

COMPOSITIONS AND METHODS FOR KALLIKREIN (*KLKB1*) GENE EDITING

[001] This patent application claims priority to US Provisional Patent Application No. 62/971,906, filed February 7, 2020; US Provisional Patent Application No. 62/981,965, filed February 26, 2020; and US Provisional Patent Application No. 63/019,076, filed May 1, 2020, the contents of each of which are incorporated herein by reference in their entirety for all purposes.

[002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 4, 2021, is named 01155-0031-00PCT_ST25.txt and is 184,584 bytes in size.

[003] Hereditary angioedema (HAE) affects one in 50,000 people and contributes to 15,000 to 30,000 emergency room visits per year. HAE is a rare autosomal, dominantly inherited blood disorder characterized by recurrent episodes of severe swelling (angioedema). The most common areas of the body to develop swelling are the limbs, face, GI tract, and airway. Minor trauma or stress may trigger an attack but swelling often occurs without a known trigger. Episodes involving the intestinal tract cause severe abdominal pain, nausea, and vomiting. Swelling in the airway can restrict breathing and lead to life-threatening obstruction of the airway or asphyxiation. Symptoms of HAE typically begin in childhood and worsen during puberty. On average, untreated individuals have an attack every 1 to 2 weeks, and most episodes last for about 3 to 4 days. There are three types of hereditary angioedema, called types I, II, and III, and the different types have similar signs and symptoms.

[004] Hereditary angioedema stems from excess bradykinin in the blood promoting vascular permeability and episodes of swelling. Most patients with HAE have a C1 inhibitor (also called C1 esterase inhibitor or C1-INH) protein deficiency. In the absence of C1-INH, bradykinin levels can rise, initiate vascular leakage, and cause swelling attacks. Its production is controlled via the kallikrein-kinin (contact) pathway which is endogenously inhibited by C1-INH. Bradykinin peptide is formed when high-molecular weight kininogen (HMWK) is cleaved by plasma kallikrein (pKal), an activated form of the protein prekallikrein. Prekallikrein is encoded by *KLKB1* and is also called KLKB1 protein. KLKB1 protein is produced in the liver and secreted into plasma where it can be activated by factor XIIa. Once KLKB1 is activated, pKal can increase bradykinin levels. An excess of bradykinin in the blood leads to fluid leakage through the walls of blood vessels into body

tissues. Excessive accumulation of fluids in body tissues causes the episodes of swelling seen in individuals with HAE.

[005] Several drugs targeting the kallikrein-kinin pathway have been developed, including C1 esterase inhibitors (Berinert®, Cinryze®), recombinant C1-INH replacement therapy (rhC1INH; conestat alfa (Rhucin®, Ruconest®)), and bradykinin receptor antagonist (Icatibant, Firazyr®). Approaches using kallikrein or prekallikrein (KLKB1) inhibitors also have been developed (ecallantide, Kalbitor®; lanadelumab, Takhzyro™).

[006] The present disclosure provides compositions and methods using the CRISPR/Cas system to knock out the *KLKB1* gene, thereby reducing the production of prekallikrein (KLKB1), reducing kallikrein, and reducing bradykinin production in subjects with HAE.

[007] Accordingly, the following embodiments are provided. In some embodiments, the present invention provides compositions and methods using a guide RNA with an RNA-guided DNA binding agent such as the CRISPR/Cas system to substantially reduce or knockout expression of the *KLKB1* gene, thereby substantially reducing or eliminating the production of bradykinin. The substantial reduction or elimination of the production of bradykinin through alteration of the *KLKB1* gene can be a long-term or permanent treatment.

[008] The following embodiments are provided herein.

[009] Embodiment A1 is a guide RNA comprising:

- a. a guide sequence comprising at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 15, 8, and 41;
- b. a guide sequence comprising at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 15, 8, and 41; or
- c. a guide sequence selected from SEQ ID NOs: 15, 8, and 41.

[0010] Embodiment A2 is the guide RNA of embodiment A1, further comprising the nucleotide sequence of SEQ ID NO: 202.

[0011] Embodiment A3 is the guide RNA of embodiment A1, wherein the guide RNA further comprises a nucleotide sequence selected from SEQ ID NO: 170, 171, 172, and 173 wherein the sequence of SEQ ID NO: 170, 171, 172, or 173 is 3' of the guide sequence.

[0012] Embodiment A4 is the guide RNA of any one of embodiments A1-A3, wherein the guide RNA further comprises a 3' tail.

[0013] Embodiment A5 is the guide RNA of any one of embodiments A1-A4, wherein the guide RNA comprises at least one modification.

[0014] Embodiment A6 is the guide RNA of embodiment A5, wherein the modification comprises a 5' end modification.

- [0015] Embodiment A7 is the guide RNA of embodiment A5 or A6, wherein the modification comprises a 3' end modification.
- [0016] Embodiment A8 is the guide RNA of any one of embodiments A1-A7, wherein the guide RNA comprises a modification in a hairpin region.
- [0017] Embodiment A9 is the guide RNA of any one of embodiments A1-A8, wherein the modification comprises a 2'-O-methyl (2'-O-Me) modified nucleotide.
- [0018] Embodiment A10 is the guide RNA of any one of embodiments A1-A9, wherein the modification comprises a phosphorothioate (PS) bond between nucleotides.
- [0019] Embodiment A11 is the guide RNA of any one of embodiments A1-A10, wherein the modification comprises a 2'-fluor (2'F) modified nucleotide.
- [0020] Embodiment A12 is the guide RNA of any one of embodiments A1 or A3-A11, further comprising the nucleotide sequence of SEQ ID NO: 171.
- [0021] Embodiment A13 is the guide RNA of embodiment A12, modified according to the pattern of nucleotide sequence of SEQ ID NO: 405.
- [0022] Embodiment A14 is the guide RNA of any one of embodiments A1 or A3-A11, further comprising the nucleotide sequence of SEQ ID NO: 173.
- [0023] Embodiment A15 is the guide RNA of embodiment A14, modified according to the pattern of SEQ ID NO: 248-255 or 450.
- [0024] Embodiment A16 is the guide RNA of any one of embodiments A12-A15, wherein the guide sequence is SEQ ID NO: 15.
- [0025] Embodiment A17 is the guide RNA of any one of embodiments A12-A15, wherein the guide sequence is SEQ ID NO: 8.
- [0026] Embodiment A18 is the guide RNA of any one of embodiments A12-A15, wherein the guide sequence is SEQ ID NO: 41.
- [0027] Embodiment A19 is the guide RNA of any one of embodiments A1 or A4-A11, wherein the guide RNA is modified according to the pattern of SEQ ID NO: 300, wherein the N's are collectively the guide sequence of embodiment A1.
- [0028] Embodiment A20 is the guide RNA of embodiment A16, wherein each N in SEQ ID NO: 300 is any natural or non-natural nucleotide.
- [0029] Embodiment A21 is the guide RNA of embodiment A19, wherein the guide sequence is SEQ ID NO: 15 and the guide RNA is modified according to mG*mG*mA*UUGCGUAUGGGACACAAGUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAA GUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUm GmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU, wherein "mA,"

“mC,” “mU,” or “mG” denote a nucleotide that has been modified with 2’-O-Me, a * denotes a phosphorothioate bond, and an N is a natural nucleotide.

