1001	Cas9 amino acid sequence with 1x NLS (RNP)
1002	mRNA encoding SpyCas9
1003	Open reading frame for SpyCas9
1005	mRNA encoding SpyCas9

SEQ ID NO	Description
1006	Open reading frame for SpyCas9
1011	Exemplary <i>EMXI</i> guide RNA (G000644)
1012	Exemplary <i>VEGFA</i> guide RNA (G000645)
1013	Exemplary SV40 NLS
1014	Exemplary SV40 NLS
1015	Exemplary nucleoplasmin NLS

DETAILED DESCRIPTION

[0034] Reference will now be made in detail to certain embodiments of the disclosure, examples of which are illustrated in the accompanying drawings. While the present teachings are described in conjunction with various embodiments, it is not intended to limit the present teachings to those embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

[0035] 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 (*e.g.*, a population of cells) and the like.

[0036] Numeric ranges are inclusive of the numbers defining the range. Measured and measurable values are understood to be approximate, taking into account significant digits and the error associated with the measurement.

[0037] The use of “comprise”, “comprises”, “comprising”, “contain”, “contains”, “containing”, “include”, “includes”, and “including” is 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. Unless specifically noted in the 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).

[0038] The term “or” is used in an inclusive sense in the specification, *i.e.*, equivalent to “and/or,” unless the context clearly indicates otherwise.

[0039] The term “about”, when used before a list, modifies each member of the list. The term “about” is understood to encompass tolerated variation or error within the art, *e.g.*, 2 standard deviations from the mean, or the sensitivity of the method used to take a measurement. When “about” is present before the first value of a series, it can be understood to modify each value in the series.

[0040] Ranges are understood to include the numbers at the end of the range and all logical values therebetween. For example, 5-10 nucleotides are understood as 5, 6, 7, 8, 9, or 10 nucleotides, whereas 5-10% is understood to contain 5% and all possible values through 10%.

[0041] At least 17 nucleotides of a 20-nucleotide sequence is understood to include 17, 18, 19, or 20 nucleotides of the sequence provided, thereby providing an upper limit even if one is not specifically provided as it would be clearly understood. Similarly, up to 3 nucleotides would be understood to encompass 0, 1, 2, or 3 nucleotides, providing a lower limit even if one is not specifically provided. When “at least”, “up to”, or other similar language modifies a number, it can be understood to modify each number in the series.

[0042] As used herein, “no more than” or “less than” is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. For example, a duplex region of “no more than 2 nucleotide base pairs” has a 2, 1, or 0 nucleotide base pairs. When “no more than” or “less than” is present before a series of numbers or a range, it is understood that each of the numbers in the series or range is modified.

[0043] As used herein, ranges include both the upper and lower limit.

[0044] In the event of a conflict between a sequence in the application and an indicated accession number or position in an accession number, the sequence in the application predominates.

[0045] As used herein, “detecting an analyte” and the like is understood as performing an assay in which the analyte can be detected, if present, wherein the analyte is present in an amount above the level of detection of the assay.

[0046] As used herein, it is understood that when the maximum amount of a value is represented by 100% (*e.g.*, 100% inhibition or 100% encapsulation) that the value is limited by the method of detection. For example, 100% inhibition is understood as inhibition to a

level below the level of detection of the assay, and 100% encapsulation is understood as no material intended for encapsulation can be detected outside the vesicles.

[0047] 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.

I. Definitions

[0048] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[0049] “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, 2' halide, or 2'-O-(2-methoxyethyl) (2'-O-moe) substitutions. An RNA may comprise one or more deoxyribose nucleotides, *e.g.* as modifications, and similarly a DNA may comprise one or more ribonucleotides. 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.*, N⁴-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, O⁶-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and O⁴-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

nucleosides with 2' methoxy substituents, or polymers containing both conventional nucleosides and one or more nucleoside analogs). Nucleic acid includes "locked nucleic acid" (LNA), an analog containing one or more LNA nucleotide monomers with a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhances hybridization affinity toward complementary RNA and DNA sequences (Vester and Wengel, 2004, *Biochemistry* 43(42):13233-41). Nucleic acid includes "unlocked nucleic acid" which enables the modulation of the thermodynamic stability and also provides nuclease stability. 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.

[0050] "Polypeptide" as used herein refers to a multimeric compound comprising amino acid residues that can adopt a three-dimensional conformation. Polypeptides include but are not limited to enzymes, enzyme precursor proteins, regulatory proteins, structural proteins, receptors, nucleic acid binding proteins, antibodies, *etc.* Polypeptides may, but do not necessarily, comprise post-translational modifications, non-natural amino acids, prosthetic groups, and the like.

[0051] "Guide RNA", "gRNA", and simply "guide" are used herein interchangeably to refer to, for example, either a single guide 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 strand (as a single guide RNA, sgRNA) or, for example, in two separate RNA strands (dual guide RNA, dgRNA). "Guide RNA" or "gRNA" refers to either an sgRNA or a dgRNA. The trRNA may be a naturally-occurring sequence, or may comprise modifications or variations. Such modifications or variations may be chemically induced.

[0052] 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 about 20 nucleotides in length, for example when used in combination with an RNA-guided DNA binding agent such as *Streptococcus pyogenes* (*i.e.*, "Spy") Cas9. Preferred guide sequence lengths for related Cas9 homologs/orthologs, including shorter or longer sequences can also be used and are known in the art.

[0053] For example, SpyCas9 guides can be 16, 17, preferably 18, 19, or 20 nucleotides in length such that, in some embodiments, the SpyCas9 guide sequence comprises 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs:1-5, optionally SEQ ID NO: 1 or 2. The degree of complementarity or identity between a guide sequence and

its corresponding target sequence is at least 80%, 85%, preferably 90%, or 95%, or is 100%. For example, in some embodiments, the guide sequence comprises a sequence of at least 16, 17, preferably 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs:1-5, optionally SEQ ID NO: 1 or 2. In some embodiments, the guide sequence and the target region is 100% complementary or identical. In other embodiments, the guide sequence and the target region may contain at least one mismatch, *i.e.*, one nucleotide that is not identical or not complementary, depending on the reference sequence. For example, the guide sequence and the target sequence may contain 1, 2, 3, or 4 mismatches within the duplex formed by the guide and the target sequence, where the total length of the target sequence is 16, 17, 18, 19, 20 nucleotides, or more. In some embodiments, the guide sequence and the target region may contain 1, 2, 3 or 4 mismatches where the guide sequence comprises at least 20 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. That is, the guide sequence and the target region may form a duplex region having 16, 17, 18, 19, 20 base pairs, or more. In certain embodiments, the duplex region may include 1, 2, 3, or 4 mismatches such that guide strand and target sequence are not fully complementary. For example, a guide strand and target sequence may be complementary over a 20-nucleotide region, including 2 mismatches, such that the guide sequence and target sequence are 90% complementary providing a duplex region of 18 base pairs out of 20. Tolerated mismatch positions are known in the art. For example, protospacer adjacent motif (PAM)-distal mismatches tend to be better tolerated than PAM-proximal matches, and mismatch tolerances at other positions have been characterized (see, *e.g.*, Sternberg et al., 2015, Nature:527:110-113).

[0054] Target sequences for RNA-guided DNA binding agents, as defined by the targeting sequence of a guide RNA, may be present on either the positive or negative strand. Tables and other disclosures provided herein may recite genomic coordinates as a target sequence. It is understood that the guide can be complementary to either the positive or negative strand of the DNA as defined by the genomic coordinates. The sequence to which the guide is complementary depends on the presence of an appropriate PAM for the RNA-guided DNA binding agent on the opposite strand. Thus, in some embodiments, the guide sequence binds the reverse complement of a target sequence, *i.e.*, the guide sequence is identical to certain nucleotides of the sense (positive) strand of the target sequence, when the PAM is present in the sense strand, except for the substitution of U for T in the guide sequence.

[0055] As used herein, an “RNA-guided DNA binding agent” or “RNA-guided DNA binding protein” 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 presence of a PAM and the sequence of the guide RNA. Exemplary RNA-guided DNA binding agents include Cas cleavases/nickases and inactivated forms thereof (*e.g.*, “dCas DNA binding agents”). “Cas nuclease”, as used herein, encompasses Cas cleavases, Cas nickases, and dCas DNA binding agents. Cas nickases include nucleases in which one of the RuvC or HNH domain of the Cas protein is mutated, such that only a single strand is cleaved by the nuclease. The dCas DNA binding agent may be a dead nuclease comprising non-functional nuclease domains (*i.e.*, a RuvC or HNH domain). In some embodiments, the Cas cleavase or Cas nickase encompasses a dCas DNA binding agent modified to permit DNA cleavage, *e.g.*, via fusion with a FokI domain.

[0056] Exemplary nucleotide and polypeptide sequences of Cas9 molecules are provided below. Methods for identifying alternate nucleotide sequences encoding Cas9 polypeptide sequences, including alternate naturally occurring variants, are known in the art. Sequences with at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to any of the Cas9 nucleic acid sequences, or nucleic acid sequences encoding the Cas9 amino acid sequences provided herein are also contemplated. In certain embodiments, the nucleotide sequence encoding the Cas9 amino acid sequence is not a naturally occurring Cas9 nucleotide sequence. Sequences with at least 95%, 96%, 97%, 98%, or 99% identity to any of the Cas9 amino acid sequences provided herein are also contemplated. In certain embodiments, the Cas9 amino acid sequence is not a naturally occurring Cas9 amino acid sequence.

[0057] Exemplary open reading frames for Cas9 are provided in **Table 12** below.

[0058] The term “linker,” as used herein, refers to a chemical group or a molecule linking two adjacent molecules or moieties. Typically, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond. In some embodiments, the linker is an amino acid or a plurality of amino acids (*e.g.*, a peptide or protein). Exemplary peptide linkers are disclosed elsewhere herein.

[0059] “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, *e.g.*, N1-methyl-psuedouridine.

[0060] “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.

[0061] 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 the target sequence 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.

[0062] As used herein, a “control” is understood as an appropriate matched sample or subject for comparison. For example, a control can be a cell population treated in the same manner as the test population except that the treatment used for the control population lacks at least one active agent, *e.g.*, a guide RNA, an mRNA encoding a nuclease, an insertion construct, or a lipid formulation. In certain embodiments, a control may be an internal control, *e.g.*, a cell population or subject prior to treatment.

[0063] In certain embodiments, a “control” as in a control subject is a comparator for a measurement, *e.g.*, a diagnostic measurement of a sign or symptom of a disease. In certain embodiments, a control can be a subject sample from the same subject at an earlier time point, *e.g.*, before a treatment intervention. In certain embodiments, a control can be a measurement from a normal subject, *i.e.*, a subject not having the disease of the treated subject, to provide a normal control, *e.g.*, an enzyme concentration or activity in a subject sample. In certain embodiments, a normal control can be a population control, *i.e.*, the average of subjects in the general population. In certain embodiments, a control can be an untreated subject with the same disease. In certain embodiments, a control can be a subject treated with a different therapy, *e.g.*, the standard of care. In certain embodiments, a control can be a subject or a population of subjects from a natural history study of subjects with the

disease of the subject being compared. In certain embodiments, the control is matched for certain factors to the subject being tested, *e.g.*, age, gender. In certain embodiments, a control may be a control level for a particular lab, *e.g.*, a clinical lab. The ability to design or select appropriate controls is within the ability of those of skill in the art. It is understood when relative values are provided, they can be considered as relative values as compared to an appropriate control.

[0064] As used herein, “subject” includes primates, including human and non-human primates, mouse, and rat. In certain embodiments, the subject is a human subject. In certain embodiments, the subject is a non-human subject. In certain embodiments, the subject is a non-human subject expressing one or more human genes, *e.g.*, a transgenic mouse expressing a human gene, or a mouse in which the liver has been repopulated with human hepatocytes. Such models are well known in the art.

[0065] As used herein, a “target sequence” refers to a sequence of nucleic acid in a target gene, in either the positive or the negative strand, that has complementarity to the guide sequence or the spacer sequence of the gRNA, *i.e.*, that is sufficiently complementary to the guide sequence to permit specific binding of the guide sequence. 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. The specific length of the target sequence and the number of mismatches possible between the target sequence and the guide sequence depend, for example, on the identity of the Cas nuclease being directed by the gRNA.

[0066] As used herein, a first sequence is considered to be “identical” or have “100% identity” with a second sequence if an alignment of the first sequence to the second sequence shows that all of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAG has 100% identity to the sequence AAGA because an alignment would give 100% identity in that there are matches, without gaps, to all three positions of the first sequence. Less than 100% identity can be calculated using routine methods. For example, ACG would have 67% identity with AAGA as two of the three positions of the first sequence are matches to the second sequence ($2/3 = 67\%$). 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.

[0067] Similarly, as used herein, a first sequence is considered to be “fully complementary” or “100% complementary” to a second sequence when all of the nucleotides of a first sequence are complementary to a second sequence, without gaps. For example, the sequence UCU would be considered to be fully complementary to the sequence AAGA as each of the nucleobases from the first sequence basepair with the nucleotides of the second sequence, without gaps. The sequence UGU would be considered to be 67% complementary to the sequence AAGA as two of the three nucleobases of the first sequence basepair with nucleobases of the second sequence. One skilled in the art will understand that algorithms are available with various parameter settings to determine percent complementarity for any pair of sequences using, *e.g.*, the NCBI BLAST interface (blast.ncbi.nlm.nih.gov/Blast.cgi) or the Needleman-Wunsch algorithm.

[0068] “Messenger RNA” or “mRNA” is used herein to refer to a polynucleotide that 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 one or more chemically modified nucleosides such as 5-methyl-cytidine (5mC), 2-thio-uridine (2sU), N1-methylpseudouridine (m1 ψU) and pseudo-uridine (ψU), or a modified cap structure as provided below.

[0069] Exemplary guide sequences useful in the guide RNA compositions and methods described herein are shown in **Table 1** and throughout the application. For example, where **Table 1** shows a guide sequence, this guide sequence may be used in a guide RNA to direct an RNA-guided DNA binding agent, *e.g.*, a nuclease, such as a Cas nuclease, such as Cas9, to a target sequence. Target sequences are provided in **Table 1** as genomic coordinates, and include both the positive and negative strands of genomic DNA (*i.e.*, the sequence given and

the sequence's reverse complement). 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, except for the substitution of U for T in the guide sequence.

[0070] 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. As used herein, when indel formation results in an insertion, the insertion is a random insertion at the site of a double-stranded break and is not directed by or based on a template sequence.

[0071] As used herein, "inhibit expression" and the like refer to a decrease in expression (*e.g.*, knockdown or knockout) of a particular gene product (*e.g.*, protein, mRNA, or both). Expression of a protein (*i.e.*, gene product) can be measured by detecting total cellular amount of the protein from a tissue sample, *e.g.*, biopsy, or cell population of interest by detecting expression of a protein in individual members of a population of cells, *e.g.*, by cell sorting to define percent of cells expressing a protein, or expression of a protein in cells in aggregate, *e.g.*, by ELISA or western blot. Inhibition of expression can result from genetic modification of a gene sequence, *e.g.*, a genomic sequence, such that the full-length gene product, or any gene product, is no longer detected, *e.g.*, knockdown of the gene. Certain genetic modifications can result in the introduction of frameshift or nonsense mutations that prevent translation of the full-length gene product. Genetic modifications at a splice site, *e.g.*, at a position sufficiently close to a splice acceptor site or a splice donor site to disrupt splicing, can prevent translation of the full-length protein. Inhibition of expression can result from a genetic modification in a regulatory sequence within the genomic sequence required for the expression of the gene product, *e.g.*, a promoter sequence, a 3' UTR sequence, *e.g.*, a capping sequence, a 5' UTR sequence, *e.g.*, a poly A sequence. Inhibition of expression may also result from disrupting expression or activity of regulatory factors required for translation of the gene product, *e.g.*, production of no gene product. For example, a genetic modification in a transcription factor sequence, inhibiting expression of the full-length transcription factor, can have downstream effects and inhibit expression of one or more gene products controlled by the transcription factor. Inhibition of expression can be predicted by changes in genomic or mRNA sequences. Mutations expected to result in inhibition of expression can be detected by known methods including next generation sequencing of DNA isolated from a tissue sample or cell population of interest. Inhibition of expression can be determined as the percent of cells in a population having a predetermined level of expression of a protein, *i.e.*, a

reduction of the percent or number of cells in a population expressing a protein of interest at least a certain level. Inhibition of expression can also be assessed by determining a decrease in overall protein level, *e.g.*, in a cell or tissue sample, *e.g.*, a biopsy sample. In certain embodiments, inhibition of expression of a secreted protein can be assessed in a fluid sample, *e.g.*, cell culture media or a body fluid. Proteins may be present in a body fluid, *e.g.*, blood or urine, to permit analysis of protein level. In certain embodiments, protein level may be determined by protein activity or the level of a metabolic product, *e.g.*, in urine or blood. In some embodiments, “inhibition of expression” may refer to some loss of expression of a particular gene product, for example a decrease in the amount of an mRNA or a protein expressed in a tissue sample or by a population of cells. In some embodiments, “inhibition” may refer to some loss of expression of a particular gene product, for example at the cell surface or secreted into a bodily fluid, *e.g.*, blood. In some embodiments, “inhibition” may refer to some loss of expression in one, or more, cell or tissue types, but not all cell or tissue types, *e.g.*, inhibition of expression in liver, but not in other organs. It is understood that the level of inhibition of expression is relative to a starting level, a reference level, or a control level, in the same type of subject sample. For example, routine monitoring of a protein level may be performed in a fluid sample from a subject, *e.g.*, blood or urine, or in a tissue sample, *e.g.*, a biopsy sample. In certain embodiments, a correlation is known, or established, wherein the level of a biomarker, *e.g.*, in blood or urine, is correlated with the level of inhibition of expression of a target gene. It is understood that the level of inhibition of expression is for the sample being assayed. Similarly, in animal studies where serial tissue samples may be obtained, *e.g.*, liver tissue, the target may be expressed in other tissues. Therefore, the level of inhibition of expression is not necessarily the level of inhibition of expression systemically, but within the tissue, cell type, or fluid being sampled.