[0030] Embodiment A22 is the guide RNA of embodiment A19, wherein the guide sequence is SEQ ID NO: 8 and the guide RNA is modified according to mU*mA*mC*CCGGGAGUUGACUUUGGGUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU, wherein “mA,” “mC,” “mU,” or “mG” denote a nucleotide that has been modified with 2’-O-Me, a * denotes a phosphorothioate bond, and N is a natural nucleotide.

[0031] Embodiment A23 is the guide RNA of embodiment A19, wherein the guide sequence is SEQ ID NO: 41 and the guide RNA is modified according to mU*mA*mU*UAUCAAUACAUAUACCGUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU, wherein “mA,” “mC,” “mU,” or “mG” denote a nucleotide that has been modified with 2’-O-Me, a * denotes a phosphorothioate bond, and N is a natural nucleotide.

[0032] Embodiment A24 is a composition comprising a guide RNA of any one of embodiments A1-A23.

[0033] Embodiment A25 is a composition of embodiment A24, further comprising an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent.

[0034] Embodiment A26 is the composition of embodiment A25, wherein the nucleic acid encoding an RNA-guided DNA binding agent comprises an mRNA comprising an open reading frame (ORF) encoding an RNA guided DNA binding agent.

[0035] Embodiment A27 is the composition of embodiment A25 or A26, wherein the RNA-guided DNA binding agent is Cas9.

[0036] Embodiment A28 is the composition of embodiment A27, wherein the Cas9 is *S. pyogenes* Cas9.

[0037] Embodiment A29 is the composition of any one of embodiments A26-A28, wherein the ORF is a modified ORF.

[0038] Embodiment A30 is the composition of any one of embodiments A24-A29, further comprising a pharmaceutical excipient.

[0039] Embodiment A31 is the composition of any one of embodiments A24-A30, wherein the guide RNA is associated with a lipid nanoparticle (LNP).

[0040] Embodiment A32 is the composition of embodiment A31, wherein the LNP comprises a cationic lipid.

[0041] Embodiment A33 is the composition of embodiment A32, 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.

[0042] Embodiment A34 is the composition of any one of embodiments A31-A33, 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.

[0043] Embodiment A35 is a pharmaceutical composition comprising a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-A34.

[0044] Embodiment A36 is a pharmaceutical composition comprising or use of a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-A34 for inducing a double stranded break or a single stranded break within a KLKB1 gene in a cell or reducing expression of KLKB1 in a cell.

[0045] Embodiment A37 is the pharmaceutical composition or use of embodiment A36, for reducing expression of the KLKB1 gene in a cell or subject.

[0046] Embodiment A38 is a pharmaceutical composition comprising or use of a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-A34 for treating a subject having hereditary angioedema (HAE).

[0047] Embodiment A39 is the pharmaceutical composition or use of embodiment A38, comprising reducing the frequency and/or severity of HAE attacks.

[0048] Embodiment A40 is a pharmaceutical composition comprising or use of a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-A34 for treating or preventing angioedema associated with HAE, bradykinin production and accumulation, bradykinin-induced swelling, angioedema obstruction of the airway, or asphyxiation.

[0049] Embodiment A41 is a pharmaceutical composition or use of a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-A34 for reducing total plasma kallikrein activity or reducing prekallikrein and/or kallikrein levels in a subject.

[0050] Embodiment A42 is the pharmaceutical composition or use of embodiment A41, wherein the total plasma kallikrein activity is reduced by more than 60%.

[0051] Embodiment A43 is a method of inducing a double stranded break or a single stranded break within a KLKB1 gene in a cell or reducing expression of KLKB1 in a cell comprising contacting a cell with a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-A34.

[0052] Embodiment A44 is the method of embodiment A43, wherein the cell is in a subject.

[0053] Embodiment A45 is a method of treating a subject having hereditary angioedema (HAE) comprising administering a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-34 thereby treating the subject.

[0054] Embodiment A46 is the method of embodiment A45, wherein treating the subject comprises reducing the frequency and/or severity of HAE attacks.

[0055] Embodiment A47 is a method of treating or preventing angioedema associated with HAE, bradykinin production and accumulation, bradykinin-induced swelling, angioedema obstruction of the airway, or asphyxiation comprising administering to the subject a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-A34, thereby treating or preventing angioedema associated with HAE, bradykinin production and accumulation, bradykinin-induced swelling, angioedema obstruction of the airway, or asphyxiation in the subject.

[0056] Embodiment A48 is a method of reducing total plasma kallikrein activity in a subject comprising administering a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-A34, thereby reducing total plasma kallikrein activity in a subject.

[0057] Embodiment A49 is the method of embodiment A48, wherein the total plasma kallikrein activity is reduced by more than 60% in the subject.

[0058] Embodiment A50 is the use of a guide RNA of any one of embodiments A1-A23 or composition of any one of embodiments A24-A34 in the preparation of a medicament for practicing any of the methods of embodiments A43-A49.

[0059] Additional embodiments are provided herein.

[0060] Embodiment 1 is a method of inducing a double-stranded break (DSB) or a single-stranded break (SSB) within the KLKB1 gene, comprising delivering a composition to a cell, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0061] Embodiment 2 is a method of reducing the expression of the KLKB1 gene comprising delivering a composition to a cell, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or

- v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
- vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
- vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
- viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0062] Embodiment 3 is a method of treating or preventing hereditary angioedema (HAE) comprising administering a composition to a subject in need thereof, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent, thereby treating or preventing HAE.

[0063] Embodiment 4 is a method of treating or preventing angioedema caused by or associated with HAE comprising administering a composition to a subject in need thereof, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent, thereby treating or preventing angioedema caused by or associated with HAE.

[0064] Embodiment 5 is a method of treating or preventing any one of bradykinin production and accumulation, bradykinin-induced swelling, angioedema obstruction of the airway, or asphyxiation comprising administering a composition to a subject in need thereof, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or

- vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent, thereby treating or preventing any one of bradykinin production and accumulation, bradykinin-induced swelling, angioedema obstruction of the airway, or asphyxiation.

[0065] Embodiment 6 is a method of reducing the frequency and/or severity of HAE attacks, comprising administering a composition to a subject in need thereof, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent, thereby reducing the frequency and/or severity of HAE attacks.

[0066] Embodiment 7 is a method for reducing the frequency and/or severity of angioedema attacks, or achieving remission of angioedema attacks in a subject, comprising administering a composition to a subject in need thereof, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent, thereby reducing the frequency and/or severity of angioedema attacks or achieving remission of angioedema attacks in a subject.

[0067] Embodiment 8 is a method of reducing total plasma kallikrein activity, comprising administering a composition to a subject in need thereof, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or

- v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent, thereby achieving remission of angioedema attacks in a subject, wherein total plasma kallikrein activity is reduced.

[0068] Embodiment 9 is the method of embodiment 8, further comprising an activation step to convert prekallikrein to its active form, pKal.