[0072] As used herein, a “genetic modification” is a change at the DNA level, *e.g.*, induced by a CRISPR/Cas9 gRNA and Cas9 system. A genetic modification may comprise an insertion, deletion, or substitution (*i.e.*, base sequence substitution, *i.e.*, mutation), typically within a defined sequence or genomic locus. A genetic modification changes the nucleic acid sequence of the DNA. A genetic modification may be at a single nucleotide position. A genetic modification may be at multiple nucleotides, *e.g.*, 2, 3, 4, 5 or more nucleotides, typically in close proximity to each other, *e.g.*, contiguous nucleotides. A genetic modification can be in a coding sequence, *e.g.*, an exon sequence. A genetic modification can be at a splice site, *i.e.*, sufficiently close to a splice acceptor site or a splice donor site to disrupt splicing. A genetic modification can include insertion of a nucleotide sequence not

endogenous to the genomic locus, *e.g.*, insertion of a coding sequence of a heterologous open reading frame or gene. As used herein, a genetic modification can be used to prevent translation of an endogenous full-length protein having an amino acid sequence of the full-length protein prior to genetic modification of the genomic locus. Prevention of translation of a full-length protein or gene product includes prevention of translation of a protein or gene product of any length. Translation of an endogenous full-length protein can be prevented, for example, by a frameshift mutation that results in the generation of a premature stop codon or by generation of a nonsense mutation. Translation of an endogenous full-length protein can be prevented by disruption of splicing. Translation of a full-length protein can be prevented by the insertion of a heterologous coding sequence. Translation of an endogenous full-length protein, *e.g.*, when the endogenous full-length protein contains an unwanted mutation, can be prevented by making a change at one or more positions to change an endogenous full-length protein coding sequence to provide a modified full-length coding sequence different from the endogenous sequence present in the cell, *e.g.*, correction of a point mutation. Translation of an endogenous full-length protein can be prevented by altering the splicing of the endogenous full-length protein to produce a different protein by alternative splicing.

[0073] “Treatment” as used herein is understood as reducing at least one sign or symptom of the disease or indication. Reduction can include to a frequency or severity such that the sign or symptom of the disease is no longer detectable. Treatment can include administration of more than one dose of the agent. Treatment can include administration with other agents. Effective treatment does not require a cure or complete elimination of the disease or indication. The rate of progression or development of a disease can be compared to the progression or development of a disease in an appropriately matched control, *e.g.*, a population control, a control from a natural history study. As used herein, “delivering” and “administering” are used interchangeably.

[0074] Co-administration, as used herein, means that a plurality of substances are administered sufficiently close together in time so that the agents act together. Co-administration encompasses administering substances together in a single formulation and administering substances in separate formulations close enough in time so that the agents act together.

[0075] As used herein, the phrase “pharmaceutically acceptable” means that which is useful in preparing a pharmaceutical composition that is generally non-toxic and is not biologically undesirable and that are not otherwise unacceptable for pharmaceutical use. Pharmaceutically acceptable generally refers to substances that are non-pyrogenic.

Pharmaceutically acceptable can refer to substances that are sterile, especially for pharmaceutical substances that are for injection or infusion.

[0076] As used herein, “*ANGPTL3*” refers to the nucleic acid sequence or protein sequence of “Angiopoietin-like protein 3” or “Angiopoietin-like 3.” The human wild-type *ANGPTL3* sequence is available at NCBI Gene ID: 27329 (available on the World Wide Web at ncbi.nlm.nih.gov/gene/?term=27329, in the version available on the date of filing the instant application); Ensembl: ENSG00000132855

MIM: 604774, Chromosome 1: 62,597,520-62,606,313. Synonyms for *ANGPTL3* include *ANL3*, *ANG-5*, *FHBL2*, and *ANGPT5*. The *ANGPTL3* gene encodes a member of a family of secreted proteins that function in angiogenesis. The encoded protein undergoes a processing event in which it formed into an N-terminal coiled-coil domain-containing chain and a C-terminal fibrinogen chain. It is expressed predominantly in the liver and is processed into domains that regulate lipid and glucose metabolism. Certain mutations of *ANGPTL3* have been associated with Hypobetalipoproteinemia Familial 2 and Atherosclerosis susceptibility. Single nucleotide polymorphisms and other variations of the human *ANGPTL3* sequence can be found, for example, on the World Wide Web at ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=27329.

[0077] As used herein, the term “within the genomic coordinates” includes the boundaries of the genomic coordinate range given. For example, if chr1: 62,597,520-62,606,313 is given, the coordinates chr1:2,597,520 and chr1:62,606,313 are encompassed. Throughout this application, the referenced genomic coordinates are based on genomic annotations in the GRCh38 (also referred to as hg38) assembly of the human genome from the Genome Reference Consortium, available at the National Center for Biotechnology Information website. Tools and methods for converting genomic coordinates between one assembly and another are known in the art and can be used to convert the genomic coordinates provided herein to the corresponding coordinates in another assembly of the human genome, including conversion to an earlier assembly generated by the same institution or using the same algorithm (*e.g.*, from GRCh38 to GRCh37), and conversion of an assembly generated by a different institution or algorithm (*e.g.*, from GRCh38 to NCBI33, generated by the International Human Genome Sequencing Consortium). Available methods and tools known in the art include, but are not limited to, NCBI Genome Remapping Service, available at the National Center for Biotechnology Information website, UCSC LiftOver, available at the UCSC Genome Browser website, and Assembly Converter, available at the Ensembl.org website.

II. Compositions

Compositions Comprising Guide RNA (gRNAs)

[0078] Provided herein are compositions useful for altering a DNA sequence, e.g., inducing a single-stranded (SSB) or double-stranded break (DSB), within a *ANGPTL3* gene, e.g., using a guide RNA with an RNA-guided DNA binding agent (e.g., a CRISPR/Cas system). Exemplary guide sequences targeting a *ANGPTL3* gene are shown in **Table 1** as SEQ ID NOs: 1-5, as are the genomic coordinates that such guide RNA targets.

[0079] Each of the guide sequences shown in **Table 1** as SEQ ID NOs: 1-5 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: 301) in 5' to 3' orientation.

[0080] In the case of an sgRNA, the above guide sequences may further comprise additional nucleotides to form an sgRNA, e.g., with the following exemplary nucleotide sequence following the 3' end of the guide sequence:

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU
GAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 303) in 5' to 3' orientation.

[0081] In the case of an sgRNA, the above guide sequences may further comprise additional nucleotides to form an sgRNA, e.g., with the following exemplary nucleotide sequence following the 3' end of the guide sequence:

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU
GAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 302) in 5' to 3' orientation.

[0082] In the case of an sgRNA, the guide sequences may be integrated into the following modified motif:

mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUAGAmGmCmUmAmGmAmAmAmU
mAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAm
AmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
(SEQ ID NO: 601), where "N" may be any natural or non-natural nucleotide, preferably an RNA nucleotide; sugar moieties of the nucleotide can be ribose, deoxyribose, or similar compounds with substitutions; m is a 2'-O-methyl modified nucleotide, and * is a phosphorothioate linkage to the adjacent nucleotide residue; and wherein the N's are collectively the nucleotide sequence of a guide sequence. In the context of a modified sequence, A, C, G, N, and U are unmodified RNA nucleotides, i.e., a 2'-OH sugar moiety with a phosphodiesterase linkage to the adjacent nucleotide residue, or a 5'-terminal PO₄.

[0083] In the case of an sgRNA, the guide sequences may further comprise a SpyCas9 sgRNA sequence. An example of a SpyCas9 sgRNA sequence is shown in the table below (SEQ ID NO: 303: GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC – “Exemplary SpyCas9 sgRNA-1”), included at the 3’ end of the guide sequence, and provided with the domains as shown in **Table 13** below. LS is lower stem. B is bulge. US is upper stem. H1 and H2 are hairpin 1 and hairpin 2, respectively. Collectively H1 and H2 are referred to as the hairpin region. A model of the structure is provided in Figure 10A of WO2019237069 which is incorporated herein by reference.

[0084] The nucleotide sequence of Exemplary SpyCas9 sgRNA-1 may serve as a template sequence for specific chemical modifications, sequence substitutions and truncations.

[0085] In certain embodiments, the gRNA is an sgRNA or a dgRNA, and it optionally comprises a chemical modification. In some embodiments, the modified sgRNA comprises a guide sequence and a SpyCas9 sgRNA sequence, *e.g.*, Exemplary SpyCas9 sgRNA-1. A gRNA, such as an sgRNA, may include modifications on the 5’ end of the guide sequence or on the 3’ end of the SpyCas9 sgRNA sequence (*e.g.*, Exemplary SpyCas9 sgRNA-1) at one or more of the terminal nucleotides, *e.g.*, at 1, 2, 3, or 4 of the nucleotides at the 5’ end or at the 3’ end. In certain embodiments, the modified nucleotide is selected from a 2’-O-methyl (2’-OME) modified nucleotide, a 2’-O-(2-methoxyethyl) (2’-O-moe) modified nucleotide, a 2’-fluoro (2’-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, or an inverted abasic modified nucleotide, or a combination thereof. In certain embodiments, the modified nucleotide includes a 2’-OME modified nucleotide. In certain embodiments, the modified nucleotide includes a PS linkage. In certain embodiments, the modified nucleotide includes a 2’-OME modified nucleotide and a PS linkage.

[0086] In certain embodiments, using SEQ ID NO: 303 (“Exemplary SpyCas9 sgRNA-1” as shown in **Table 13**) as an example, the Exemplary SpyCas9 sgRNA-1 further includes one or more of:

A. a shortened hairpin 1 region, or a substituted and optionally shortened hairpin 1 region, wherein

1. at least one of the following pairs of nucleotides are substituted in the substituted and optionally shortened hairpin 1 region with Watson-Crick pairing nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, or H1-4 and H1-9, and the hairpin 1 region optionally lacks

- a. any one or two of H1-5 through H1-8,
 - b. one, two, or three of the following pairs of nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, and H1-4 and H1-9, or
 - c. 1-8 nucleotides of the hairpin 1 region; or
 - 2. the shortened hairpin 1 region lacks 4-8 nucleotides, preferably 4-6 nucleotides; and
 - a. one or more of positions H1-1, H1-2, or H1-3 is deleted or substituted relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 303) or
 - b. one or more of positions H1-6 through H1-10 is substituted relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 303); or
 - 3. the shortened hairpin 1 region lacks 5-10 nucleotides, preferably 5-6 nucleotides, and one or more of positions N18, H1-12, or N is substituted relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 303); or
- B. a shortened upper stem region, wherein the shortened upper stem region lacks 1-6 nucleotides and wherein the 6, 7, 8, 9, 10, or 11 nucleotides of the shortened upper stem region include less than or equal to 4 substitutions relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 303); or
- C. a substitution relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 303) at any one or more of LS6, LS7, US3, US10, B3, N7, N15, N17, H2-2 and H2-14, wherein the substituent nucleotide is neither a pyrimidine that is followed by an adenine, nor an adenine that is preceded by a pyrimidine; or
- D. an Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 303) with an upper stem region, wherein the upper stem modification comprises a modification to any one or more of US1-US12 in the upper stem region, wherein
- 1. the modified nucleotide is optionally selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, an inverted abasic modified nucleotide, or a combination thereof; or
 - 2. the modified nucleotide optionally includes a 2'-OMe modified nucleotide.

[0087] In certain embodiments, the Exemplary SpyCas9 sgRNA-1 lacks 6 nucleotides in shortened hairpin 1.

[0088] In certain embodiments, the Exemplary SpyCas9 sgRNA-1 lacks 8 nucleotides in shortened hairpin 1.

[0089] In certain embodiments, in the Exemplary SpyCas9 sgRNA-1, H-1 and H-3 are deleted.

[0090] In certain embodiments, the Exemplary SpyCas9 sgRNA-1 further comprises a 3' tail. In certain embodiments, the 3' tail is 1-4 nucleotides in length, optionally 1 nucleotide in length.

[0091] In certain embodiments, the Exemplary SpyCas9 sgRNA-1 comprises an upper stem region comprising a modification to any one or more of US1-US12 in the upper stem region.

[0092] In certain embodiments, Exemplary SpyCas9 sgRNA-1, or an sgRNA, such as an sgRNA comprising an Exemplary SpyCas9 sgRNA-1, further includes a 3' tail, *e.g.*, a 3' tail of 1, 2, 3, 4, or more nucleotides. In certain embodiments, the tail includes one or more modified nucleotides. In certain embodiments, the modified nucleotide is selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a 2' deoxy (2'H-) modified nucleotide, an abasic nucleotide, a locked nucleic acid (LNA) nucleotide, an unlocked nucleic acid (UNA) nucleotide, a phosphorothioate (PS) linkage between nucleotides, a terminal inverted abasic modified nucleotide, or a combination thereof. In certain embodiments, the modified nucleotide includes a 2'-OMe modified nucleotide. In certain embodiments, the modified nucleotide includes a PS linkage between nucleotides. In certain embodiments, the modified nucleotide includes a 2'-OMe modified nucleotide and a PS linkage between nucleotides.

[0093] In certain embodiments, the hairpin region includes one or more modified nucleotides. In certain embodiments, the modified nucleotide is selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, an inverted abasic modified nucleotide, or a combination thereof. In certain embodiments, the modified nucleotide includes a 2'-OMe modified nucleotide.

[0094] In certain embodiments, the upper stem region includes one or more modified nucleotides. In certain embodiments, the modified nucleotide selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides,

an inverted abasic modified nucleotide, or a combination thereof. In certain embodiments, the modified nucleotide includes a 2'-OMe modified nucleotide.

[0095] In certain embodiments, the Exemplary SpyCas9 sgRNA-1 comprises one or more YA dinucleotides, wherein Y is a pyrimidine, optionally wherein the YA dinucleotide includes a modified nucleotide. In certain embodiments, the modified nucleotide selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, an inverted abasic modified nucleotide, or a combination thereof. In certain embodiments, the modified nucleotide includes a 2'-OMe modified nucleotide.