[0069] Embodiment 10 is the method of embodiment 8, wherein the total plasma kallikrein activity is reduced by more than 60%, more than 85%, or more than 60-80%.

[0070] Embodiment 11 is a method of reducing total plasma kallikrein levels, comprising administering a composition to a subject in need thereof, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally

- b. an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent, thereby total plasma kallikrein levels.

[0071] Embodiment 12 is a method of reducing prekallikrein and/or kallikrein levels, comprising administering a composition to a subject in need thereof, wherein the composition comprises:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent, thereby reducing prekallikrein and/or kallikrein.

[0072] Embodiment 13 is the method of any one of the preceding embodiments, wherein there is a dose dependent increase in percent editing.

[0073] Embodiment 14 is the method of embodiment 13, wherein there is a dose dependent reduction in total plasma kallikrein levels.

[0074] Embodiment 15 is the method of embodiment 13 or 14, wherein there is a dose dependent reduction in plasma kallikrein activity.

[0075] Embodiment 16 is the method of any one of the preceding embodiments wherein the effect is durable for at least 1 month, 2 months, 4 months, 6 months, 1 year, 2 years, 5 years, 10 years or more after the administration.

[0076] Embodiment 17 is the method of any one of the preceding embodiments wherein the effect is durable for at least 6 months.

[0077] Embodiment 18 is the method of any one of the preceding embodiments wherein the effect is durable for at least 1 year.

[0078] Embodiment 19 is the method of embodiment 6, wherein the frequency of HAE attacks is reduced.

[0079] Embodiment 20 is the method of embodiment 19, wherein the frequency is reduced by at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 60-80%, or at least 40-90%.

[0080] Embodiment 21 is the method of embodiment 20, wherein the frequency is reduced by at least 60-80%.

[0081] Embodiment 22 is the method of embodiment 20, wherein the frequency is reduced by at least 40-90%.

[0082] Embodiment 23 is the method of any one of the preceding embodiments, wherein the effect is durable for at least 1 month, 2 months, 4 months, 6 months, 1 year, 2 years, 5 years, 10 years or more after the administration.

[0083] Embodiment 24 is the method of any one of the preceding embodiments, wherein the effect is durable for at least 6 months after the administration.

[0084] Embodiment 25 is the method of any one of the preceding embodiments, wherein the effect is durable for at least 1 year after the administration.

[0085] Embodiment 26 is the method of any one of the preceding embodiments, wherein the effect is compared to a basal level.

[0086] Embodiment 27 is the method of any one of the preceding embodiments, wherein the effect is compared to a subject's basal level.

[0087] Embodiment 28 is the method of any one of the preceding embodiments, wherein an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent is administered.

[0088] Embodiment 29 is a composition comprising:

- a. a guide RNA comprising
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or

- v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
- vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
- vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
- viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and optionally
- b. an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent.

[0089] Embodiment 30 is a composition comprising a short-single guide RNA (short-sgRNA), comprising:

- a. a guide sequence comprising:
 - i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and
- b. a conserved portion of an sgRNA comprising a hairpin region, wherein the hairpin region lacks at least 5-10 nucleotides and optionally wherein the short-sgRNA comprises one or more of a 5' end modification and a 3' end modification.

[0090] Embodiment 31. The composition of embodiment 29, comprising the sequence of SEQ ID NO: 202.

- [0091] Embodiment 32 is the composition of embodiment 29 or embodiment 30, comprising a 5' end modification.
- [0092] Embodiment 33 is the composition of any one of embodiments 29-32, wherein the short-sgRNA comprises a 3' end modification.
- [0093] Embodiment 34 is the composition of any one of embodiments 29-33, wherein the short-sgRNA comprises a 5' end modification and a 3' end modification.
- [0094] Embodiment 35 is the composition of any one of embodiments 29-34, wherein the short-sgRNA further comprises a 3' tail.
- [0095] Embodiment 36 is the composition of embodiment 35, wherein the 3' tail comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides.
- [0096] Embodiment 37 is the composition of embodiment 35, wherein the 3' tail comprises about 1-2, 1-3, 1-4, 1-5, 1-7, 1-10, at least 1-2, at least 1-3, at least 1-4, at least 1-5, at least 1-7, or at least 1-10 nucleotides.
- [0097] Embodiment 38 is the composition of any one of embodiments 29-37, wherein the short-sgRNA does not comprise a 3' tail.
- [0098] Embodiment 39 is the composition of any one of embodiments 29-38, comprising a modification in the hairpin region.
- [0099] Embodiment 40 is the composition of any one of embodiments 29-39, comprising a 3' end modification, and a modification in the hairpin region.
- [00100] Embodiment 41 is the composition of any one of embodiments 29-40, comprising a 3' end modification, a modification in the hairpin region, and a 5' end modification.
- [00101] Embodiment 42 is the composition of any one of embodiments 29-41, comprising a 5' end modification, and a modification in the hairpin region.
- [00102] Embodiment 43 is the composition of any one of embodiments 29-42, wherein the hairpin region lacks at least 5 consecutive nucleotides.
- [00103] Embodiment 44 is the composition of any one of embodiments 29-43, wherein the at least 5-10 lacking nucleotides:
- a. are within hairpin 1;
 - b. are within hairpin 1 and the "N" between hairpin 1 and hairpin 2;
 - c. are within hairpin 1 and the two nucleotides immediately 3' of hairpin 1;
 - d. include at least a portion of hairpin 1;
 - e. are within hairpin 2;
 - f. include at least a portion of hairpin 2;

- g. are within hairpin 1 and hairpin 2;
- h. include at least a portion of hairpin 1 and include the “N” between hairpin 1 and hairpin 2;
- i. include at least a portion of hairpin 2 and include the “N” between hairpin 1 and hairpin 2;
- j. include at least a portion of hairpin 1, include the “N” between hairpin 1 and hairpin 2, and include at least a portion of hairpin 2;
- k. are within hairpin 1 or hairpin 2, optionally including the “N” between hairpin 1 and hairpin 2;
- l. are consecutive;
- m. are consecutive and include the “N” between hairpin 1 and hairpin 2;
- n. are consecutive and span at least a portion of hairpin 1 and a portion of hairpin 2;
- o. are consecutive and span at least a portion of hairpin 1 and the “N” between hairpin 1 and hairpin 2;
- p. are consecutive and span at least a portion of hairpin 1 and two nucleotides immediately 3' of hairpin 1;
- q. consist of 5-10 nucleotides;
- r. consist of 6-10 nucleotides;
- s. consist of 5-10 consecutive nucleotides;
- t. consist of 6-10 consecutive nucleotides; or
- u. consist of nucleotides 54-58 of SEQ ID NO: 400.

[00104] Embodiment 45 is the composition of any one of embodiments 29-44, comprising a conserved portion of an sgRNA comprising a nexus region, wherein the nexus region lacks at least one nucleotide.

[00105] Embodiment 46 is the composition of embodiment 45, wherein the nucleotides lacking in the nexus region comprise any one or more of:

- a. at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in the nexus region;
- b. at least or exactly 1-2 nucleotides, 1-3 nucleotides, 1-4 nucleotides, 1-5 nucleotides, 1-6 nucleotides, 1-10 nucleotides, or 1-15 nucleotides in the nexus region; and
- c. each nucleotide in the nexus region.

[00106] Embodiment 47 is a composition comprising a modified single guide RNA (sgRNA) comprising

- a. a guide sequence comprising:
- i. a guide sequence selected from SEQ ID NOs: 1-149; or
 - ii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149; or
 - iii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-149; or
 - iv. a guide sequence comprising any one of SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
 - v. a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
 - vi. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
 - vii. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (vi); or
 - viii. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (vi); and

further comprising

- b. one or more modifications selected from:
1. a YA modification at one or more guide region YA sites;
 2. a YA modification at one or more conserved region YA sites;
 3. a YA modification at one or more guide region YA sites and at one or more conserved region YA sites;
 4. i) a YA modification at two or more guide region YA sites;
ii) a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and
iii) a YA modification at one or more of conserved region YA sites 1 and 8; or
 5. i) a YA modification at one or more guide region YA sites, wherein the guide region YA site is at or after nucleotide 8 from the 5' end of the 5' terminus;
ii) a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and optionally;
iii) a YA modification at one or more of conserved region YA sites 1 and 8; or

6.
 - i) a YA modification at one or more guide region YA sites, wherein the guide region YA site is within 13 nucleotides of the 3' terminal nucleotide of the guide region;
 - ii) a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and
 - iii) a YA modification at one or more of conserved region YA sites 1 and 8; or
7.
 - i) a 5' end modification and a 3' end modification;
 - ii) a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and
 - iii) a YA modification at one or more of conserved region YA sites 1 and 8; or
8.
 - i) a YA modification at a guide region YA site, wherein the modification of the guide region YA site comprises a modification that at least one nucleotide located 5' of the guide region YA site does not comprise;
 - ii) a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and
 - iii) a YA modification at one or more of conserved region YA sites 1 and 8; or
9.
 - i) a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and
 - ii) a YA modification at conserved region YA sites 1 and 8; or
10.
 - i) a YA modification at one or more guide region YA sites, wherein the YA site is at or after nucleotide 8 from the 5' terminus;
 - ii) a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and
 - iii) a modification at one or more of H1-1 and H2-1; or
11.
 - i) a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; ii) a YA modification at one or more of conserved region YA sites 1, 5, 6, 7, 8, and 9; and iii) a modification at one or more of H1-1 and H2-1; or
12. i) a modification, such as a YA modification, at one or more nucleotides located at or after nucleotide 6 from the 5' terminus;

- ii) a YA modification at one or more guide sequence YA sites;
 - iii) a modification at one or more of B3, B4, and B5, wherein B6 does not comprise a 2'-OMe modification or comprises a modification other than 2'-OMe;
 - iv) a modification at LS10, wherein LS10 comprises a modification other than 2'-fluoro; and/or
 - v) a modification at N2, N3, N4, N5, N6, N7, N10, or N11; and
- wherein at least one of the following is true:

- i. a YA modification at one or more guide region YA sites;
- ii. a YA modification at one or more conserved region YA sites;
- iii. a YA modification at one or more guide region YA sites and at one or more conserved region YA sites;
- iv. at least one of nucleotides 8-11, 13, 14, 17, or 18 from the 5' end of the 5' terminus does not comprise a 2'-fluoro modification;
- v. at least one of nucleotides 6-10 from the 5' end of the 5' terminus does not comprise a phosphorothioate linkage;
- vi. at least one of B2, B3, B4, or B5 does not comprise a 2'-OMe modification;
- vii. at least one of LS1, LS8, or LS10 does not comprise a 2'-OMe modification;
- viii. at least one of N2, N3, N4, N5, N6, N7, N10, N11, N16, or N17 does not comprise a 2'-OMe modification;
- ix. H1-1 comprises a modification;
- x. H2-1 comprises a modification; or
- xi. at least one of H1-2, H1-3, H1-4, H1-5, H1-6, H1-7, H1-8, H1-9, H1-10, H2-1, H2-2, H2-3, H2-4, H2-5, H2-6, H2-7, H2-8, H2-9, H2-10, H2-11, H2-12, H2-13, H2-14, or H2-15 does not comprise a phosphorothioate linkage.

[00107] Embodiment 48 is the composition of embodiment 47, comprising SEQ ID NO: 450.

- [00108] Embodiment 49 is the composition of any one of embodiments 29-48, for use in inducing a double-stranded break (DSB) or a single-stranded break within the KLKB1 gene in a cell or subject.
- [00109] Embodiment 50 is the composition of any one of embodiments 29-48, for use in reducing the expression of the KLKB1 gene in a cell or subject.
- [00110] Embodiment 51 is the composition of any one of embodiments 29-48, for use in treating or preventing HAE in a subject.
- [00111] Embodiment 52 is the composition of any one of embodiments 29-48, for use in reducing serum and/or plasma bradykinin concentration in a subject.
- [00112] Embodiment 53 is the composition of any one of embodiments 29-48, for use in reducing bradykinin-mediated vasodilation concentration in a subject.
- [00113] Embodiment 54 is the composition of any one of embodiments 29-48, for use in treating or preventing bradykinin production and accumulation, bradykinin-mediated vasodilation, swelling, or angioedema, obstruction of the airway, or asphyxiation.
- [00114] Embodiment 55 is the composition of any one of embodiments 29-48, for use in treating or preventing angioedema caused by or associated with HAE.
- [00115] Embodiment 56 is the composition of any one of embodiments 29-48, for use in reducing the frequency of angioedema attacks.
- [00116] Embodiment 57 is the composition of any one of embodiments 29-48, for use in reducing the severity of angioedema attacks.
- [00117] Embodiment 58 is the composition of any one of embodiments 29-48, for use in reducing the frequency and/or severity of attacks.
- [00118] Embodiment 59 is the composition of any one of embodiments 29-48, for use in achieving remission of angioedema attacks.
- [00119] Embodiment 60 is the composition of any one of embodiments 29-48, for use in reducing the frequency and/or severity of HAE attacks.
- [00120] Embodiment 61 is the composition of embodiment 60, for use in reducing the frequency of HAE attacks.
- [00121] Embodiment 62 is the composition of embodiment 61, wherein the frequency is reduced by at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 60-80%, or at least 40-90%.
- [00122] Embodiment 63 is the method of embodiment 61, wherein the frequency is reduced by at least 60-80%.