Table 13: Exemplary spyCas9 sgRNA-1 (SEQ ID NO: 303)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
G	U	U	U	U	A	G	A	G	C	U	A	G	A	A	A	U	A	G	C	A	A	G	U	U	A	A	A	A	U
LS1-LS6						B1-B2		US1-US12								B3-B6				LS7-LS12									
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
A	A	G	G	C	U	A	G	U	C	C	G	U	U	A	U	C	A	A	C	U	U	G	A	A	A	A	A	G	U
Nexus (N1-N18)																		H1-1 through H1-12											
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76														
G	G	C	A	C	C	G	A	G	U	C	G	G	U	G	C														
H2-1 through H2-15																													
N																													

Table 1: *ANGPTL3* guide sequences and chromosomal coordinates

Guide ID	Strand	Guide Target Sequence	SEQ ID NO	Genomic Coordinates (hg38)
G016571	-	UUGUUUAUAUUGGUCUCCA	1	chr1:62598744-62598764
G028723	-	UUGUUUAUAUUGGUCUCCA	1	chr1:62598744-62598764
G016568	+	CAAAGACCUUCUCCAGACCG	2	chr1:62598724-62598744
G028720	+	CAAAGACCUUCUCCAGACCG	2	chr1:62598724-62598744
G016569	-	GGUCUCCACGGUCUGGAGA	3	chr1:62598733-62598753
G028721	-	GGUCUCCACGGUCUGGAGA	3	chr1:62598733-62598753
G016551	-	CUAUGGAGUAUAUCUUCUCU	4	chr1:62603987-62604007
G016554	-	UGUUUCCCGGGAUUGCAUUG	5	chr1:62604146-62604166

Table 2: Exemplary unmodified and modified sgRNA sequences targeting *ANGPTL3*

Guide ID	Unmodified guide	SEQ ID NO	Modified guide	SEQ ID NO
G016571	UUGUUUAUAUUG GUCUCCAGUUU UAGAGCUAGAAA UAGCAAGUAAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	101	mU*mU*mG*UUUAUAUUGG UCUCCAGUUUUAGAmGmC mUmAmGmAmAmUmAmG mCAAGUAAAAUAAGGCUA GUCCGUUAUCAmAmCmUmU mGmAmAmAmAmAmGmUmG mGmCmAmCmCmGmAmGmU mCmGmGmUmGmCmU*mU*m U*mU	201
G028723	UUGUUUAUAUUG GUCUCCAGUUU UAGAGCUAGAAA UAGCAAGUAAAA AUAAGGCUAGUC CGUUAUCACGAA AGGGCACCGAGU CGGUGCU	102	mU*mU*mG*UUUAUAUUGG UCUCCAGUUUUAGAmGmC mUmAmGmAmAmUmAmG mCAAGUAAAAUAAGGCUA GUCCGUUAUCACGAAAGGG CACCGAGUCGmU*mG*mC* mU	202
G016568	CAAAGACCUUCU CCAGACCGGUUU UAGAGCUAGAAA UAGCAAGUAAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	103	mC*mA*mA*AGACCUUCUCC AGACCGGUUUUAGAmGmCm UmAmGmAmAmUmAmGm CAAGUAAAAUAAGGCUAG UCCGUUAUCAmAmCmUmU mGmAmAmAmAmAmGmUmG mGmCmAmCmCmGmAmGmU mCmGmGmUmGmCmU*mU*m U*mU	203
G028720	CAAAGACCUUCU CCAGACCGGUUU UAGAGCUAGAAA UAGCAAGUAAAA AUAAGGCUAGUC CGUUAUCACGAA AGGGCACCGAGU CGGUGCU	104	mC*mA*mA*AGACCUUCUCC AGACCGGUUUUAGAmGmCm UmAmGmAmAmUmAmGm CAAGUAAAAUAAGGCUAG UCCGUUAUCACGAAAGGGC ACCGAGUCGmU*mG*mC* mU	204
G016569	GGUCUCCACGG UCUGGAGAGUUU UAGAGCUAGAAA UAGCAAGUAAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	105	mG*mG*mU*CUUCCACGGUC UGGAGAGUUUUAGAmGmC mUmAmGmAmAmUmAmG mCAAGUAAAAUAAGGCUA GUCCGUUAUCAmAmCmUmU mGmAmAmAmAmAmGmUmG mGmCmAmCmCmGmAmGmU mCmGmGmUmGmCmU*mU*m U*mU	205

Guide ID	Unmodified guide	SEQ ID NO	Modified guide	SEQ ID NO
G028721	GGUCUUCCACGG UCUGGAGAGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCACGAA AGGGCACCGAGU CGGUGCU	106	mG*mG*mU*CUUCCACGGUC UGGAGAGUUUAGAmGmC mUmAmGmAmAmUmAmG mCAAGUUAAAAUAAGGCUA GUCCGUUAUCACGAAAGGG CACCGAGUCGmU*mG*mC* mU	206
G016551	CUAUGGAGUAUA UCUUCUCUGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	107	mC*mU*mA*UGGAGUAUAU CUUCUCUGUUUAGAmGmC mUmAmGmAmAmUmAmG mCAAGUUAAAAUAAGGCUA GUCCGUUAUCAmAmCmUmU mGmAmAmAmAmGmUmG mGmCmAmCmCmGmAmGmU mCmGmGmUmGmCmU*mU*m U*mU	207
	CUAUGGAGUAUA UCUUCUCUGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCACGAA AGGGCACCGAGU CGGUGCU	108	mC*mU*mA*UGGAGUAUAU CUUCUCUGUUUAGAmGmC mUmAmGmAmAmUmAmG mCAAGUUAAAAUAAGGCUA GUCCGUUAUCACGAAAGGG CACCGAGUCGmU*mG*mC* mU	208
G016554	UGUUUCCGGGA UUGCAUUGGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCAACUU GAAAAAGUGGCA CCGAGUCGGUGC UUUU	109	mU*mG*mU*UUUCCGGGAU UGCAUUGGUUUUAGAmGmC mUmAmGmAmAmUmAmG mCAAGUUAAAAUAAGGCUA GUCCGUUAUCAmAmCmUmU mGmAmAmAmAmGmUmG mGmCmAmCmCmGmAmGmU mCmGmGmUmGmCmU*mU*m U*mU	209
	UGUUUCCGGGA UUGCAUUGGUUU UAGAGCUAGAAA UAGCAAGUUAAA AUAAGGCUAGUC CGUUAUCACGAA AGGGCACCGAGU CGGUGCU	110	mU*mG*mU*UUUCCGGGAU UGCAUUGGUUUUAGAmGmC mUmAmGmAmAmUmAmG mCAAGUUAAAAUAAGGCUA GUCCGUUAUCACGAAAGGG CACCGAGUCGmU*mG*mC* mU	210

[0096] Within the above tables, in the context of an unmodified sequence, A, C, G, U, and N are, independently, any natural or non-natural adenine, cytosine, guanine, uridine, and any nucleotide (e.g., A, C, G, or U), respectively. In the context of a modified sequence, m is

indicative of a 2'-O-methyl modified nucleotide; * is indicative of a phosphorothioate internucleotide linkage; and A, C, G, U, and N are RNA nucleotides, *i.e.*, 2'-OH and phosphodiesterase linkage to the 3' nucleotide, when present.

[0097] In some embodiments, a composition comprising one or more guide RNAs (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 *ANGPTL3* is provided. In some embodiments, an engineered cell comprising a genetic modification in a human *ANGPTL3* sequence within genomic coordinates of chr1: 62598724...62604166 is provided. In some embodiments, an engineered cell comprising a genetic modification in a human *ANGPTL3* sequence is provided, wherein the genetic modification comprises a modification of at least one nucleotide within the genomic coordinates corresponding to a *ANGPTL3* guide sequence selected from SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2. In some embodiments, an engineered cell comprising a genetic modification in a human *ANGPTL3* sequence is provided, wherein the genetic modification comprises a modification of at least one nucleotide within the genomic coordinates selected from **Table 1**.

[0098] In some embodiments comprising a gRNA, the gRNA may comprise a crRNA comprising a guide sequence shown in **Table 1**. In some embodiments, the gRNA comprises a guide sequence shown in **Table 1**, *e.g.*, as an sgRNA. In some embodiments, the gRNA may comprise a guide sequence selected from SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2.

[0099] The gRNA may comprise a guide sequence comprising 18, 19, or 20 contiguous nucleotides of a guide sequence shown in **Table 1**, *e.g.*, SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2. In some embodiments, the gRNA comprises a guide sequence with at least 90%, or 95%, or 100% identity to a guide sequence shown in **Table 1**, *e.g.*, SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2. In each embodiment described herein, the gRNA may comprise a crRNA and trRNA associated as a single RNA (sgRNA) or 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.

[00100] In each embodiment described herein, the guide RNA may comprise two non-covalently linked RNA strands 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 an RNA duplex via the base pairing between portions of the crRNA and the trRNA.

[00101] In each embodiment 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**, or a guide sequence selected from SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2, covalently linked to a trRNA.

[00102] The sgRNA may comprise 18, 19, or 20 contiguous nucleotides of a guide sequence shown in **Table 1**, or a guide sequence selected from SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2. 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.

[00103] The guide RNA compositions provided herein are designed to recognize (*e.g.*, hybridize to) a target sequence in a *ANGPTL3* gene. For example, the *ANGPTL3* 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 a *ANGPTL3* 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.

[00104] In some embodiments, the Spy guide sequence is at least 90% or 95%; or 100% identical to the reverse complement of a target sequence present in a human *ANGPTL3* gene. In some embodiments, the target sequence is complementary to the guide sequence of the Spy guide RNA. In some embodiments, the degree of complementarity or identity between a guide sequence of a Spy guide RNA and its corresponding target sequence is at least 90%, or 95%; or 100%. In some embodiments, the target sequence and the guide sequence of the Spy gRNA is 100% complementary or identical.

[00105] In some embodiments, the target sequence and the guide sequence of the Spy gRNA may contain at least one mismatch. For example, the target sequence and the guide sequence of the Spy gRNA may contain 1 or 2 mismatches, where the total length of the guide sequence is 20.

[00106] In some embodiments, the Spy guide sequence comprises a sequence of at least 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2.

[00107] In some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding a SpyCas9 cleavase as described herein. In some embodiments, an mRNA comprising an ORF encoding a SpyCas9 cleavase, is provided, used, or administered.

Modified gRNAs and mRNAs

[00108] As provided herein, the gRNA may be 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 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 that is synthesized with a non-canonical nucleoside or nucleotide, is herein 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 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) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); and (iv) modification of nucleotides at the 3' end or 5' end of the oligonucleotide, *e.g.*, to provide exonuclease stability, *e.g.*, with 2' O-me, 2' halide, or 2' deoxy substituted ribose; or inverted abasic terminal nucleotide, or replacement of phosphodiester with phosphothioate.

[00109] Chemical modifications such as those listed above can be combined to provide modified gRNAs 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 certain embodiments, phosphate groups of a 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.

[00110] In some embodiments, the gRNA comprises one, two, three or more modified residues. In some embodiments, at least 5% (*e.g.*, at least 5%, 10%, 15%, preferably at least 20%, 25%, 30%, 35%, 40%, 45%, or 50%) of the positions in a modified gRNA are modified

nucleosides or nucleotides. In some embodiments, at least 5% of the positions in the modified guide RNA are modified nucleotides or nucleosides. In some embodiments, at least 10% of the positions in the modified guide RNA are modified nucleotides or nucleosides. In some embodiments, at least 15% of the positions in the modified gRNA are modified nucleotides or nucleosides. In some embodiments, preferably at least 20% of the positions in the modified gRNA are modified nucleotides or nucleosides. In some embodiments, no more than 80% of the positions in the modified gRNA are modified nucleotides. In some embodiments, no more than 70% of the positions in the modified gRNA are modified nucleotides. In some embodiments, no more than 60% of the positions in the modified gRNA are modified nucleotides. In some embodiments, 10-80% of the positions in the modified gRNA are modified nucleotides. In some embodiments, 20-80% of the positions in the modified gRNA are modified nucleotides.

[00111] 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 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.

[00112] Examples of modified phosphate groups include, phosphorothioate, borano phosphate esters, methyl phosphonates, phosphoroamidates, phosphodithioate, 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), or carbon (bridged methylenephosphonates). The replacement can occur at either linking oxygen or at both of the linking oxygens.

[00113] The phosphate group can be replaced by non-phosphorus containing connectors in certain backbone modifications, *e.g.*, an amide linkage. 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, carboxymethyl, carbamate, amide, and thioether. Further examples of moieties which can replace the phosphate group can include, without limitation, *e.g.*, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, methyleneimino, methylenemethylimino, methylenehydrazo, methylenedimethylhydrazo, and methyleneoxymethylimino.

[00114] 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.

[00115] The modified nucleosides and modified nucleotides can include one or more modifications to the sugar group, *i.e.*, a 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.

[00116] 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, or 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 the 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 the amino can be, *e.g.*, NH_2 ; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino,

heteroaryl-amino, or diheteroaryl-amino, ethylenediamine, or poly-amino). In some embodiments, the 2' hydroxyl group modification can include "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), (OCH₂CH₂OCH₃, *e.g.*, a PEG derivative). 2' modifications can include hydrogen (*i.e.*, deoxyribose sugars); halo (*e.g.*, bromo, chloro, fluoro, or iodo); amino (wherein the amino can be, *e.g.*, NH₂; alkyl-amino, dialkyl-amino, heterocycl-yl, aryl-amino, diaryl-amino, heteroaryl-amino, diheteroaryl-amino, or amino acid); NH(CH₂CH₂NH)_nCH₂CH₂-amino (wherein the amino can be, *e.g.*, an amino 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.

[00117] 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. As used herein, a single abasic sugar is not understood to result in a discontinuity of a duplex.

[00118] In certain embodiments, 2' modifications include, for example, 2'-OMe, 2'-F, or 2'-H, optionally 2'-O-Me.

[00119] 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 modified nucleobase. Examples of nucleobases include, but are not limited to, adenine (A), guanine (G), cytosine (C), and uridine (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 a pyrimidine analog. In some embodiments, the nucleobase can include, for example, naturally-occurring and synthetic derivatives of a base.

[00120] In some embodiments, the guide RNAs disclosed herein comprise one of the modification patterns disclosed in WO2018/107028, the contents of which are herein incorporated by reference in their entirety. In some embodiments, the guide RNAs disclosed

herein comprise one of the structures/modification patterns disclosed in US20170114334, the contents of which are herein incorporated by reference in their entirety. In some embodiments, the guide RNAs disclosed herein comprise one of the structures/modification patterns disclosed in WO2017/136794, the contents of which are herein incorporated by reference in their entirety. In some embodiments, the guide RNAs disclosed herein comprise one of the structures/modification patterns disclosed in WO2018/107028 or WO2019/237069, the contents of each of which are herein incorporated by reference in their entirety. In some embodiments, the guide RNAs disclosed herein comprise one of the structures/modification patterns disclosed in WO2021/119275, the contents of which are herein incorporated by reference in their entirety.

[00121] In some embodiments, the sgRNA comprises any of the modification patterns shown herein, where N is any natural or non-natural nucleotide, and wherein the totality of the N's comprise an *ANGPTL3* guide sequence as described herein in **Table 1**. In some embodiments, the modified sgRNA comprises the following sequence:
 mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU (SEQ ID NO: 601), wherein the totality of N's comprise an *ANGPTL3* guide sequence as described in **Table 1**, for example, wherein the N's are replaced with any of the guide sequences disclosed herein in **Table 1**, optionally wherein the N's are replaced with SEQ ID NOs: 1-5, optionally with SEQ ID NO: 1 or 2.

[00122] In some embodiments, the sgRNA comprises any of the modification patterns shown herein, wherein N is any natural or non-natural nucleotide, and wherein the totality of the N's comprise an *ANGPTL3* guide sequence as described herein in **Table 1**. In some embodiments, the modified sgRNA comprises the following sequence:
 mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCG G*mU*mG*mC (SEQ ID NO: 607), wherein the totality of N's comprise an *ANGPTL3* guide sequence as described in **Table 1**, for example, wherein the N's are replaced with any of the guide sequences disclosed herein in **Table 1**, optionally wherein the N's are replaced with SEQ ID NO: 1-5, optionally with SEQ ID NO: 1 or 2.

[00123] In some embodiments, the sgRNA comprises any of the modification patterns shown herein, where N is any natural or non-natural nucleotide, and wherein the totality of the N's comprise an *ANGPTL3* guide sequence as described herein in **Table 1**. In some

embodiments, the modified sgRNA comprises the following sequence:

mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUm

AmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCACGAAAG

GGCACCGAGUCGmU*mG*mC*mU (SEQ ID NO: 612), wherein the totality of N's comprise an *ANGPTL3* guide sequence as described in **Table 1**, for example, wherein the N's are replaced with any of the guide sequences disclosed herein in **Table 1**, optionally wherein the N's are replaced with SEQ ID NOs: 1-5, optionally with SEQ ID NO: 1 or 2.

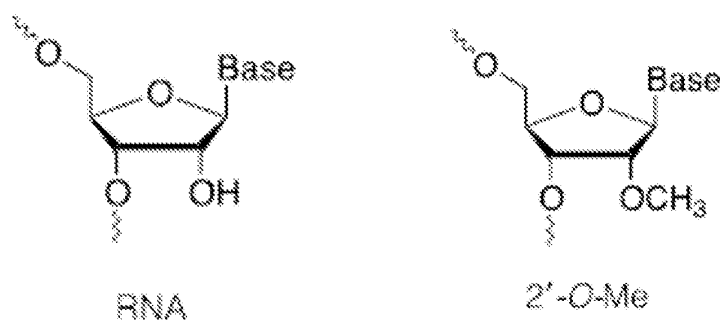
[00124] In some embodiments, the sgRNA comprises (*e.g.*, consists of) a sequence selected from **Table 2**. In some embodiments, the sgRNA comprises (*e.g.*, consists of) the nucleotide sequence selected from SEQ ID NOs: 102, 101, 104, and 103. In some embodiments, the guide RNA comprises (*e.g.*, consists of) the modified nucleotide sequence selected from SEQ ID NOs: 202, 201, 204, and 203.

[00125] Any of the modifications described below may be present in the gRNAs and the mRNAs described herein.

[00126] In the context of chemically modified sequences, "A," "C," "G," "U," or "N" denote, respectively, an adenine, cytosine, guanine, uridine, or any nucleobase containing RNA nucleotide, *i.e.*, 2'-OH with a phosphodiesterase linkage to the 3' nucleotide.

[00127] The terms "mA," "mC," "mU," or "mG" are used to denote a nucleotide that has been modified with 2'-O-Me.

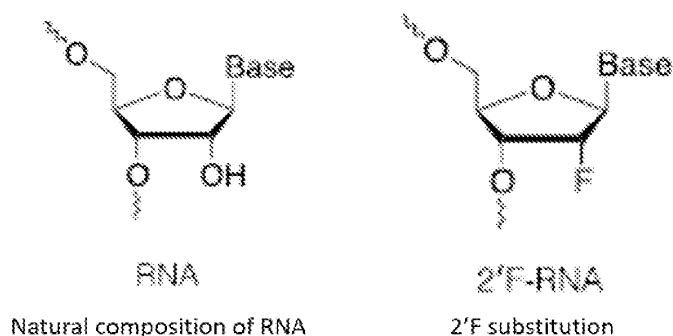
[00128] Modification with 2'-O-methyl can be depicted as follows:



[00129] 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.

[00130] In this application, the terms "fA," "fC," "fU," or "fG" are used to denote a nucleotide that has been modified with 2'-F.

[00131] Substitution with 2'-F can be depicted as follows:

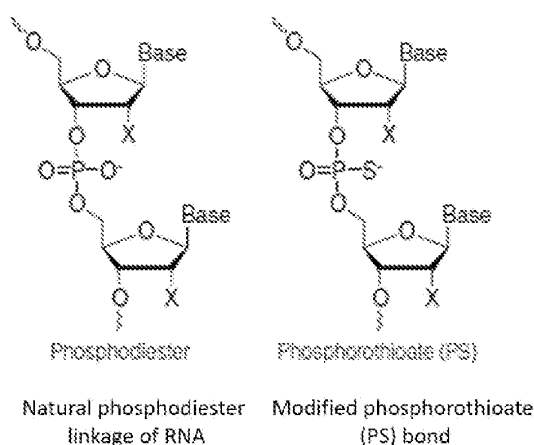


[00132] Phosphorothioate (PS) linkage or bond refers to a bond where a sulfur is substituted for one non-bridging phosphate oxygen in a phosphodiester linkage, for example in the bonds between nucleotide bases. When phosphorothioates are used to generate oligonucleotides, the modified oligonucleotides may also be referred to as S-oligos.

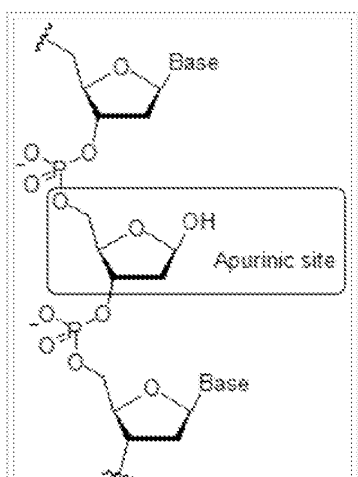
[00133] A “*” is used to denote a PS modification. In this application, the terms A*, C*, U*, or G* may be used to denote a nucleotide that is linked to the next (*e.g.*, 3') nucleotide with a PS bond.

[00134] In this application, the terms “mA*,” “mC*,” “mU*,” or “mG*” are used to denote a nucleotide that has been modified with 2'-O-Me and that is linked to the next (*e.g.*, 3') nucleotide with a PS bond.

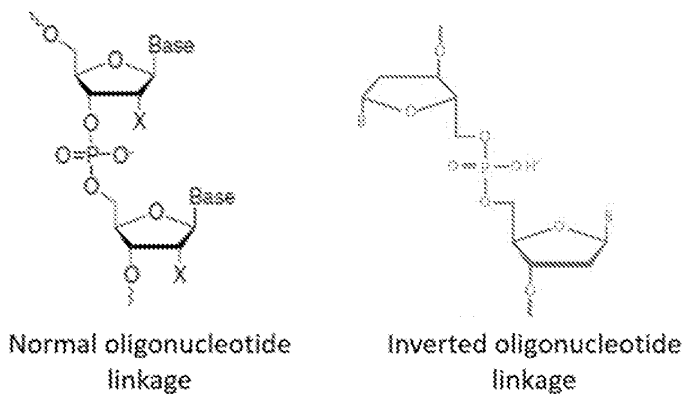
[00135] The diagram below shows the substitution of S- into a non-bridging phosphate oxygen, generating a PS bond in lieu of a phosphodiester bond:



[00136] 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. As used herein, the presence of a single abasic site is not considered to disrupt a duplex, *e.g.*, a duplex formed between the targeting sequence of a guide RNA and a target site in the genome:



[00137] 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). Such inverted bases can only be present as a terminal nucleotide. In chemical synthesis methods performed 3' to 5', inverted bases do not have 5' hydroxy available to grow the chain. For example:



[00138] 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.

[00139] 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 or performance.

[00140] 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.

[00141] 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.

[00142] In some embodiments, the guide RNA comprises a modified sgRNA. In some embodiments, the sgRNA comprises the modification pattern shown in mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU (SEQ ID NO: 601), wherein N is any natural or non-natural nucleotide, and wherein the totality of the N's comprise a guide sequence that directs a nuclease to a target sequence in *ANGPTL3*, e.g., the genomic coordinates shown in **Table 1**, e.g., SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2.

[00143] In some embodiments, the guide RNA comprises an sgRNA comprising any one of the guide sequences of SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2, and a conserved portion of an sgRNA, for example, the conserved portion of the sgRNA shown as Exemplary SpyCas9 sgRNA-1 or the conserved portions of the gRNAs shown in **Tables 3-4** and throughout the specification. In some embodiments, the guide RNA comprises an sgRNA comprising any one of the guide sequences of SEQ ID NOs: 1-5, optionally SEQ ID NO: 1 or 2, and the nucleotides of

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU
GAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 302) or the nucleotides of
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAA
AGGGCACCGAGUCGGUGCU (SEQ ID NO: 309), wherein the nucleotides are on the 3'
end of the guide sequence, and wherein the sgRNA may be modified as shown herein or in

the sequence

mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmU
mAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAm
AmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
(SEQ ID NO: 601) or in the sequence

mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmU
mAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCG
GmU*mG*mC*mU (SEQ ID NO: 612). In some embodiments, the sgRNA comprises
Exemplary SpyCas9 sgRNA-1 or the modified versions thereof provided herein, or a version
as provided in **Table 3B or 4B**, wherein the totality of the N's comprise a guide sequence
that directs a nuclease to a target sequence. Each N is independently modified or unmodified.
In certain embodiments, in the absence of an indication of a modification, the nucleotide is an
unmodified RNA nucleotide residue, *i.e.*, having a ribose sugar and a phosphodiester
backbone.

Table 3A: Exemplary unmodified SpyCas9 scaffold sequences

#mer	Unmodified nucleotide sequence	SEQ ID NO
42	GUUUUAGAGCUAUGCUGUUUUG	301
100	GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAG GCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCG AGUCGGUGCUUUU	302
96	GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAG GCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCG AGUCGGUGC	303
97	GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAG GCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCG AGUCGGUGCU	304
88	GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAG GCUAGUCCGUUAUCAACUUGGCACCGAGUCGGUG C	305
88	GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAG GCUAGUCCGUUAUCAAAAUGGCACCGAGUCGGUG C	306
89	GGUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAA GGCUAGUCCGUUAUCAAAAUGGCACCGAGUCGGU GC	307
90	GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAG GCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG UGC	308
91	GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAG GCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG UGCU	309

Table 3B: Exemplary unmodified SpyCas9 guide RNA sequences

#mer	Unmodified nucleotide sequence	SEQ ID NO
100	(N)20GUUUUAGAGCUAGAAAUAGCAAGUUAAAAU AAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUUUU	401
96	(N)20GUUUUAGAGCUAGAAAUAGCAAGUUAAAAU AAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGC	402
97	(N)20GUUUUAGAGCUAGAAAUAGCAAGUUAAAAU AAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCU	403
88	(N)20GUUUUAGAGCUAGAAAUAGCAAGUUAAAAU AAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCG GUGC	404
88	(N)20GUUUUAGAGCUAGAAAUAGCAAGUUAAAAU AAGGCUAGUCCGUUAUCAAUAUGGCACCGAGUCG GUGC	405
89	(N)20GGUUUUAGAGCUAGAAAUAGCAAGUUAAAA UAAGGCUAGUCCGUUAUCAAUAUGGCACCGAGUC GGUGC	406
90	(N)20GUUUUAGAGCUAGAAAUAGCAAGUUAAAAU AAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGU CGGUGC	407
91	(N)20GUUUUAGAGCUAGAAAUAGCAAGUUAAAAU AAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGU CGGUGCU	408

[00144] Wherein the Ns collectively are a guide sequence provided herein. Within the table, in the context of an unmodified sequence, A, C, G, U, and N are, independently, any natural or non-natural adenine, cytosine, guanine, uridine, and any nucleotide (e.g., A, C, G, or U), respectively.

Table 4A: Exemplary modified SpyCas9 guide scaffold sequences

#mer	Modified sequence	SEQ ID NO
100	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAm CmUmUmGmAmAmAmAmAmGmUmGmGmCmAmC mCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*m U	501
96	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAm CmUmUmGmAmAmAmAmAmGmUmGmGmCmAmC mCmGmAmGmUmCmGmGmUmGmC	502
97	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAm	503

#mer	Modified sequence	SEQ ID NO
	CmUmUmGmAmAmAmAmAmGmUmGmGmCmAmC mCmGmAmGmUmCmGmGmUmGmCmU	
88	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUA AAAUAAGGCUAGUCCGUUAUCAACU UGGCACCGAGUCGG*mU*mG*mC	504
88	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUA AAAUAAGGCUAGUCCGUUAUCAA UGGCACCGAGUCGG*mU*mG*mC	505
88	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAm AmAmUmGmGmCmAmCmGmAmGmUmCmGmG *mU*mG*mC	506
90	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUA AAAUAAGGCUAGUCCGUUAUCACGA AAGGGCACCGAGUCGG*mU*mG*mC	507
90	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUA AAAUAAGGCUAGUCCGUUAUCAmCm GmAmAmAmGmGmGmCmAmCmGmAmGmUmC mGmG*mU*mG*mC	508
91	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUA AAAUAAGGCUAGUCCGUUAUCACGA AAGGGCACCGAGUCGGU*mG*mC*mU	509
91	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUA AAAUAAGGCUAGUCCGUUAUCACGA AAGGGCACCGAGUCGGmUmGmC*mU	510
91	GUUUfUAGmAmGmCmUmAmGmAmAmUmAmG mCmAmAGUfUmAfAmAfAmUAmAmGmGmCmUmA GUmCmCGUfUAmUmCmCmGmAmAmAmGmGmG mCmAmCmCmGmAmGmUmCmGmGmU*mG*mC*m U	511
91	GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUA AAAUAAGGCUAGUCCGUUAUCACGA AAGGGCACCGAGUCGGmU*mG*mC*mU	512

Table 4B: Exemplary modified SpyCas9 guide sequences

#mer	Modified sequence	SEQ ID NO
100	mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUA AAAUAAAGGCUAGUCCGUUAUCAmAmCmUmUmG mAmAmAmAmAmGmUmGmGmCmAmCmCmGmAm GmUmCmGmGmUmGmCmU*mU*mU*mU	601
96	mN*mN*mN*NNNNNNNNNNNNNNNNNN GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAm CmUmUmGmAmAmAmAmAmGmUmGmGmCmAmC mCmGmAmGmUmCmGmGmUmGmC	602

#mer	Modified sequence	SEQ ID NO
97	mN*mN*mN*NNNNNNNNNNNNNNNNNN GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAm CmUmUmGmAmAmAmAmGmUmGmGmCmAmC mCmGmAmGmUmCmGmGmUmGmCmU	603
88	mN*mN*mN*NNNNNNNNNNNNNNNNNN GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACU UGGCACCGAGUCGG*mU*mG*mC	604
88	mN*mN*mN*NNNNNNNNNNNNNNNNNN GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCAA UGGCACCGAGUCGG*mU*mG*mC	605
88	mN*mN*mN*NNNNNNNNNNNNNNNNNN GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAm AmAmUmGmGmCmAmCmCmGmAmGmUmCmGmG *mU*mG*mC	606
90	mN*mN*mN*NNNNNNNNNNNNNNNNNNGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUA AAAUAAAGGCUAGUCCGUUAUCACGAAAGGGCA CCGAGUCGG*mU*mG*mC	607
90	mN*mN*mN*NNNNNNNNNNNNNNNNNN GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCAmCm GmAmAmAmGmGmGmCmAmCmCmGmAmGmUmC mGmG*mU*mG*mC	608
91	mN*mN*mN*NNNNNNNNNNNNNNNNNN GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCACGA AAGGGCACCGAGUCGGU*mG*mC*mU	609
91	mN*mN*mN*NNNNNNNNNNNNNNNNNN GUUUUAGAmGmCmUmAmGmAmAmUmAmGm CAAGUUAAAAUAAGGCUAGUCCGUUAUCACGA AAGGGCACCGAGUCGGmUmGmC*mU	610
91	mN*mN*mN*NNNNNNNNNNNNNNNNNN GUUUfUAGmAmGmCmUmAmGmAmAmUmAmG mCmAmAGUfUmAfAmAfAmUAmAmGmGmCmUmA GUmCmCGUfUmUmCmCmGmAmAmAmGmGmG mCmAmCmCmGmAmGmUmCmGmGmU*mG*mC*m U	611
91	mN*mN*mN*NNNNNNNNNNNNNNNNNNGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUA AAAUAAAGGCUAGUCCGUUAUCACGAAAGGGCA CCGAGUCGGmU*mG*mC*mU	612

[00145] wherein “m” indicates a 2’-O-Me modification, “f” indicates a 2’-fluoro modification, a “*” indicates a phosphorothioate linkage between nucleotides, and no

modification in the context of a modified sequence indicates an RNA (2'-OH) and a phosphodiesterase linkage to the 3' nucleotide when one is present.

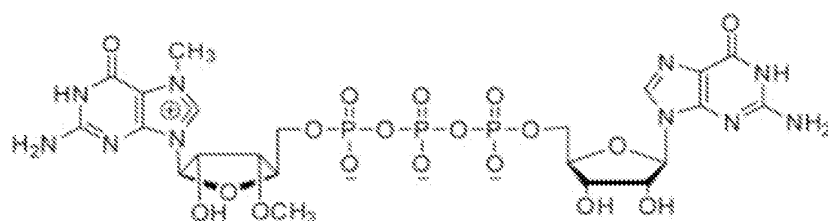
[00146] In certain embodiments, the chemically modified scaffold sequences of **Table 4A** further comprise a chemically modified targeting sequence. In certain embodiments, the chemically modified guide sequence is (mN*)₃(N)₁₃₋₁₇. In certain embodiments, the guide sequence is (mN*)₃(N)₁₇, *i.e.*, mN*mN*mN*NNNNNNNNNNNNNNNNNNNN. In certain embodiments, each N of the (N)₁₃₋₁₇ or the (N)₁₇ is unmodified. In certain embodiments, each N in the (N)₁₃₋₁₇ or the (N)₁₇ is independently modified, *e.g.*, independently modified with a 2'-O-methyl modification.

[00147] 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, *e.g.*, Cas9 nuclease, as described in **Table 12**. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, *e.g.*, Cas9 nuclease, is provided, used, or administered. In some embodiments, the ORF encoding an RNA-guided DNA binding agent 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.

[00148] In some embodiments, the mRNA or modified ORF may comprise a modified uridine at at least 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.

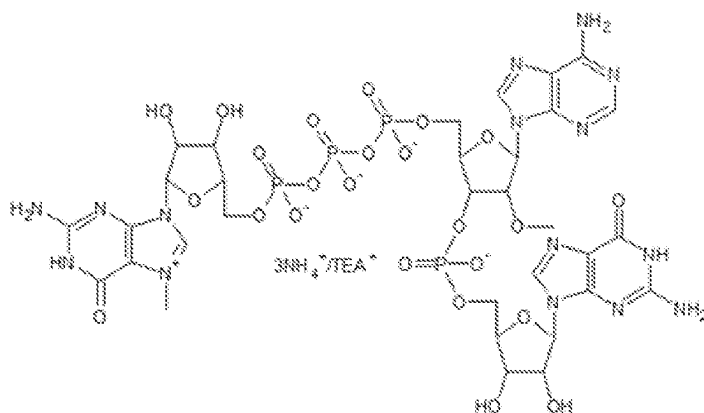
[00149] In some embodiments, an mRNA disclosed herein comprises a 5' cap, such as a Cap0, Cap1, or Cap2. A 5' cap is generally a 7-methylguanine ribonucleotide (which may be further modified, as discussed below *e.g.*, with respect to ARCA) linked through a 5'-triphosphate to the 5' position of the first 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; and 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.

[00150] 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.



[00151] 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, or CleanCap AU: TriLink Biotechnologies as Cat. Nos. N-7114. The CleanCap™ AG structure is shown below.



[00152] 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; and Mao, X. and Shuman, S. (1994) *J. Biol. Chem.* 269, 24472-24479.