- [00123] Embodiment 64 is the method of embodiment 61, wherein the frequency is reduced by at least 40-90%.
- [00124] Embodiment 65 is the composition of embodiment 60, for use in reducing total plasma kallikrein activity.
- [00125] Embodiment 66 is the composition of embodiment 60, for use in reducing total plasma kallikrein levels.
- [00126] Embodiment 67 is the composition of embodiment 60, for use in reducing prekallikrein and/or kallikrein levels.
- [00127] Embodiment 68 is the composition of any one of embodiments 65-67, wherein there is a dose dependent increase in percent editing.
- [00128] Embodiment 69 is the composition of any one of embodiments 65-68, wherein there is a dose dependent reduction in total plasma kallikrein levels.
- [00129] Embodiment 70 is the composition of any one of embodiments 65-69, wherein there is a dose dependent reduction in plasma kallikrein activity.
- [00130] Embodiment 71 is the composition of any one of embodiments 29-70, wherein the effect is durable for at least 1 month, 2 months, 4 months, 6 months, 1 year, 2 years, 5 years, 10 years or more after the administration.
- [00131] Embodiment 72 is the composition of any one of embodiments 29-71, wherein the effect is durable for at least 6 months.
- [00132] Embodiment 73 is the composition of any one of embodiments 29-72, wherein the effect is durable for at least 1 year.
- [00133] Embodiment 74 is the method of any of embodiments 1-28, further comprising:
- a. inducing a double-stranded break (DSB) within the *KLKB1* gene in a cell or subject;
 - b. reducing the expression of the *KLKB1* gene in a cell or subject;
 - c. treating or preventing HAE in a subject;
 - d. reducing serum and/or plasma bradykinin concentration in a subject;
 - e. reducing bradykinin production;
 - f. reducing bradykinin-mediated vasodilation;
 - g. treating or preventing bradykinin-mediated swelling and angioedema; and/or
 - h. treating or preventing obstruction of the airway or asphyxiation caused by swelling.

[00134] Embodiment 75 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition decreases KLKB1 mRNA production.

[00135] Embodiment 76 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition decreases prekallikrein protein levels in plasma or serum.

[00136] Embodiment 77 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition decreases total kallikrein (prekallikrein and pKal) protein levels in plasma or serum.

[00137] Embodiment 78 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition decreases the proportion of circulating cleaved HMWK (cHMWK) compared to total HMWK in citrated serum or citrated plasma.

[00138] Embodiment 79 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition reduces a subject's proportion of cHMWK in citrated plasma to below 30% of total HMWK.

[00139] Embodiment 80 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition decreases the spontaneous pKal activity in serum or plasma.

[00140] Embodiment 81 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition decreases kallikrein activity.

[00141] Embodiment 82 is the method, composition, or composition for use of any one the preceding embodiments, wherein the kallikrein activity comprises total kallikrein activity, prekallikrein activity, and/or pKal activity.

[00142] Embodiment 83 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition reduces a subject's pKal activity by at least about 40% prior to the method or use of the composition.

[00143] Embodiment 84 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition reduces a subject's pKal activity by at least about 50% prior to the method or use of the composition.

[00144] Embodiment 85 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition reduces a subject's pKal activity by at least about 60% prior to the method or use of the composition.

[00145] Embodiment 86 is the method, composition, or composition for use of any one the preceding embodiments, wherein the composition reduces a subject's pKal activity to less than about 40% of basal levels.

[00146] Embodiment 87 is the method, composition, or composition for use of any one of the preceding embodiments, wherein the composition reduces a subject's pKal activity to about 40-50% of basal levels.

[00147] Embodiment 88 is the method, composition, or composition for use of any one of the preceding embodiments, wherein the composition reduces a subject's pKal activity to 20-40% or 20-50% of basal levels.

[00148] Embodiment 89 is the method, composition, or composition for use of any one of the preceding embodiments, wherein the composition increases serum and/or plasma bradykinin levels.

[00149] Embodiment 90 is the method, composition, or composition for use of any one of the preceding embodiments, wherein the composition results in editing of the KLKB1 gene.

[00150] Embodiment 91 is the method, composition, or composition for use of embodiment 90, wherein the editing is calculated as a percentage of the population that is edited (percent editing).

[00151] Embodiment 92 is the method, composition, or composition for use of embodiment 91, wherein the percent editing is between 30 and 99% of the population.

[00152] Embodiment 93 is the method, composition, or composition for use of embodiment 91, wherein the percent editing is between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the population.

[00153] Embodiment 94 is the method, composition, or composition for use of any one of the preceding embodiments, wherein the composition reduces serum and/or plasma bradykinin concentration.

[00154] Embodiment 95 is the method, composition, or composition for use of any one of the preceding embodiments, wherein the composition reduces serum and/or plasma bradykinin concentration, and wherein a reduction in serum and/or plasma bradykinin results in decreased swelling in organ tissues, including limbs, face, GI tract, or airway.

[00155] Embodiment 96 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide sequence is selected from

- a. SEQ ID NOs: 1-149; or
- b. SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
- c. any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
- d. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or

- e. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (d); or
- f. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (d).

[00156] Embodiment 97 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprises a sgRNA comprising:

- a. SEQ ID NOs: 1, 7, 8, 15, 26, 27, 28, 41, 42, 46, 51, 52, 53, 56, 69, 71; or
- b. any one of SEQ ID Nos: 8, 15, 41, 51, 69; or
- c. a sequence that comprises 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 1; or
- d. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (c); or
- e. a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (c).

[00157] Embodiment 98 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the target sequence is in exon 1, exon 3, exon 4, exon 5, exon 6, or exon 8, exon 9, exon 10, exon 11, exon 12, exon 13, exon 14, or exon 15 of the human KLKB1 gene.

[00158] Embodiment 99 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 1 of the human KLKB1 gene.

[00159] Embodiment 100 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 3 of the human KLKB1 gene.

[00160] Embodiment 101 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 4 of the human KLKB1 gene.

[00161] Embodiment 102 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 5 of the human KLKB1 gene.

[00162] Embodiment 103 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 6 of the human KLKB1 gene.

[00163] Embodiment 104 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 8 of the human KLKB1 gene.

[00164] Embodiment 105 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 9 of the human KLKB1 gene.

[00165] Embodiment 106 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 10 of the human KLKB1 gene.

[00166] Embodiment 107 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 11 of the human KLKB1 gene.

- [00167] Embodiment 108 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 12 of the human KLKB1 gene.
- [00168] Embodiment 109 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 13 of the human KLKB1 gene.
- [00169] Embodiment 110 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 14 of the human KLKB1 gene.
- [00170] Embodiment 111 is the method, composition for use, or composition of embodiment 98, wherein the target sequence is in exon 15 of the human KLKB1 gene.
- [00171] Embodiment 112 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide sequence is complementary to a target sequence in the positive strand of KLKB1.
- [00172] Embodiment 113 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide sequence is complementary to a target sequence in the negative strand of KLKB1.
- [00173] Embodiment 114 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the first guide sequence is complementary to a first target sequence in the positive strand of the KLKB1 gene, and wherein the composition further comprises a second guide sequence that is complementary to a second target sequence in the negative strand of the KLKB1 gene.
- [00174] Embodiment 115 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide RNA comprises a guide sequence selected from any one of SEQ ID Nos 1-149 and further comprises a nucleotide sequence of SEQ ID NO: 170, wherein the nucleotides of SEQ ID NO: 170 follow the guide sequence at its 3' end.
- [00175] Embodiment 116 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide RNA comprises a guide sequence selected from any one of SEQ ID Nos: 1-149 and further comprises a nucleotide sequence of SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, or any one of SEQ ID Nos: 400-450, wherein the nucleotides of SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, or any one of conserved portions of sgRNA from Table 4 follow the guide sequence at its 3' end.
- [00176] Embodiment 117 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide RNA is a single guide RNA (sgRNA).