[00153] 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 embodiments, the poly-A tail includes non-adenine nucleotides, *i.e.*, is an interrupted poly-A tail. In certain embodiments, the poly-A tail is interrupted by a non-adenine nucleotide about every 40, 50, 60, 70, 80, or 90 nucleotides. In certain embodiments, the poly-A tail is interrupted by a non-adenine nucleotide about every 50 nucleotides.

Ribonucleoprotein complex

[00154] In some embodiments, a composition is encompassed comprising one or more gRNAs comprising (1) one or more guide sequences from **Table 1**, or one or more sgRNAs from **Table 2**; and (2) a SpyCas9 cleavase.

[00155] 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 Cas nuclease is the Cas9 protein from the Spy CRISPR/Cas system. In some embodiments, the gRNA together with Cas9 is called a Cas9 RNP.

[00156] 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 or more than one HNH domain. In some embodiments, the Cas9 protein is a wild type Cas9. In each of the composition, system, kit, use, and method embodiments, the wild type Cas induces a double strand break in a target DNA.

[00157] In some embodiments, the RNA-guided DNA-binding agent comprises one or more heterologous functional domains (*e.g.*, is or comprises a fusion polypeptide).

[00158] 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 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1, 2, or 3NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with two 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 NLS is not linked to the C-terminus. In some embodiments, the NLS is inserted within the RNA-guided DNA binding agent sequence. In certain circumstances, at least two NLSs comprised in the RNA-guided DNA-binding agent are the same (*e.g.*, two SV40 NLSs). In certain embodiments, at least two different NLSs are present the RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA-binding agent is fused to two SV40 NLS sequences linked at the C-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: 1013) or PKKKRRV (SEQ ID NO: 1014). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 1015). In a specific embodiment, a single

PKKKRKV NLS (SEQ ID NO: 1013) 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.

[00159] 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).

Determination of efficacy of gRNAs

[00160] In some embodiments, the efficacy of a gRNA is determined when delivered or expressed together with other components, including a SpyCas9 cleavase, forming an RNPSpyCas9. In some embodiments, the gRNA is delivered to a cell as part of an RNP. In some embodiments, the gRNA is delivered to a cell along with an mRNA encoding a Cas9 cleavase.

[00161] As described herein, use of an RNA-guided DNA binding 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. In some embodiments, the efficacy of particular

gRNAs is determined based on *in vitro* models. In some embodiments, the *in vitro* model is a primary cell line, *e.g.*, primary hepatocytes. In some embodiments, the primary hepatocytes are primary human hepatocytes. With respect to using primary cells, commercially available primary cells 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 a primary hepatocyte) is determined, *e.g.*, by analyzing genomic DNA from cells transfected *in vitro* with Cas9 mRNA and the guide RNA. In some embodiments, such a determination comprises analyzing genomic DNA from the cells transfected *in vitro* with Cas9 mRNA, the guide RNA, and a donor oligonucleotide. Exemplary procedures for such determinations are provided in the working examples in which primary hepatocytes are used.

[00162] 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.

[00163] In some embodiments, the efficacy of a guide RNA is measured by percent indels or percent genetic modifications of *ANGPTL3*. In some embodiments, the efficacy of a guide RNA is measured by percent indels or percent genetic modifications at an *ANGPTL3* locus. In some embodiments, the efficacy of a guide RNA is measured by percent indels or percent genetic modifications of *ANGPTL3* at genomic coordinates of **Table 1**. In some embodiments, the percent editing of *ANGPTL3* is compared to the percent indels or genetic modifications necessary to achieve reduction, *e.g.*, knockdown, of the *ANGPTL3* protein products. In some embodiments, the efficacy of a guide RNA is measured by reduced expression of *ANGPTL3* protein. In some embodiments, said reduced expression of *ANGPTL3* protein is as measured by ELISA, *e.g.*, as described herein.

[00164] In some embodiments, the *ANGPTL3* protein expression is reduced in a population of cells using the methods and compositions disclosed herein. In some embodiments, the level of protein as determined, *e.g.*, by ELISA, is reduced by at least 55%, 60%, 65%, 70%, 75%, preferably at least 80%, 85%, 90%, or 95% relative to a control population of unmodified cells.

[00165] An “unmodified cell” (or “unmodified cells”) refers to a control cell (or cells) of the same type of cell in an experiment or test, wherein the “unmodified” control cell has not been contacted with an *ANGPTL3* guide. Therefore, an unmodified cell (or cells) may be a cell that

has not been contacted with a guide RNA, or a cell that has been contacted with a guide RNA that does not target *ANGPTL3*.

[00166] In some embodiments, the efficacy of a guide RNA is measured by the number or frequency of indels or genetic modifications at off-target sequences within the genome of the target cell type, such as a primary hepatocyte cell. 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 or relative to the frequency of indel creation at the target site. Thus, the disclosure provides guide RNAs which do not exhibit off-target indel formation in the target cell type (*e.g.*, a primary hepatocyte cell), or which produce a frequency of off-target indel formation of <5% in a cell population 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.*, primary hepatocyte cell) as compared to a control cell. In some embodiments, guide RNAs are provided which produce indels at less than 5 validated off-target sites, *e.g.*, as evaluated by one or more methods provided herein. In some embodiments, guide RNAs are provided which produce indels at less than or equal to 4, 3, 2, or 1 validated off-target site(s), *e.g.*, as evaluated by one or more methods provided 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.

Genetic modification for inhibition of target gene expression

[00167] In some embodiments, provided herein are engineered cells or population of cells comprising a genetic modification, *e.g.*, of an endogenous nucleic acid sequence encoding *ANGPTL3*.

[00168] In some embodiments, the engineered cells or population of cells comprise a genetic modification of an *ANGPTL3* gene as assessed by sequencing, *e.g.*, NGS, wherein at least 50%, 55%, 60%, 65%, 70%, 75%, preferably at least 80%, 85%, or 90% of cells comprise an insertion, deletion, or substitution in the endogenous *ANGPTL3* sequence. In some embodiments, at least 50% of cells in the population comprise a modification selected from an insertion, a deletion, and a substitution in the endogenous *ANGPTL3* sequence. In some embodiments, at least 80% of cells in the population comprise a modification selected from an insertion, a deletion, and a substitution in the endogenous *ANGPTL3* sequence. In some embodiments, at least 85% of cells in the population comprise a modification selected from an insertion, a deletion, and a substitution in the endogenous *ANGPTL3* sequence. In some embodiments, at least 90% of cells in the population comprise a modification selected

from an insertion, a deletion, and a substitution in the endogenous *ANGPTL3* sequence. In some embodiments, the cells in a population comprise hepatocytes in a liver. In some embodiments, *ANGPTL3* expression is decreased by at least 50%, 55%, 60%, 65%, 70%, 75%, preferably at least 80%, 85%, or 90%, as compared to a suitable control, *e.g.*, wherein the *ANGPTL3* gene has not been modified. In some embodiments, expression of *ANGPTL3* is decreased by at least 70%, or to below the limit of detection of the assay as compared to a suitable control, *e.g.*, wherein the *ANGPTL3* gene has not been modified. In some embodiments, expression of *ANGPTL3* is decreased by at least 75%, or to below the limit of detection of the assay as compared to a suitable control, *e.g.*, wherein the *ANGPTL3* gene has not been modified. In some embodiments, expression of *ANGPTL3* is decreased by at least 80%, or to below the limit of detection of the assay as compared to a suitable control, *e.g.*, wherein the *ANGPTL3* gene has not been modified. In some embodiments, expression of *ANGPTL3* is decreased by at least 85%, or to below the limit of detection of the assay as compared to a suitable control, *e.g.*, wherein the *ANGPTL3* gene has not been modified. In some embodiments, expression of *ANGPTL3* is decreased by at least 90%, or to below the limit of detection of the assay as compared to a suitable control, *e.g.*, wherein the *ANGPTL3* gene has not been modified. In some embodiments, expression of *ANGPTL3* is decreased by no more than 95%, as compared to a suitable control, *e.g.*, wherein the *ANGPTL3* gene has not been modified. Assays for *ANGPTL3* protein and mRNA expression are known in the art.

[00169] The target gene is genetically modified using a guide RNA with an RNA-guided DNA binding agent, resulting in inhibition of expression in a cell. In some embodiments, disclosed herein are cells engineered by inducing a break (*e.g.*, double-stranded break (DSB)) within target genes in the cells, *e.g.*, using a guide RNA with a SpyCas9 cleavase (*i.e.*, a CRISPR/Cas system). The methods may be used *in vitro*, *e.g.*, for screening guides.

III. Methods and Uses Including Therapeutic Methods and Uses of Genome Editing Agents

[00170] The gRNAs and associated systems, uses, methods, and compositions disclosed herein are useful for making genome editing therapeutic agents.

[00171] In some embodiments, the gRNAs comprising the guide sequences of **Table 1** together with a SpyCas9 cleavase induce DSBs, and non-homologous end joining (NHEJ) during repair leads to a modification, *e.g.*, a mutation, in an *ANGPTL3* gene. In some

embodiments, NHEJ leads to a deletion or insertion of a nucleotide(s), which induces a frame shift or nonsense mutation in an *ANGPTL3* gene. In certain embodiments, gRNAs comprising guide sequences targeted to target genomic sequences are also delivered to the cell together with a SpyCas9 cleavase, either together or separately, to make a genetic modification in a target genomic sequence to inhibit the expression of a full-length expression product from the target gene. In certain embodiments, the gRNAs are sgRNAs.

[00172] In some embodiments, the guide RNAs, compositions, systems, and formulations are used to produce a cell *in vivo*, *e.g.*, liver cell, *e.g.*, a hepatocyte with a genetic modification in an *ANGPTL3* gene. In some embodiments, the cell is in a subject.

[00173] In some embodiments, the subject is a mammalian. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human primate.

[00174] In some embodiments, the subject has or is at risk of having an *ANGPTL3*-related disease or condition. An "*ANGPTL3*-associated disease or condition", or an "*ANGPTL3*-related disease or condition" as used herein, is intended to include any disease or condition associated with the *ANGPTL3* gene or protein expression or activity. Such a disease or condition may be caused, for example, by excess production of the *ANGPTL3* protein, by *ANGPTL3* gene mutations, by abnormal cleavage of the *ANGPTL3* protein, or by abnormal interactions between *ANGPTL3* and other proteins or other endogenous or exogenous substances. Exemplary *ANGPTL3*-associated diseases include Hypobetalipoproteinemia Familial 2 and Atherosclerosis susceptibility. In some embodiments, an *ANGPTL3*-related disease or condition is selected from the group consisting of a cardiovascular disease and a metabolic disorder. In some embodiments, an *ANGPTL3*-related disease or condition includes, but is not limited to, hypercholesterolemia (*e.g.*, total blood cholesterol levels > 190mg/dl, or LDL-cholesterol levels > 100 mg/dl), familial hypercholesterolemia (FH), autosomal dominant hypercholesterolemia (ADH), autosomal recessive hypercholesterolemia (ARH), hyperlipidemia, hypertriglyceridemia, coronary artery disease, stroke, myocardial infarction, obesity, xanthoma, atherosclerosis, aortic stenosis, liver steatosis, high blood pressure, type 2 diabetes, and insulin resistance.

[00175] In certain embodiments, methods comprise instructing an end user, *e.g.*, a healthcare provider, or a subject, to administer an additional agent, such as that provided above, in conjunction with administration of a gRNA provided herein. In certain embodiments, an additional agent, *i.e.*, one or more additional agents, is administered in conjunction with, *e.g.*, before, at the time of, or after administration of, the gRNA and

SpyCas9 cleavase, until a desired clinical outcome is reached, *e.g.*, reduction of serum cholesterol or lipid.

[00176] In one aspect, the invention provides a method of treating a patient by selecting a patient on the basis that the patient is in need of LDL lowering, LDL lowering without lowering HDL, or total cholesterol lowering. The method includes administering to the patient a gRNA in an amount sufficient to lower the patient's LDL levels, *e.g.*, without substantially lowering HDL levels.

[00177] Genetic predisposition plays a role in the development of target gene associated diseases, *e.g.*, hypobetalipoproteinemia.

[00178] A healthcare provider, such as a doctor, nurse, or geneticist can take a family history before prescribing or administering a gRNA and SpyCas9 cleavase of the invention. In addition, a test may be performed to determine a genotype or phenotype. For example, a DNA test may be performed on a sample from the patient, *e.g.*, a blood sample, to identify the *ANGPTL3* genotype or phenotype before a *ANGPTL3* gRNA is administered to the patient. Variants in *ANGPTL3*, both pathogenic and benign, can be found, for example in the NCBI SNP database available on the World Wide Web at ncbi.nlm.nih.gov/snp/?LinkName=gene_snp&from_uid=27329.

Delivery of gRNA Compositions

[00179] Lipid nanoparticles (LNPs) are a well-known means for delivery of nucleotides and protein cargoes and may be used for delivery of the guide RNAs and Cas9 cleavases, and compositions disclosed herein *in vivo* and *in vitro*. In some embodiments, the LNPs deliver a nucleic acid cargo, a protein cargo, or a nucleic acid together with protein cargo.

[00180] In some embodiments, a method for delivering any one of the cells or populations of cells disclosed herein to a subject is provided, wherein the gRNA is delivered via an LNP *in vivo*. In some embodiments, the gRNA/LNP is also associated with a Cas9 or an mRNA encoding Cas9.

[00181] In some embodiments, a composition comprising any one of the gRNAs disclosed herein and an LNP is provided. In some embodiments, the composition further comprises a Cas9 or an mRNA encoding Cas9.

[00182] In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in preparing a medicament for treating a disease or disorder (*e.g.*, an *ANGPTL3*-associated disease or disorder).

[00183] In some embodiments, a method for delivering any one of the gRNAs disclosed herein *in vivo* is provided, wherein the gRNA is associated with an LNP. In some embodiments, the gRNA is not associated with an LNP. In some embodiments, the gRNA/LNP or gRNA is also associated with a Cas9 or an mRNA encoding Cas9.

[00184] 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 (LNP); see *e.g.*, WO2017/173054 and WO2021/222287, the contents of each of which are herein incorporated by reference in their entirety.

[00185] 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 and tolerances accepted within the art. 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.

IV. EXAMPLES

[00186] 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

1.1. *In vitro* transcription (“IVT”) of nuclease mRNA

[00187] Capped and polyadenylated mRNA containing N1-methyl pseudo-U was generated by *in vitro* transcription using routine methods. Typically, a DNA plasmid containing a T7 promoter, a sequence for transcription, and a polyadenylation region was linearized with XbaI per manufacturer’s protocol. The XbaI was inactivated by heating. The linearized plasmid was purified from enzyme and buffer salts. The IVT reaction to generate modified mRNA was performed by incubating at 37°C: 50 ng/μL linearized plasmid; 2-5 mM each of GTP, ATP, CTP, and N1-methyl pseudo-UTP (Trilink); 10-25 mM ARCA (Trilink);

5 U/μL T7 RNA polymerase; 1 U/μL murine RNase inhibitor (NEB); 0.004 U/μL inorganic *E. coli* pyrophosphatase (NEB); and 1x reaction buffer. TURBO DNase (Thermo Fisher) was added to a final concentration of 0.01U/μL, and the reaction was incubated at 37°C to remove the DNA template.

[00188] The mRNA was purified using a MegaClear Transcription Clean-up kit (Thermo Fisher) or a RNeasy Maxi kit (Qiagen) per the manufacturers' protocols. Alternatively, the mRNA was purified through a precipitation protocol, which in some cases was followed by HPLC-based purification. Briefly, after the DNase digestion, mRNA was purified using LiCl precipitation, ammonium acetate precipitation, and sodium acetate precipitation. 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. In a further alternative method, mRNA was purified with a LiCl precipitation method followed by further purification by tangential flow filtration. RNA concentrations were determined by measuring the light absorbance at 260 nm (Nanodrop), and transcripts were analyzed by capillary electrophoresis by Bioanalyzer (Agilent).

[00189] *Streptococcus pyogenes* ("Spy") Cas9 mRNA was generated from plasmid DNA encoding an open reading frame according to SEQ ID Nos: 1003 and 1006 (see sequences in **Table 14**). When the sequences cited in this paragraph are referred to below with respect to RNAs, it is understood that Ts should be replaced with Us (which can be modified nucleosides as described above). Messenger RNAs used in the Examples include a 5' cap and a 3' polyadenylation sequence, *e.g.*, up to 100 nts. Guide RNAs were chemically synthesized by commercial vendors or using standard *in vitro* synthesis techniques with modified nucleotides.

1.2. Preparation of LNP formulation containing sgRNA and Cas9 mRNA

[00190] In general, the lipid nanoparticle components were dissolved in 100% ethanol at various molar ratios. The RNA cargos (*e.g.*, Cas9 mRNA and sgRNA) 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 used contained ionizable lipid ((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), also called herein Lipid A, cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2K-DMG) (e.g., catalog # GM-020 from NOF, Tokyo, Japan) in a molar ratio of 50% Lipid A, 38% cholesterol, 9% DSPC, and 3% PEG2k-DMG. The LNPs were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. The LNPs used comprised a single RNA species such as Cas9 mRNA or an sgRNA. LNPs were similarly prepared with a mixture of Cas9 mRNA and a guide RNA.

[00191] The LNPs were prepared using a cross-flow technique utilizing impinging jet mixing of the lipid in ethanol with two volumes of RNA solution and one volume of water. First, the lipid in ethanol was mixed through a mixing cross with the two volumes of RNA solution. Then, a fourth stream of water was 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 buffer exchanged into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS) and concentrated as needed by methods known in the art. The resulting mixture was then filtered using a 0.2 μ m sterile filter. The final LNPs were characterized to determine the encapsulation efficiency, polydispersity index, and average particle size. The final LNP was stored at 4°C or -80°C until further use.

1.3. DNA isolation

[00192] Cells were harvested post-transfection at 72 hours. DNA was extracted from each well of a 96-well plate using 50 μ L/well QuickExtract DNA Extraction solution (Epicentre, Cat. QE09050) or Quick Extract (Lucigen, Cat. SS000035-D2) according to manufacturer's protocol.

1.4. Next-generation sequencing (“NGS”) and analysis for editing efficiency

[00193] 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. PCR primers were designed around the target site within the gene of interest (*e.g.*, *ANGPTL3*), and the genomic area of interest was amplified. Primer sequence design was done as is standard in the field.

[00194] Additional PCR was performed according to the manufacturer's protocols (Illumina) to add chemistry for sequencing. The amplicons were sequenced on an Illumina MiSeq instrument. The reads were aligned to the reference genome (*e.g.*, hg38) 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 or deletion (“indel”) was calculated.

[00195] The editing percentage (*e.g.*, the “editing efficiency” or “percent editing”) is defined as the total number of sequence reads with insertions or deletions (“indels”) over the total number of sequence reads, including wild type reads.

Example 2. *In vitro* editing efficiency and protein knockdown of ANGPTL3 guides in primary human hepatocytes

[00196] Five guide RNAs targeting ANGPTL3 were tested for editing efficiency and protein knockdown in primary human hepatocytes (PHH) (Donor 1) (Gibco/Thermo Fisher Lot: HU8284) using a dilution series in two separate experiments.

2.1. PHH Cell preparation

[00197] PHH were thawed in hepatocyte thawing medium. Thawed cells were centrifuged and supernatant was discarded. The pelleted cells were resuspended in Plating Media consisting of William's E Medium (Gibco, Cat. A12176-01) containing plating supplements dexamethasone + cocktail supplement A (Gibco, Cat. A15563, Combo Kit Cat. #CM3000) and FBS content (Gibco, Cat. A13450). Cells were counted and plated with a density of 33,000 cells/well on Bio-coat collagen I-coated 96-well plates (Corning, Ref #354407). Plated cells were allowed to settle and adhere for 4-6 hours in a tissue culture incubator at 37°C and 5% CO₂ atmosphere. After incubation, cells were checked for monolayer formation and were washed once with hepatocyte maintenance medium consisting of William's E Medium (Gibco, Cat. A12176-01) containing Cell Maintenance supplements dexamethasone + cocktail supplement B (Gibco, Cat. A15564, Combo Kit Cat. #CM4000) and incubated in Maintenance Media for 24 hours.

2.2. LNP treatment

[00198] LNPs were generally prepared as described in Example 1. The LNPs contained 50% Lipid A, 38% cholesterol, 9% DSPC, and 3% PEG2k-DMG by molar ratio. The LNPs were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA (SEQ ID NO: 1002) of 1:2 by weight. Each LNP was applied to cells using a 7-point dose response curve starting at 300 ng mRNA/100 μ l.

[00199] Upon treatment with LNPs, cells were incubated for 24 hours at 37°C in William's E Medium (Gibco, A1217601) with maintenance supplements and 3% fetal bovine serum. Samples were run in duplicate. After 72 hours, cells were harvested and analyzed by NGS as described in Example 1. EC50 values and mean percent editing results are shown in **Table 5A**. Dose response curves are plotted in **Fig. 1A**.

Table 5A: Percent editing of ANGPTL3 Guides in PHH donor 1 for selecting five guides

	G016551				G016554				G016568			
Guide concentration (nM)	Mean	SD	N	EC50	Mean	SD	N	EC50	Mean	SD	N	EC50
32.25	78.5	3.0	2	0.50	75.4	4.9	2	0.68	64.3	0.1	2	0.48
10.75	60.9	15.4	2		74.0	N/A	1		61.9	1.1	2	
3.58	68.5	5.7	2		56.2	N/A	1		49.9	5.7	2	
1.19	50.0	N/A	1		47.8	0.1	2		44.7	3.9	2	
0.40	32.9	N/A	1		33.2	2.3	2		35.0	1.2	2	
0.13	13.5	0.1	2		13.4	1.3	2		20.5	1.6	2	
0.04	2.7	0.1	2		4.9	1.3	2		12.1	0.1	2	
0.00	0.0	0.0	2		0.1	0.1	2		0.1	0.0	2	
	G016569				G016571							
Guide concentration (nM)	Mean	SD	N	EC50	Mean	SD	N	EC50				
32.25	60.6	2.5	2	0.62	65.0	0.5	2	0.49				
10.75	55.4	0.2	2		60.3	2.0	2					
3.58	48.5	3.1	2		52.7	2.5	2					
1.19	41.2	6.0	2		44.0	1.8	2					
0.40	25.4	5.3	2		31.8	0.6	2					
0.13	15.2	0.4	2		19.4	2.6	2					
0.04	7.9	2.3	2		7.6	2.5	2					
0.00	0.0	0.0	2		0.0	0.0	2					

2.3. Protein Knockdown in Dose Response

[00200] The edited cells were cultured with Cellartis Power Primary HEP Medium (Takara, Cat. Y20020) for 14 days with media changes every 2-3 days. Secreted ANGPTL3

levels in culture media were determined using a Human Angiopoietin-like 3 (ANGPTL3) DuoSet ELISA Kit (R&D Systems, Cat. DY3829) according to the manufacturer's protocol using 4 µg/mL final concentration of capture antibody. The plates were read on a Clariostar plate reader at an absorbance of 450 nm and a wavelength correction of 570 nm. ANGPTL3 levels were calculated by using a four-parameter logistic curve fit off the standard curve. Dose response curve for reduction of secreted ANGPTL3 protein (pg/mL) after treatment with guides targeted to ANGPTL3 are shown in **Fig. 1B**, and the data are shown in **Table 5B**. The samples were run in duplicate.

Table 5B: Secreted ANGPTL3 (huANGPTL3 pg/mL) in PHH donor 1 for selected five guides

	G016551			G016554			G016568		
Guide concentration (nM)	Mean	SD	N	Mean	SD	N	Mean	SD	N
46.50	250.4	164.7	2	523.9	74.9	2	92.7	131.1	2
15.50	146.1	206.7	2	276.7	47.6	2	176.5	99.9	2
5.17	404.8	112.5	2	469.0	92.3	2	247.1	144.3	2
1.72	3750.4	556.1	2	2266.5	235.1	2	2731.7	819.4	2
0.57	5409.6	869.2	2	3459.2	168.6	2	4014.2	661.7	2
0.06	3802.7	969.0	2	3947.5	391.0	2	4183.9	84.6	2
0.02	4152.5	106.3	2	3735.5	478.5	2	4223.2	15.0	2
	G016569			G016571					
Guide concentration (nM)	Mean	SD	N	Mean	SD	N			
46.50	96.2	136.1	2	284.8	23.0	2			
15.50	167.0	74.2	2	372.7	47.8	2			
5.17	144.8	81.0	2	299.8	37.9	2			
1.72	1523.1	298.1	2	1247.2	345.6	2			
0.57	2487.5	510.0	2	3540.7	700.9	2			
0.06	3908.2	9.0	2	4057.8	78.2	2			
0.02	3990.6	34.3	2	4098.3	19.7	2			

Example 3. *In vitro* editing and protein knockdown in primary human hepatocytes with dose response curve

[00201] Guide RNAs targeting ANGPTL3 were tested for editing efficacy and protein knockdown in primary human hepatocytes (PHH) (Gibco/Thermo Fisher Lot: HU8290 (donor 2), HU8300 (donor 3), HU8317 (donor 4)).

3.1. PHH Cell preparation

[00202] PHH were generally prepared as in Example 2.1.

3.2. LNP treatment

[00203] LNPs were generally prepared as described in Example 1. The LNPs contained 50% Lipid A, 38% cholesterol, 9% DSPC, and 3% PEG2k-DMG by molar ratio. The LNPs were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA (SEQ ID NO: 1002) of 1:2 by weight. Each LNP was applied to cells using an 8-point 3-fold dilution curve starting at 300 ng mRNA/100 μ l as shown in **Table 6**.

Table 6: Concentrations of guide and mRNA for dose response curve.

Guide concentration (nM)	mRNA concentration (ng)
46.50	300
15.50	100
5.17	33.33
1.72	11.11
0.57	3.70
0.19	1.24
0.06	0.41
0.02	0.14

[00204] Upon treatment with LNPs, cells were incubated for 24 hours at 37°C in William's E Medium (Gibco, A1217601) with maintenance supplements and 3% fetal bovine serum. Samples were run in quadruplet. After 72 hours, cells were harvested and analyzed by NGS as described in Example 1. EC50 values, calculated excluding the 46.50 nM dose of guide, and mean percent editing results are shown in **Tables 7A-C**. Dose response curves are plotted in **Figs. 2A-C**.

Table 7A: Editing efficiency and EC50 (nM) for selected guides in PHH donor 2

	G016571				G016568			
Guide concentration (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)
46.50	67.9	1.8	4	1.29	76.5	2.6	4	0.89
15.50	86.2	1.4	4		85.6	2.5	4	
5.17	82.5	3.2	4		83.4	1.8	4	
1.72	51.4	2.9	4		60.7	2.3	4	
0.57	21.6	1.4	4		29.8	0.9	4	
0.19	6.3	1.1	4		10.8	0.6	4	
0.06	1.2	0.2	4		2.7	0.5	4	
0.02	0.4	0.1	4		0.9	0.2	4	

Table 7B: Editing efficiency and EC50 (nM) for selected guides in PHH donor 3

	G016571				G016568			
Guide concentration (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)
46.50	62.2	3.0	4	0.99	72.5	4.9	4	0.38
15.50	79.3	6.6	3		73.3	1.5	3	
5.17	72.3	2.4	4		71.3	3.2	4	
1.72	50	3.3	4		59.3	3.1	4	
0.57	32.2	1.6	4		43.2	1.9	4	
0.19	13	0.5	4		24.2	0.8	4	
0.06	3.4	0.8	4		8.4	0.5	4	
0.02	0.9	0.2	4		2.7	0.9	4	

Table 7C: Editing efficiency and EC50 (nM) for selected guides in PHH donor 4

	G016571				G016568			
Guide concentration (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)
46.50	69.4	1.9	4	0.61	74.9	1.8	4	0.54
15.50	86.6	1.1	4		83.6	1.6	4	
5.17	83.4	0.6	4		79.5	0.5	4	
1.72	65.1	0.8	4		62.6	0.6	4	
0.57	40.9	1	4		42.8	1.7	4	
0.19	15.9	1.1	4		19	1.9	4	
0.06	3.6	0.5	4		5	0.2	4	
0.02	0.8	0.2	4		1.2	0.2	4	

3.3. Protein Knockdown in Dose Response

[00205] The edited cells were cultured as described in Example 2.3. Secreted ANGPTL3 levels in culture media were determined and read as in Example 2.3. ANGPTL3 levels were calculated by using a four-parameter logistic curve fit off the standard curve. Dose response curve for reduction of secreted ANGPTL3 protein (pg/mL) after treatment with guides targeted to ANGPTL3 are shown in **Figs. 3A-C**, and the data are shown in **Tables 8A-C**. The samples were run in quadruplet. EC50 values were calculated excluding the 46.50 nM dose of guide.

Table 8A: Secreted ANGPTL3 (huANGPTL3 pg/mL) in PHH donor 2 for selected guides

Guide (nM)	G016571				G016568			
	Mean	SD	N	EC50 (nM)	Mean	SD	N	EC50 (nM)
46.50	811.8	230.6	4	0.38	651.5	19.4	4	0.51
15.50	1166.0	54.7	4		714.0	281.2	4	
5.17	910.8	17.4	4		800.4	80.8	4	
1.72	1495.8	63.1	4		1302.2	140.9	4	
0.57	2740.1	66.2	4		2607.7	66.6	4	
0.19	2749.2	95.8	4		2832.4	108.2	4	
0.06	3647.2	51.7	4		3662.1	41.0	4	
0.02	4106.3	44.7	4		4100.4	15.3	4	

Table 8B: Secreted ANGPTL3 (huANGPTL3 pg/mL) in PHH donor 3 for selected guides

Guide (nM)	G016571				G016568			
	Mean	SD	N	EC50 (nM)	Mean	SD	N	EC50 (nM)
46.50	899.7	107.5	4	0.24	649.0	43.7	4	0.31
15.50	741.3	42.2	4		486.7	22.1	4	
5.17	855.6	37.6	4		529.7	10.6	4	
1.72	1250.9	10.9	4		1086.5	51.2	4	
0.57	1883.4	34.4	4		1789.4	62.0	4	
0.19	2749.2	95.8	4		2832.4	108.2	4	
0.06	3647.2	51.7	4		3662.1	41.0	4	
0.02	4106.3	44.7	4		4100.4	15.3	4	

Table 8C: Secreted ANGPTL3 (huANGPTL3 pg/mL) in PHH donor 4 for selected guides

Guide (nM)	G016571				G016568			
	Mean	SD	N	EC50 (nM)	Mean	SD	N	EC50 (nM)
46.50	59.7	73.3	4	0.35	0.0	0.0	4	0.31
15.50	322.2	33.2	4		271.7	47.6	4	

	G016571				G016568			
Guide (nM)	Mean	SD	N	EC50 (nM)	Mean	SD	N	EC50 (nM)
5.17	419.5	30.1	4		211.3	41.9	4	
1.72	964.1	109.7	4		1021.2	106.6	4	
0.57	1938.5	59.8	4		1498.7	52.1	4	
0.19	2749.2	95.8	4		2832.4	108.2	4	
0.06	3647.2	51.7	4		3662.1	41.0	4	
0.02	4106.3	44.7	4		4100.4	15.3	4	

Example 4. Evaluating Potency of 91-mer and 100-mer ANGPTL3 guides in a dilution series

[00206] Guide RNAs formatted as 91-mer or 100-mer targeting *ANGPTL3* were tested for editing efficacy in primary human hepatocytes (PHH) (Gibco/Thermo Fisher Lot: HU8300 (donor 3), HU8373A (donor 5), HU8284(donor 1)).

4.1. PHH Cell preparation

[00207] PHH cells were prepared as in Example 2.1.

4.2. LNP Treatment and Editing

[00208] LNPs were generally prepared as described in Example 1. The LNPs contained 50% Lipid A, 38% cholesterol, 9% DSPC, and 3% PEG2k-DMG by molar ratio. The LNPs were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA (SEQ ID NO: 1002) of 1:2 by weight. Each LNP was applied to cells using a 12-point dose response curve starting with a LNP dose of 450 ng.

[00209] Upon treatment with LNPs, cells were incubated for 24 hours at 37°C in William's E Medium (Gibco, A1217601) with maintenance supplements and 3% fetal bovine serum. Samples were run in quadruplet or sextuplicate. After 72 hours, cells were harvested and analyzed by NGS as described in **Example 1.4**. Dose response curves are plotted in **Figs. 4A-C**. EC50 values, calculated excluding the 46.50 nM dose of guide, and mean percent editing results are shown in **Tables 9A-C**.