[00177] Embodiment 118 is the method, composition for use, or composition of embodiment 117, wherein the sgRNA comprises a guide sequence comprising any one of SEQ ID Nos: 8, 15, 41, 51, 69.

[00178] Embodiment 119 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide RNA is modified according to the pattern of SEQ ID NO: 300, wherein the N's are collectively any one of the guide sequences of Table 1 (SEQ ID Nos: 1-149).

[00179] Embodiment 120 is the method, composition for use, or composition of embodiment 119, wherein each N in SEQ ID NO: 300 is any natural or non-natural nucleotide, wherein the N's form the guide sequence, and the guide sequence targets Cas9 to the KLKB1 gene.

[00180] Embodiment 121 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 1-149 and the nucleotides of SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, or any of the conserved portions of sgRNA from Table 4, wherein the nucleotides of SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, or any of the conserved portions of sgRNA from Table 4 follow the guide sequence at its 3' end.

[00181] Embodiment 122 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the sgRNA comprises a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID Nos: 1-149.

[00182] Embodiment 123 is the method, composition for use, or composition of embodiment 122, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 8, 15, 41, 51, 69.

[00183] Embodiment 124 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide RNA comprises at least one modification.

[00184] Embodiment 125 is the method, composition for use, or composition of embodiment 124, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

[00185] Embodiment 126 is the method, composition for use, or composition of any one of embodiments 124-125, comprising a phosphorothioate (PS) bond between nucleotides.

[00186] Embodiment 127 is the method, composition for use, or composition of any one of embodiments 124-126, comprising a 2'-fluoro (2'-F) modified nucleotide.

[00187] Embodiment 128 is the method, composition for use, or composition of any one of embodiments 124-127, comprising a modification at one or more of the first five nucleotides at the 5' end of the guide RNA.

[00188] Embodiment 129 is the method, composition for use, or composition of any one of embodiments 124-128, comprising a modification at one or more of the last five nucleotides at the 3' end of the guide RNA.

[00189] Embodiment 130 is the method, composition for use, or composition of any one of embodiments 124-129, comprising a PS bond between the first four nucleotides of the guide RNA.

[00190] Embodiment 131 is the method, composition for use, or composition of any one of embodiments 124-130, comprising a PS bond between the last four nucleotides of the guide RNA.

[00191] Embodiment 132 is the method, composition for use, or composition of any one of embodiments 124-131, comprising a 2'-O-Me modified nucleotide at the first three nucleotides at the 5' end of the guide RNA.

[00192] Embodiment 133 is the method, composition for use, or composition of any one of embodiments 124-132, comprising a 2'-O-Me modified nucleotide at the last three nucleotides at the 3' end of the guide RNA.

[00193] Embodiment 134 is the method, composition for use, or composition of any one of embodiments 124-133, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 300.

[00194] Embodiment 135 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition further comprises a pharmaceutically acceptable excipient.

[00195] Embodiment 136 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide RNA is associated with a lipid nanoparticle (LNP).

[00196] Embodiment 137 is the method, composition for use, or composition of embodiment 136, wherein the LNP comprises a cationic lipid.

[00197] Embodiment 138 is the method, composition for use, or composition of embodiment 137, 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.

- [00198] Embodiment 139 is the method, composition for use, or composition of any one of embodiments 136-138, wherein the LNP comprises a neutral lipid.
- [00199] Embodiment 140 is the method, composition for use, or composition of embodiment 139, wherein the neutral lipid is DSPC.
- [00200] Embodiment 141 is the method, composition for use, or composition of any one of embodiments 136-140, wherein the LNP comprises a helper lipid.
- [00201] Embodiment 142 is the method, composition for use, or composition of embodiment 141, wherein the helper lipid is cholesterol.
- [00202] Embodiment 143 is the method, composition for use, or composition of any one of embodiments 136-142, wherein the LNP comprises a stealth lipid.
- [00203] Embodiment 144 is the method, composition for use, or composition of embodiment 143, wherein the stealth lipid is PEG2k-DMG.
- [00204] Embodiment 145 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition further comprises an RNA-guided DNA binding agent.
- [00205] Embodiment 146 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition further comprises an mRNA that encodes an RNA-guided DNA binding agent.
- [00206] Embodiment 147 is the method, composition for use, or composition of embodiment 145 or 146, wherein the RNA-guided DNA binding agent is Cas9.
- [00207] Embodiment 148 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.
- [00208] Embodiment 149 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprises a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 1.
- [00209] Embodiment 150 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 2.
- [00210] Embodiment 151 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 3.

[00211] Embodiment 152 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 4.

[00212] Embodiment 153 is the method, composition for use, or composition of any one of embodiments 1-89, wherein the sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 5.

[00213] Embodiment 154 is the method, composition for use, or composition of any one of embodiments 1-89, wherein the sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 6.

[00214] Embodiment 155 is the method, composition for use, or composition of any one of embodiments 1-89, wherein the sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 7.

[00215] Embodiment 156 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprises a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 8.

[00216] Embodiment 157 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 9.

[00217] Embodiment 158 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 10.

[00218] Embodiment 159 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 11.

[00219] Embodiment 160 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 12.

[00220] Embodiment 161 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 13.

[00221] Embodiment 162 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 14.

[00222] Embodiment 163 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 15.

[00223] Embodiment 164 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 16.

[00224] Embodiment 165 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 17.

[00225] Embodiment 166 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 18.

[00226] Embodiment 167 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 19.

[00227] Embodiment 168 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 20.

[00228] Embodiment 169 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 21.

[00229] Embodiment 170 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 22.

[00230] Embodiment 171 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 23.

[00231] Embodiment 172 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 24.

[00232] Embodiment 173 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 25.

[00233] Embodiment 174 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 26.

[00234] Embodiment 175 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 27.

[00235] Embodiment 176 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 28.

[00236] Embodiment 177 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 29.

[00237] Embodiment 178 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 30.

[00238] Embodiment 179 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 31.

[00239] Embodiment 180 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 32.

[00240] Embodiment 181 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 33.

[00241] Embodiment 182 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 34.

[00242] Embodiment 183 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 35.

[00243] Embodiment 184 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 36.

[00244] Embodiment 185 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 37.

[00245] Embodiment 186 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 38.

[00246] Embodiment 187 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 39.

[00247] Embodiment 188 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 40.

[00248] Embodiment 189 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 41.

[00249] Embodiment 190 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 42.

[00250] Embodiment 191 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 43.

[00251] Embodiment 192 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 44.

[00252] Embodiment 193 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 45.

[00253] Embodiment 194 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 46.

[00254] Embodiment 195 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 47.

[00255] Embodiment 196 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 48.

[00256] Embodiment 197 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 49.

[00257] Embodiment 198 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 50.

[00258] Embodiment 199 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 51.

[00259] Embodiment 200 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 52.

[00260] Embodiment 201 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 53.

[00261] Embodiment 202 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 54.

[00262] Embodiment 203 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 55.

[00263] Embodiment 204 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 56.