Table 9A: Editing efficiency and EC50 (nM) for 91-mer and 100-mer guides in PHH donor 3

	G016571 (100-mer)				G028723 (91-mer)				Control			
Guide Concentration (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)
46.50	73.57	5.0	6	0.84	78.43	9.0	6	0.18	65.58	5.0	4	0.56
15.50	81.97	3.0	6		74.07	13.0	6		80.65	5.0	4	
5.17	72.50	12.0	6		80.70	6.0	6		77.10	4.0	4	
1.72	58.30	3.0	6		74.57	4.0	6		58.60	6.0	4	
0.57	32.87	5.0	6		63.78	5.0	6		44.48	2.0	4	
0.19	8.43	0.0	6		42.15	1.0	6		23.75	2.0	4	
0.06	1.72	0.0	6		13.68	2.0	6		8.25	1.0	4	
0.02	0.38	0.0	6		4.05	0.0	6		1.65	0.0	4	
0.007	0.15	0.0	6		0.57	0.0	6		0.40	0.0	4	
0.002	0.10	0.0	6		0.23	0.0	6		0.23	0.0	4	
0.001	0.12	0.0	6		0.12	0.0	6		0.23	0.0	4	
0.00	0.13	0.0	6		0.10	0.0	6		0.20	0.0	4	

Table 9B: Editing efficiency and EC50 (nM) for 91-mer and 100-mer guides in PHH donor 5

	G016571 (100-mer)				G028723 (91-mer)				Control			
Guide Concentration (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)
46.50	78.98	2.48	6	0.75	89.83	0.88	6	0.19	68.98	2.16	4	0.55
15.50	91.42	1.24	6		93.17	0.79	6		89.38	0.39	4	
5.17	89.18	0.78	6		92.70	0.76	6		85.90	0.95	4	
1.72	71.87	2.19	6		88.97	0.65	6		71.45	0.69	4	
0.57	37.77	4.14	6		77.37	1.80	6		47.30	0.67	4	
0.19	11.15	0.94	6		46.78	1.59	6		21.05	1.30	4	
0.06	2.12	0.40	6		16.53	0.88	6		6.98	0.92	4	
0.02	0.47	0.10	6		3.77	0.16	6		1.68	0.26	4	
0.007	0.13	0.05	6		0.72	0.15	6		0.43	0.13	4	
0.002	0.13	0.05	6		0.20	0.0	6		0.28	0.05	4	
0.001	0.10	0.0	6		0.10	0.0	6		0.23	0.05	4	
0.00	0.12	0.04	6		0.12	0.04	6		0.23	0.05	4	

Table 9C: Editing efficiency and EC50 (nM) for 91-mer and 100-mer guides in PHH donor 1

	G016571 (100-mer)				G028723 (91-mer)				Control			
Guide Concentration (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)
46.50	78.53	1.70	6	0.68	84.25	0.83		0.16	67.18	1.84	4	0.54
15.50	84.18	1.72	6		84.92	1.76	6		87.70	0.48	4	
5.17	81.47	0.96	6		86.15	1.59	6		84.88	1.25	4	
1.72	63.78	1.66	6		80.73	1.87	6		69.53	0.78	4	
0.57	40.13	2.40	6		68.53	1.30	6		47.38	2.45	4	
0.19	13.58	0.53	6		50.05	1.00	6		21.40	1.21	4	

	G016571 (100-mer)				G028723 (91-mer)				Control			
Guide Concentration (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)	Editing (%)	SD	N	EC50 (nM)
0.06	2.77	0.29	6		20.72	1.17	6		7.53	1.00	4	
0.02	0.50	0.11	6		5.62	0.27	6		2.05	0.45	4	
0.007	0.17	0.05	6		1.17	0.23	6		0.53	0.13	4	
0.002	0.15	0.05	6		0.33	0.08	6		0.23	0.05	4	
0.001	0.12	0.04	6		0.13	0.05	6		0.23	0.05	4	
0.00	0.12	0.04	6		0.12	0.04	6		0.20	0.0	4	

Example 5. Off-Target Analysis of ANGPTL3 Guides

5.1. Biochemical Off-Target Analysis

[00210] A biochemical method (See, *e.g.*, Cameron et al., *Nature Methods*. 6, 600-606; 2017) was used to determine potential off-target genomic sites cleaved by Cas9 using specific guides targeting *ANGPTL3*. Single guide RNAs targeting human *ANGPTL3* were screened using genomic DNA reference material NA24385 from the Coriell Institute alongside two control guides with known off-target profiles. The number of potential off-target sites was detected using a guide concentration of 48 nM and Cas9 protein concentration of 16 nM in the biochemical assay for which results are shown in **Table 10**.

Table 10: Biochemical Off-Target Analysis

Guide ID	Target Gene	Sites
G016568	<i>ANGPTL3</i>	14
G016554	<i>ANGPTL3</i>	40
G016569	<i>ANGPTL3</i>	52
G016571	<i>ANGPTL3</i>	55
G016551	<i>ANGPTL3</i>	56
G000644	<i>EMXI</i>	99
G000645	<i>VEGFA</i>	1021

Example 6. Validation of Off-Target Sites for Selected ANGPTL3 Guides

[00211] Test guides were further evaluated for possible off-target indel formation using amplicon sequencing at potential off-target sites following editing in cells. Each guide's respective potential off-target sites were identified by the biochemical assay described in **Example 5** or by *in silico* prediction.

6.1. Validation of Off-Target Sites for the 100-mer ANGPTL3 Guide

[00212] The 100-mer sgRNA targeting human *ANGPTL3* was evaluated for off-target validation with two primer sets in triplicates. Primary human hepatocytes (PHH, Gibco, Lot: Hu8284) were cultured as in Example 2.1 and were plated and transfected with LNPs comprising Cas9 mRNA and sgRNA. Each cell plate was treated via single dose transfection of 38.2 nM of guide (equivalent to 250 ng mRNA) to achieve dose saturation as required for further downstream off-target assays. DNA was isolated from the cells by lysing and subjected to NGS. Some potential off-target sites failed quality metrics and were not counted in the “sites characterized” tally for **Table 11**. Repair structures were manually inspected at loci with statistically relevant indel rates at the off-target cleavage sites to confirm indel repair structures.

Table 11: Evaluation of potential off-target editing sites for G016571

Guide ID	Sites evaluated	Sites characterized	Sites with statistically significant %indels
G016571 Primer Set 1	47	28	0
G016571 Primer Set 2	45	27	0

6.2. Validation of Off-Target Sites for the 91-mer *ANGPTL3* Guide

[00213] The 91-mer sgRNA targeting human *ANGPTL3* was evaluated for off-target validation in triplicates in two PHH donors. Primary human hepatocytes from two donors (PHH, Gibco, Lot: Hu8284, Hu8300) were plated and transfected with LNPs comprising Cas9 mRNA and sgRNA. Each cell plate was treated via single dose transfection of 38.2 nM of guide (equivalent to 250 ng mRNA) to achieve dose saturation as required for further downstream off-target assays. DNA was isolated from the cells by lysing and subjected to NGS. Some potential off-target sites failed quality metrics and were not counted in the “sites characterized” tally for **Table 12**. Repair structures were manually inspected at loci with statistically relevant indel rates at the off-target cleavage sites to confirm indel repair structures.

Table 12: Evaluation of potential off-target editing sites for G028723

Guide ID	Sites evaluated	Sites characterized	Sites with statistically significant %indels
G028723 Donor 1	129	120	0
G028723 Donor 3	129	119	0

	AGAUCCUGACCUUCCGGAUCCCUACUACGUGGGCCCCUUGGCCCGGGCAACUCCCGUUCGCUUGGAUACCCG GAAGUCCGAGGAGACCAUACACCCUUGGAACUUCGAGGAGGUGGUGACAAAGGCGCCUCCGCCACGACUUCUACU GAGCGAUAGACCAACUUCGACAAGAACUCCGCCAACGAGAGGUGUCUCCCAAGCAUCCUUGCUACGAGUACU UCACCGUACAAACGAGUGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGCCCGCCUUCUUGUCCGGCGA GCAGAAAGAGGCCAUUGGACCGUGUUAAGACCAACCGGACCGUGAACGAGCAGUAGAGGAGACUA CUUCAAAGAUUCGAGUUCGACUCCGUGGAGAUACCGGUGGGCGGCGUUCGCCGGAAGCUCAACGCCUCCUGGAC UACCAGACCUUGAAGAUCAUCAAAGGACAAGGACUUCUGGACAAACGAGGAGAACGAGGACAUCCUGGAGGACA UCGUGUGACCCUGACCCUUGCAGGACCGGAGAUCAAGAGGCGGUGAGAGCCUAGGACCUACGCCACCCUGUUCGA CGACAAGGUGAUGAAGAGCUGAAGCGGCGGCGUACACCGUGGGCGGCGUUCGCCGGAAGCUCAACGGC AUCCGGGACAAGCAGUCCGGCAAGACCAUCCUGGACUUCUGAAGUCCGACGGCUUCGCCAACCCGGAACUUCU AGCUGAUCCACGACGACUCCUGACCUUCAAAGGAGGACUCCAGAAAGCCAGGUGUCCGGCCAGGGCGACUCCU GCACGAGCAUUCGCCAACUUGGCCGCUCCCGCCAUCAAAGAGGGCAUCCUGCAGACCGUGAAGGUGGUGGAC GAGCUGGUGAAGGUGGCGGCAAGCCGAGAACAUUGUAGCAGAUUGGCGGGAGAACAGACCC AGAGGGCCAGAAACUCCCGGAGCGGAUAAACGGAUCGAGGAGGGAUCAAAGGAGCUUGGCUCCAGAUCC UGAAGGAGACCCCGUGGAGAACCCAGCUGCAGAACGAGAAAGCUGAACCUACCUAGCAGAACGGCCGGGA CAUGUACGUGGACAGGAGCUGGACAUCAACCGGCUUCGACUACGACGUGGACCAUUCGUGCCCCAGUCCUUC CUGAAGGACGACUCCAUCCGACAACAAAGGUGCUGACCCGUGCCGACAAGAACCGGGGCAAGUCCGACAACGUGCCU CCGAGGAGGUGGUGAAGAGAUAGAAACUACUGGGCGAGCUGAACGAGAACCUACCUAGCAGAACGCCGGAAGU UCGACAAACUGACCAAGGCCGAGCGGGCGCCUUGUCCGAGCUGGACAAGGCCGCUUCAAACGCGGACGUGGU GGAGACCCGGCAGAUCAACAGCAGUGGGCCAGAUCCUGGACUCCCGGAUGAACACCAAGUACGACGAGACGAG AAGCUGAUCCGGGAGGUGAAGGUGAUACCCUGAAGUCCAAAGCUGGUGUCCGACUCCGGAAGACUCCAGUUCU ACAAGGUGCGGGAGAUCAACACUACCAACCGCCACGACGCCUACCUAAGCCGUGGUGGGCACCGCCCGUAGU CAAGAAGUACCCAAACUGGAGUCCGAGUUCGUGUACGGGCAUACAAGGUGUACGACGUGCGGGAAGAUAGUCGCC AAGUCCGAGCAGGAGUCCGCAAGGCCACCGCCAGUACUUCUACUCCAAACUACAAGACUUCUCAAAGACCG AGAUACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCUUGAUCCGAGACCAACGCGGAGACCGCGAGAUCCGUG GGAACAAGGGCCGGAUUCGCCACCGUGCGGAAGGUGUCCUAGCCCGAGGUAACUCCGUAAGAAAGACCGAG GUGCAGACCGCGCGCUUCUCCAAAGAGUCCAUCCUGCCCAAGCGGAACUCCGACAAGCUGAUCCGCCGGAAGAG ACUGGGACCCCAAGAAUACGGCGGUUCGACUCCCGCCACCGUGGCCUACUCCGUGGUGGGCCAAAGGUGGA GAAAGGCAAGUCCAAAGAGCUGAAUCCGUGAAGGAGCUGUGGGCAUACAUAUAGGAGCGGUCCUUCUUCGAG AAGAACCCCAUCGACUUCUGGAGGCCAAGGGCUACAAGGAGGUGAAGAGGACCUCAUAACUGGCCAAGU ACUCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGAUGGUGUCCGCGCGGCGAGCUGCAGAAGGGCAACGAGCU GGCCUUGCCUCCAGUACGUGAACUUCUACCUUGCCUCCACUACGAGAAAGCUGAAGGGCUCCCGCGAGGAC AACGAGCAGAAAGCUGUUCGUGGAGCAGCAAGCAUACUCCGAGCAGAUCAUCGAGCAGAUCCCGAGUUCU CCAGCGGGUGAUCCUGGCCGACGCCAACCCUGGACAAGGUGCUGUCCGCCUACAACAAAGCACCGGGACAAGCCAU CCGGGAGCAGGCCGAGAAUACAUCACCUUGUUAACCCUGACCAACCGUGGCCCGCCCGCCUUAAGUACUUC GACACCAUCCGACCGGAAGCGGUAACCUCCACAAGGAGGUGGAGCGCACCCUGAUCCACAGUCCAGUCCAUA CCGGCCUGUACGAGACCCGGAUCGACCUUCUCCAGCUGGGCGGCGACGGCGCGGCUCCCAAGAAAGACGGAA GGUGUGACUACGACCGCCUCAAAGAACACCCGGAUGGAGUCUCAAAGCUACAUAUACCAACUACACUUAACAA AUGUUGUCCCCCAAAAUAGUAGCCAUUCGUAUCUGCUCUCAAUAAAAAGAAAGUUUUCUACAUUCUCUCGAGAAA

[illegible]

1005	mRNA encoding SpyCas9	GGGUCCCGCAGUCCAGCGGCUUCGUUGUUCGUGUGUUGUUGCGGCUUAUUCGGAUCCGCCACC AUGGACAAAGAUACAGCAUCGGACUUGGAAACAACAGCGUCCGGAUUGGCGAUCAUCACAGACGAAUAC AAGUCCCGAGCAAGAAGUUAAGGUCCUGGAAACACAGACAGACACAGCAUACAAGAAACCUUGAUCGGAGCAC UGGUUUCGACACGGGAAACAGCAGAGAACAAACAGACUAGAAAGACACGAAGAAAGAAACACAAGAAAGAA AGAACAAUUCUGUACCUUGCAGGAAAUUCUUCAGCAACGAAUUGGCAAGGUCAGCAGCAAGUUCUCCACAGACU GGAAGAAAGCUUCUGGUUGAAGAGACAAAGAACACGAAAGACACCCGACUUCUGGAAACAUCGUCGACGAAGU CGCAUACCAAGAAAGUACCCGACAAUUCACCCUGAGAGAAAGCUGGUCGACAGCACAGCAAGGCACGACCUG AGACUGAUUACCUUGGCACUGGCACACAUCAAGUUCAGAGGACAUUCUUGAUUGAAGGAGACUUGAACCCGG ACAAACGCGACGUCGACAAGCUUGUUAUCCAGCUGGUCCAGACAUAACAACAGCGUUGUUGAAGAAACCCGAUCAA CGAAAGCGGAGUCGACGCAAGGCAAUCCUGAGCGCAAGAGCAGAGCAAGAGCAAGCAAAACCCUGAUCGCA CAGUCUCCGGAGAAAAGAAACGGAACUGUUCGGAAACUUGAUCGCAUCGAGCCUGGACUGACACCCGAAACUUA AGAGCAACUUCGACCUUGGCAGAAAGCGCAAGCGUCGAGCAGCAAGCAGCAAGCAGCAGCAGCAGCAGCAGCAGC GUGGCACAGAUCCGGAGACCAGUACGACGACCGUUCUGGACGCAAGAACCCUGAGCGACGCAAUCCUGGAGC GACUUCUGAGAUCAACACAGAAAUCAAAAGGCAACCGUGAGCGCAAGCAUGAUCAAGAGAUACAGCAACACCC ACGAGGACCUAGACUGUGAAGGCACUGGUCAGACAGCAGUGGCCGGAAGAAUACAAGGAAAUUCUUCUUCGACCA GAGCAAGAAACGGAUACGCGAGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCACAAGUUAUCAAGCCGAU CCUGGAAAGAUUGGACGGAAACAGAAAGAACUGUCUGGCAAGCUAGAAACAGAGAAACCCUGGAGAAAGCAGAGAAC AUUCGACAAACGGAAGCAUCCCGCACCAAGAUCCACUUGGAGAACUGCAGCGAAUCCUGAGAAAGACAGGAAGAUUC UACCCGUUCUGAAGGACAACAGAGAAAGAUUCGAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGACCCGC UGGCAAGAGGAAACAGCAAUUCGAUUGGAUACAAAGAAAGAGCGAAGAAACAUAACACCGUGGAACUUCGAAAG AAGUCUGACAAAGGAGCAAGCGCACAGAGCUUAUCGAAAGAAUAGACAAACUUCGACAAAGAACUCCGCCGAACCGA AAAGUCCUGCCGAAGCACAGCCUGCUGUACGAUAUUAUCAGUUAACAACGAAACUGACAAAGGUAAGUAACGUC ACAGAAAGAAUAGAGAAACCGGCAUUCUGAGCGGAGAACAGAAAGGCAAGGCAUUCGACCGUUGUUCGAAGACA ACAGAAAGGUCACAGUACAAGCAGUAGGAAGAAUUAUUAAGAAAGAAUUGCUUAGAAAGCAGUAGAGAAAGAAU ACGCGGAGUCGAAAGACAGAUUACAACGCAAGCCUGGAAACAACACGACCCUGGAAAGAUCAUACAAGGACAAGGACU UCCUGGACAACGAAAGAAACGAAGACAUCCUGGAAAGACAUCCUGCACUGACACUGACACUGUUGGAAAGACAGAGAAA UGAUUCGAAAGAAAGCUGAAGACUAUCGCACACCUUUGCAGCAGCAAGGUUAUGAAGCAGUAGAGAAAGAAAGAU ACACAGGAUUGGGGAAGACUGAGCAGAAAGCUGAUACAACGGAUUCAGAGACAACGACAGCGGAAAGACAUCUUGG ACUUCUGAAGAGCGGAGUUCGCAACAGAAAACUUAUCAGCUGCUUCCACGACGACGACGCGUAGACUUCGACUUCAGGA AGCAUCCAGAAAGGCACAGGUCAGCGGACAGGAGACUCCUGCAGCAACAUUCGCAAAACUUGGCAAGGAAAGCCCG GCAUUCAGAAAGGGAUCCUGCAGACAGUCAAGGUCGACCAACUUGGUAAGGUAAGGUAAGGAAAGACACAAGCCG GAAAACAUUCGUAUCGAAUUGGCAAGAGAAAACAGACAACACAGAAAGGCAAGAAAGCAAGCAGAGAAAGAAUUG AAGAGAAUUCGAAAGAAUCAAAGGAACUGGGGAAGCCAGUAUCUUGAAGGAACAACCCGUGCGAAAAACACACAGCUG CAGAACGAAAGACUUAACCUAGACUACCGAAGACGGAAGAGACAUUAGCUGACCCAGGAAACUGGACAUCAACA GACUGAGCGACUACGACGUCGACCCACUUCGUCCGACAGCUUCUUGAAGGACGACAGCAUCGACACAAGGUCCU GACAGAAAGCAGCAAGAACAGAGAAAGAGCGACAACUGUCCCGAGCGAAGAAAGUUCGUCAAGAAAGAAAGAAACUA CUGGAGACAGCUGUAACGCAAGAGUACACAGAGAAAUUCGACAAACUUCGACAAACUUCGACAAAGGACAGAGGAGG ACUGAGCGAAACUGGACAAGGCGAGUUAUCAAGAGACAGCUGGUCGAAACAAGACAGAUCAACAAGCACGUCGCG ACAGAUCCUGGACAGCAGAAUAGACACAAGAACGAGGAAAACGCAAGCGUAGUACAGAGAAAGUACAAGGUAUCAC
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73