[00264] Embodiment 205 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 57.

[00265] Embodiment 206 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 58.

[00266] Embodiment 207 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 59.

[00267] Embodiment 208 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 60.

[00268] Embodiment 209 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 61.

[00269] Embodiment 210 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 62.

[00270] Embodiment 211 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 63.

[00271] Embodiment 212 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 64.

[00272] Embodiment 213 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 65.

[00273] Embodiment 214 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 66.

[00274] Embodiment 215 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 67.

[00275] Embodiment 216 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 68.

[00276] Embodiment 217 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 69.

[00277] Embodiment 218 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 70.

[00278] Embodiment 219 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 71.

[00279] Embodiment 220 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 72.

[00280] Embodiment 221 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 73.

[00281] Embodiment 222 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 74.

[00282] Embodiment 223 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 75.

[00283] Embodiment 224 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 76.

[00284] Embodiment 225 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 77.

[00285] Embodiment 226 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 78.

[00286] Embodiment 227 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 79.

[00287] Embodiment 228 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 80.

[00288] Embodiment 229 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 81.

[00289] Embodiment 230 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 82.

[00290] Embodiment 231 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 83.

[00291] Embodiment 232 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 84.

[00292] Embodiment 233 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 85.

[00293] Embodiment 234 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 86.

[00294] Embodiment 235 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 87.

[00295] Embodiment 236 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 88.

[00296] Embodiment 237 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 89.

[00297] Embodiment 238 is the method, composition for use, or composition of any one of embodiments 1-89, wherein the sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 90.

[00298] Embodiment 239 is the method, composition for use, or composition of any one of embodiments 1-89, wherein the sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 91.

[00299] Embodiment 240 is the method, composition for use, or composition of any one of embodiments 1-89, wherein the sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 92.

[00300] Embodiment 241 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 93.

[00301] Embodiment 242 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 94.

[00302] Embodiment 243 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 95.

[00303] Embodiment 244 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 96.

[00304] Embodiment 245 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 97.

[00305] Embodiment 246 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 98.

[00306] Embodiment 247 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 99.

[00307] Embodiment 248 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 100.

[00308] Embodiment 249 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 101.

[00309] Embodiment 250 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 102.

[00310] Embodiment 251 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 103.

[00311] Embodiment 252 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 104.

[00312] Embodiment 253 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 105.

[00313] Embodiment 254 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 106.

[00314] Embodiment 255 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 107.

[00315] Embodiment 256 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 108.

[00316] Embodiment 257 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 109.

[00317] Embodiment 258 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 110.

[00318] Embodiment 259 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 111.

[00319] Embodiment 260 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 112.

[00320] Embodiment 261 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 113.

[00321] Embodiment 262 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 114.

[00322] Embodiment 263 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 115.

[00323] Embodiment 264 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 116.

[00324] Embodiment 265 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 117.

[00325] Embodiment 266 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 118.

[00326] Embodiment 267 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 119.

[00327] Embodiment 268 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 120.

[00328] Embodiment 269 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 121.

[00329] Embodiment 270 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 122.

[00330] Embodiment 271 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 123.

[00331] Embodiment 272 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 124.

[00332] Embodiment 273 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 125.

[00333] Embodiment 274 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 126.

[00334] Embodiment 275 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 127.

[00335] Embodiment 276 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 128.

[00336] Embodiment 277 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 129.

[00337] Embodiment 278 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 130.

[00338] Embodiment 279 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 131.

[00339] Embodiment 280 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 132.

[00340] Embodiment 281 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 133.

[00341] Embodiment 282 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 134.

[00342] Embodiment 283 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 135.

[00343] Embodiment 284 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 136.

[00344] Embodiment 285 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 137.

[00345] Embodiment 286 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 138.

[00346] Embodiment 287 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 139.

[00347] Embodiment 288 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 140.

[00348] Embodiment 289 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 141.

[00349] Embodiment 290 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 142.

[00350] Embodiment 291 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 143.

[00351] Embodiment 292 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 144.

[00352] Embodiment 293 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 145.

[00353] Embodiment 294 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 146.

[00354] Embodiment 295 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 147.

[00355] Embodiment 296 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 148.

[00356] Embodiment 297 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the composition comprising a sequence selected from SEQ ID NOs: 1-149 is SEQ ID NO: 149.

[00357] Embodiment 298 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide sequence is selected from SEQ ID NO: 310-386.

[00358] Embodiment 299 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide sequence is selected from SEQ ID NO: 310-311, 313-326, 329-337, 339-342, 344-346, 348, 350, 352-356, 361, 362, 364, 365, 366, 367, 369-374, 376-380, and 382-386.

[00359] Embodiment 300 is the method, composition for use, or composition of any one of the preceding embodiments, wherein the guide sequence is selected from SEQ ID NO: 310-386 is SEQ ID NO: 310.

[00360] Embodiment 301 is the method, composition for use, or composition of any one of the preceding embodiments, comprising an sgRNAs comprising the guide sequence of any one of SEQ ID NOs: 1-149 and any one of the conserved portions of sgRNA of Table 4, optionally having the modification pattern of SEQ ID NO: 450 or any one of the modification patterns of Table 4, optionally wherein the sgRNA comprises a 5' and 3' end modification.

[00361] Embodiment 302 is the method, composition, or composition for use of any one of embodiments 1-301, wherein the composition is administered as a single dose.

[00362] Embodiment 303 is the method, composition, or composition for use of any one of embodiments 1-301, wherein the composition is administered one time.

[00363] Embodiment 304 is the method, composition, or composition for use of any one of embodiments 302 or 303, wherein the single dose or one time administration:

- a. inducing a double-stranded break (DSB) within the *KLKB1* gene in a cell or subject; and/or
- b. reducing expression of the *KLKB1* gene in a cell or subject; and/or
- c. treating or preventing HAE in a subject; and/or

- d. treating or preventing angioedema caused by or associated with HAE in a subject; and/or
- e. reducing serum and/or plasma bradykinin concentration in a subject;
- f. reducing bradykinin-mediated vasodilation;
- g. treating or preventing bradykinin-mediated swelling and angioedema; and/or
- h. treating or preventing obstruction of the airway or asphyxiation caused by swelling.

[00364] Embodiment 305 is the method or composition of embodiment 304, wherein the single dose or one time administration achieves any one or more of a) – h) for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks.

[00365] Embodiment 306 is the method or composition of embodiment 304, wherein the single dose or one time administration achieves a durable effect.

[00366] Embodiment 307 is the method, composition, or composition for use of any one of embodiments 1-306, further comprising achieving a durable effect.

[00367] Embodiment 308 is the method, composition, or composition for use of embodiment 307, wherein the durable effect persists at least 1 month, at least 3 months, at least 6 months, at least one year, or at least 5 years.

[00368] Embodiment 309 is the method, composition, or composition for use of any one of embodiments 1-308, wherein administration of the composition results in a therapeutically relevant reduction of kallikrein activity, total plasma kallikrein levels, prekallikrein and/or kallikrein levels, or bradykinin in serum and/or plasma.