	UGGCAAGAGGAAACAGCAGAUUCGCAUGGAUGACAAAGAAAGCGAAGAAACAAUACACCCGUGGAAACUUCGAAG AAGUCGUCGACAAAGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUACAAACUUCGACAAAGAACUUCGCCGAACGA AAAGGUCCUGCCGAAGCACAGCCUGCUUACGAAUACUUCACAGUACUACACGAAACUACAGAAAGGUCAAGUACGUC ACAGAAGGAUUGAGAAAGCCGGCAUUCUGAGCGGAGAACAGAAAGGCAUUCGUCGACCCUGCUUCAAAGACA AACAGAAAGGUACAGUCAAGCAGCUGAAGGAAGACUACUUAAGAAUUCGAAUUCUUCGACAGCUGCGAAUUC AGCGGAGUCGAAGACAGAUUACCGCAAGCGAAGAAUACUACCCAGCCUUCGUAAGAUCAUCAAAGGACAAAGGACU UCCUGGACACGAAAGAAACGAAAGACAUCCUGGAAGACAUCCUGUUCGACAGCAGGUAAGAGAGAAAGAAAGAAU UGAUCGAAAGAAAGACUAGAGACAUACGCACACCUUUCGACAGCAGAACGUAAGGUAAGAGAGAAAGAAAGAAU ACACAGGAUUGGGAGAGCUGAGCAGAAAGCUGAUCAACGGAAUCAGAGAAAGCAGAGAGCGGAAAGACAUCUUGG ACUUCUGAAGAGCGAUCGGAUUCGCAACACAGAAACUUCUUGCAGCUGAUCCACGACGACGCCUGACAUUCAAAGGA AGACAUCCAGAAAGCACAGGUCAGCGACAGGAGACAGCCUGCACGAACACAUCCGAAACCCUGGACGGAAGCCCG GCAUCAAAGAAAGGAAUCCUGCAGACAGACAGUCAAAGGUCGUCAGAAACUGGUAAGGUCAUUGGGAAGACACAAGCCG GAAACAUUCGUCAUCGAAUUGGCAAGAGAAACACGACAAACAGAGAAAGGACAGAGAAAGAAAGAAUUG AAGAGAAUUCGAAAGGAUUAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCCGAAACACACAGCUG CAGAACGAAAGGCUUACCUAGUACUACCUUGCAGAACGGAAGAGACAUUACGUCGACCGAGGAAACUGGACAUCAACA GACUGAGCGACUACGACGUCGACCAUUCGUCCCGCAGAGCUUCCUGAAGGAGCAGCAGCAUCCGACAAACAGGUCCU GACAAAGACGCAAGAACAGAGGAAGAGCGACACGUCUCCGAGCGAAGAAUGUCUCAAAGAAUGAAGAAACUA CUGGAGACAGCUGGAAACGCAAGCUGAUCAACAGAGAAAGUUCGACAAACCCUGACAAAGGCAGAGAGAGGAGG ACUGAGCGAAACUGGACAAAGCAGGAUUCUCAAAGAGACACGUGGUGGAAACAAAGACAGACAAAGCACGUCGC ACAGAUCCUGGACAGCAGAAUACACAAAGUACGAGAAACGCAACGACAGCUUACAGAGAAUGUCAAAGGUCAUCAC ACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCAGUUCUACAAGGUCAGAGAAUCAAACACUACCCAC CACGCACACGACGCAUACCUAGAACGACAGUCGUCGGAACAGCACUAGUCAAAGUACCCGAAAGCUGGAAAGCGAAU UCGUCAACGGAGACUACAAGGUCAACGACGUCAGAAAGAUUGAUCGCAAGAGAGCGAAACAGGAAUUCGGAAAGGCAA CAGCAAGUACUUCUACAGCAACAUCAUGAACUUCUCAAAGACAGAAUACACUGGCAACCGGAAACGGAAGAAUCAG AAAGAGACCCGUGAUCGAAACAAACGGAGAAACAGGAGAAUUCUGGGGACAAAGGAAAGAGACUUCGCAACAGU CAGAAAGGUCCUGAGCAUCCCGCAGGUCAACUUCGUAAGAAAGACAGAAAGUCCAGACAGGAGGAUUCAGCAAGGA AAGCAUCCUGCCGAAAGAAACAGCGACAAAGCUGAUCGCAAGAAAGAGACUUGGACCCGAAAGAAUACCGGAGG AUUCGACAGCCCGACAGUCGAUACAGCGUCCUGGUCGCAAGGUGGAAAGGUGGAAAGGAAAGAGCAAGCAAGCUGAA GAGCGUCAAGGAACUGCUGGGAUUCACAAUCAAUGGAAAGAAAGCAGCUUCGAAAGAAACCCGUAUCGACUUCUGGA AGCAAGGGAUACAAGGAAGUCAAGAAAGGACCUGAUCAAGCUGCCGAAAGUACAGCCUGUUCGAAACUGGAAAA CGGAAGAAAGAGAAUUCGUGGCAAGCGCAGGAGAAACUGCAGAAAGGAAACGAAACUGGCACUGCCGAGCAAGUACGU CAACUUCUGUACCUGGCAAGCCACUACGAAAGCUGAAGGGAAGCCCGAAGACAAACGAAAGCAGAGCAGCUGUUC GUCGAACAGCACAAAGCAUCCUGGACGAAUUAUCGAAAGACAGAUACGCGAAUUCAGCAAGAGACAGUACCCUGGCAG ACGCAAAACCUGGACAAAGUCCUGAGCGCAUACAAACAGCACAGACAAAGCCGAUCAGAGAACAGGCAGAAACAU CAUCCACCUUGUUCACACUGACAAACCUUGGAGCACCCGGCAGCAUCAAAGUACUUCGACACAACAAUCGACAGAAAG AGAUACAAAGCAAAAGGAAGUCCUGGACGCAACUUGAUCCACAGAGCAUACAGGACUGUACGAAACAAAGAA UCGACCUAGCCAGCCAGCGGAGACGGAGGAGAAAGCCCGAAAGAAAGAGAAAGGUCAAG

1011	Exemplary <i>EMVI</i> guide RNA (G000644)	mG*mA*mG*UCCGAGCAGAGAAAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAAGGCU AGUCCGUUAUUCAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmGmAmGmUmCmGmUmGmCmUm*mU *mU*mU
1012	Exemplary <i>VEGFA</i> guide RNA (G000645)	mG*mA*mC*CCCCUCCACCCCGCCUCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAAGGCUA GUCCGUUAUUCAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmUmGmCmUm*mU* mU*mU
1013	Exemplary SV40 NLS	PKKKRKV
1014	Exemplary SV40 NLS	PKKKRRV
1015	Exemplary nucleoplasmin NLS	KRPAATKKAGQAKKKK

What is claimed is:

1. A system comprising:
 - A. a modified *S. pyogenes* Cas9 (SpyCas9) guide RNA comprising:
 1. a targeting sequence comprising a sequence at least 95%, or 90%, identical to the nucleotide sequence of SEQ ID NOs: 1, 2, 3, 4, or 5;
 2. a targeting sequence comprising a sequence identical to at least 18, 19, or 20 contiguous nucleotides of the nucleotide sequence of SEQ ID NOs: 1, 2, 3, 4, or 5; or
 3. a targeting sequence comprising a sequence identical to the nucleotide sequence of SEQ ID NOs: 1, 2, 3, 4, or 5; and
 - B. an *S. pyogenes* Cas9 (SpyCas9) cleavase.
2. The system of claim 1, wherein the SpyCas9 guide RNA comprises a targeting sequence identical to the nucleotide sequence of SEQ ID NOs: 1, 2, 3, 4, or 5.
3. The system of claim 1 or 2, wherein the SpyCas9 guide RNA further comprises one or more of:
 - A. a shortened hairpin 1 region, or a substituted and optionally shortened hairpin 1 region relative to SEQ ID NO: 303, wherein
 1. at least one of the following pairs of nucleotides are substituted in the substituted and optionally shortened hairpin 1 region with Watson-Crick pairing nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, or H1-4 and H1-9, and the hairpin 1 region optionally lacks
 - a. any one or two of H1-5 through H1-8,
 - b. one, two, or three of the following pairs of nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, and H1-4 and H1-9, or
 - c. 1-8 nucleotides of the hairpin 1 region; or
 2. the shortened hairpin 1 region lacks 4-8 nucleotides, preferably 4-6 nucleotides; and
 - a. one or more of positions H1-1, H1-2, or H1-3 is deleted or substituted relative to SEQ ID NO: 303; or
 - b. one or more of positions H1-6 through H1-10 is substituted relative to SEQ ID NO: 303; or
 3. the shortened hairpin 1 region lacks 5-10 nucleotides, preferably 5-6 nucleotides, and one or more of positions N18, H1-12, or N is substituted relative to SEQ ID NO: 303; or

B. a shortened upper stem region, wherein the shortened upper stem region lacks 1-6 nucleotides and wherein the 6, 7, 8, 9, 10, or 11 nucleotides of the shortened upper stem region include less than or equal to 4 substitutions relative to SEQ ID NO: 303; or

C. a substitution relative to SEQ ID NO: 303 at any one or more of LS6, LS7, US3, US10, B3, N7, N15, N17, H2-2 and H2-14, wherein the substituent nucleotide is neither a pyrimidine that is followed by an adenine, nor an adenine that is preceded by a pyrimidine; or

D. SEQ ID NO: 303 with an upper stem region, wherein the upper stem modification comprises a modification to any one or more of US1-US12 in the upper stem region.

4. The system of claim 3, wherein the SpyCas9 guide RNA lacks 6 nucleotides in the shortened hairpin 1.

5. The system of claim 3, wherein the SpyCas9 guide RNA lacks 8 nucleotides in the shortened hairpin 1.

6. The system of any one of claims 3-5, wherein H-1 and H-3 are deleted.

7. The system of any one of claims 3-6, wherein the SpyCas9 guide RNA further comprises a 3' tail.

8. The system of claim 7, wherein the 3' tail is 1-4 nucleotides in length, optionally 1 nucleotide in length.

9. The system of any one of claims 3-8, wherein the SpyCas9 guide RNA comprises an upper stem region comprising a modification to any one or more of US1-US12 in the upper stem region.

10. The system of any one of claims 1-9, wherein the targeting sequence comprises a modified nucleotide sequence according to the pattern (mN*)₃(N)₁₃₋₁₇, wherein "m" is indicative of a 2'-O-methyl modification, * is indicative of a phosphorothioate bond, and N is indicative of a 2'-OH and a phosphodiester bond.

11. The system of claim 1 or 2, wherein the SpyCas9 guide RNA comprises a modified nucleotide sequence selected from the sequence of SEQ ID NO: 501-512, wherein the modified nucleotide sequence is at 3' of the guide sequence.

12. The system of claim 11, wherein the SpyCas9 guide RNA is modified according to the pattern of a nucleotide sequence selected from SEQ ID NO: 601-612, wherein the (mN*)₃N₁₇ refers to the targeting sequence of claim 1 or 2.

13. The system of any one of claims 1-12, wherein the SpyCas9 guide RNA comprises the nucleotide sequence selected from SEQ ID NOs: 102, 101, 104, and 103.

14. The system of claim 13, wherein each nucleotide is a natural or non-natural nucleotide.
15. The system of claim 14, wherein the SpyCas9 guide RNA comprises the modified nucleotide sequence selected from SEQ ID NOs: 202, 201, 204, and 203.
16. The system of any one of claims 1-15, wherein the SpyCas9 cleavase comprises a SpyCas9 cleavase polypeptide or a nucleic acid encoding a SpyCas9 cleavase polypeptide.
17. The system of claim 16, wherein the nucleic acid encoding the SpyCas9 cleavase comprises an mRNA comprising an open reading frame (ORF) encoding the SpyCas9 cleavase.
18. The system of claim 17, wherein the SpyCas9 cleavase comprises an amino acid sequence having at least 95% identity to SEQ ID NO: 1001; or wherein the ORF encoding the SpyCas9 cleavase comprises a nucleotide sequence having at least 95% identity to a sequence selected from SEQ ID NOs: 1003 and 1006.
19. The system of claim 17 or 18, wherein the ORF is a modified ORF.
20. A composition comprising the system of any one of claims 1-19.
21. The composition of claim 20, further comprising a pharmaceutical excipient.
22. The composition of claim 20 or 21, wherein the SpyCas9 guide RNA is associated with a lipid nanoparticle (LNP).
23. The composition of claim 22, wherein the LNP comprises a cationic lipid.
24. The composition of claim 23, wherein the cationic lipid 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.
25. The composition of any one of claims 22-24, wherein the LNP comprises a helper lipid.
26. The composition of claim 25, wherein the helper lipid is cholesterol.
27. The composition of any one of claims 22-26, wherein the LNP comprises a neutral lipid.
28. The composition of claim 27, wherein the neutral lipid is 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC).
29. The composition of any one of claims 22-28, wherein the LNP comprises a stealth lipid.
30. The composition of claim 29, wherein the stealth lipid is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol 2000 (PEG2k-DMG).

31. The composition of any one of claims 22-30, wherein the LNP comprises (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, DSPC, cholesterol, and PEG2k-DMG.
32. A pharmaceutical composition comprising the composition of any one of claims 20-31.
33. The pharmaceutical composition of claim 32 or use of a pharmaceutical composition of claim 32 for inducing a double-stranded break within an *ANGPTL3* gene in a cell.
34. The pharmaceutical composition or use of claim 33, for reducing expression of an *ANGPTL3* gene in a cell.
35. The pharmaceutical composition or use of claim 33 or 34, wherein the cell is a liver cell.
36. The pharmaceutical composition or use of claim 35, wherein the cell is in a subject.
37. The pharmaceutical composition or use of claim 32 or 33, for treating a subject having an *ANGPTL3*-related disease.
38. A kit comprising the system of any one of claims 1-19, the composition of any one of claims 20-31, or the pharmaceutical composition of any one of claims 32-37.
39. A method of inducing a double-stranded break within an *ANGPTL3* gene in a cell comprising contacting the cell with the system of any one of claims 1-19, the composition of any one of claims 20-31, or the pharmaceutical composition of any one of claims 32-37.
40. Use of the system of any one of claims 1-19, the composition of any one of claims 20-31, or the pharmaceutical composition of any one of claims 32-37 in the preparation of a medicament for practicing the method of claim 39.
41. A method of modifying a genomic locus in a human liver cell, comprising contacting a human liver cell with the system of any one of claims 1-19, the composition of any one of claims 20-31, or the pharmaceutical composition of any one of claims 32-37.
42. The pharmaceutical composition, use, method, or kit of any one of claims 33-41, wherein the cell or the liver cell is a hepatocyte.
43. The pharmaceutical composition, use, method, or cell of claim 42, wherein the cell or the liver cell is in a subject with an *ANGPTL3*-related disease.
44. A method of treating an *ANGPTL3*-related disease in a subject, comprising administering to the subject the system of any one of claims 1-19, the composition of any one of claims 20-31, or the pharmaceutical composition of any one of claims 32-37.

45. The pharmaceutical composition, use, method, or kit of any one of claims 36-44, further comprising determining the ANGPTL3 protein level in a blood or serum sample from the subject.

46. Use of the system of any one of claims 1-19, the composition of any one of claims 20-31, or the pharmaceutical composition of any one of claims 32-37 in the preparation of a medicament for practicing the method of any one of claims 39, 41, or 44.