[00369] Embodiment 310 is the method, composition, or composition for use of any one of embodiments 1-309, wherein administration of the composition results in serum and/or plasma bradykinin levels within a therapeutic range.

[00370] Embodiment 311 is the method, composition, or composition for use of any one of the preceding embodiments, wherein administration of the composition results in serum and/or plasma bradykinin levels within 100, 120, or 150% of normal range.

[00371] Embodiment 312 is use of a composition of any of the preceding composition embodiments for the preparation of a medicament for treating a human subject having HAE.

[00372] Embodiment 313 is use of a composition of any of the preceding composition embodiments for the preparation of a medicament for treating and preventing bradykinin production and accumulation, bradykinin-induced swelling, angioedema obstruction of the airway, or asphyxiation.

[00373] Embodiment 314 is use of a composition of any of the preceding composition embodiments for the preparation of a medicament for treating or preventing angioedema caused by or associated with HAE.

[00374] Embodiment 315 is use of a composition of any of the preceding composition embodiments for the preparation of a medicament for reducing the frequency of angioedema attacks.

[00375] Embodiment 316 is use of a composition of any of the preceding composition embodiments for the preparation of a medicament for reducing the severity of angioedema attacks.

[00376] Embodiment 317 is use of a composition of any of the preceding composition embodiments for the preparation of a medicament for reducing the frequency and/or severity of HAE attacks.

[00377] Embodiment 318 is use of a composition of any of the preceding composition embodiments for the preparation of a medicament for achieving remission of angioedema attacks.

[00378] Embodiment 319 is use of a composition of any of the preceding composition embodiments for the preparation of a medicament for achieving durable remission, e.g. maintained for at least 1 month, 2 months, 4 months, 6 months, 1 year, 2 years, 5 years, 10 years or more.

BRIEF DESCRIPTION OF THE DRAWINGS

[00379] Figs. 1A-1D shows percent editing (indel frequency) detected at various sites within the *KLKB1* locus using guide RNAs in primary human hepatocytes (PHH) (FIGS. 1A-1B) and primary cynomolgus hepatocytes (PCH) (FIGS. 1C-1D).

[00380] Figs. 2A-2D show percent editing (indel frequency) of *KLKB1* sgRNAs in PHH (FIGS. 2A-2B) and PCH (FIGS. 2C-2D).

[00381] Figs. 3A-3E show percent editing (indel frequency) (FIG. 3A), secreted KLKB1 protein levels (FIG. 3B), and correlation plots (FIGS. 3C-E), after transfection of PHH with *KLKB1*-targeting guide RNAs in three different PHH lots (HU8300, HU8284, and HU8296).

[00382] Figs. 4A-4D show percent editing of the *KLKB1* guides in primary human hepatocytes (PHH) (FIGS. 4A-4B), and percent editing of the *KLKB1* guides in primary cynomolgus hepatocytes (PCH) (FIGS. 4C-4D).

- [00383] Figs. 5A-5J show dose response data for percent editing and secreted kallikrein for certain guide sequences in PHH (FIGS. 5A-5D) and PCH (FIGS. 5E-5H), and correlation plots of percent editing and secreted protein in PHH and PCH (FIGS. 5I-5J).
- [00384] Figs. 6A-6D provide dose response curve data for indel frequency for certain guide sequences in PHH (FIGS. 6A-6B) and PCH (FIGS. 6C-6D).
- [00385] Figs. 7A-7E show dose response curve data for indel frequency (FIGS. 7A and 7B) and KLKB1 secretion (FIGS. 7C and 7D) for certain guide sequences in PHH (FIGS. 7A and 7C) and PCH (FIGS. 7B and 7D) and western blot analysis to measure secreted protein (FIG. 7E).
- [00386] FIG. 8A shows *KLKB1* editing % for various modified sgRNAs *in vivo* in Hu KLKB1 mice.
- [00387] Figs. 8B and 8C show KLKB1 protein levels measured using the ELISA and electrochemiluminescence-based array respectively in Hu KLKB1 mice (Example 6).
- [00388] Fig. 8D shows the fold change of KLKB1 mRNA levels for each sequence in Hu KLKB1 mice.
- [00389] Figs. 9A-9D show levels of *KLKB1* editing (FIG. 9A), serum KLKB1 protein (prekallikrein and kallikrein) (FIG. 9B), percent TSS (FIG. 9C) in treated mice, and the correlation of percent liver editing to percent KLKB1 protein (FIG. 9D).
- [00390] Fig. 10 shows dose-dependent levels of KLKB1 gene editing, percent knockdown of KLKB1 mRNA, and plasma kallikrein in Hu KLKB1 mouse model.
- [00391] Fig. 11A shows levels of KLKB1 gene editing and plasma kallikrein in a dose response assay at after treatment with the indicated doses of sgRNA in Hu KLKB1 mouse model.
- [00392] Fig. 11B shows levels of absorbance at 600 nm light to detect Evans blue (EB) dye from colon samples in a dose response vascular permeability assay in response to treatment with permeabilizing agents at after treatment with the indicated doses of sgRNA in Hu KLKB1 mouse model.
- [00393] Figs. 12A-12B show *in vivo* dose-dependent reductions in circulating total kallikrein activity (FIG. 12A) and protein levels (FIG. 12B), respectively, after a single dose administration of CRISPR/Cas9 components at 1.5 mg/kg, 3 mg/kg, or 6 mg/kg with G013901 in cynomolgus monkeys.
- [00394] Figs. 13A-13B show *in vivo* reductions in circulating total kallikrein activity (FIG. 13A) and protein levels (FIG. 13B), respectively, after a single dose administration of

CRISPR/Cas9 components at the indicated dosages with G012267 in cynomolgous monkeys.

[00395] Fig. 14 labels the 10 conserved region YA sites in an exemplary sgRNA sequence (SEQ ID NO: 201) from 1 to 10. The numbers 25, 45, 50, 56, 64, 67, and 83 indicate the position of the pyrimidine of YA sites 1, 5, 6, 7, 8, 9, and 10 in an sgRNA with a guide region indicated as (N)x, e.g., wherein x is optionally 20.

[00396] Fig. 15 shows an exemplary sgRNA (SEQ ID NO: 401; not all modifications are shown) in a possible secondary structure with labels designating individual nucleotides of the conserved region of the sgRNA, including the lower stem, bulge, upper stem, nexus (the nucleotides of which can be referred to as N1 through N18, respectively, in the 5' to 3' direction), hairpin 1, and hairpin 2 regions. A nucleotide between hairpin 1 and hairpin 2 is labeled n. A guide region may be present on an sgRNA and is indicated in this figure as "(N)x" preceding the conserved region of the sgRNA.

DETAILED DESCRIPTION

[00397] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying drawings. While the invention is described in conjunction with the illustrated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the invention as defined by the appended claims and included embodiments.

[00398] Before describing the present teachings in detail, it is to be understood that the disclosure is not limited to specific compositions or process steps, as such may vary. It should be noted that, as used in this specification and the appended claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a conjugate" includes a plurality of conjugates and reference to "a cell" includes a plurality of cells and the like.

[00399] 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. Also, the use of "comprise", "comprises", "comprising", "contain", "contains", "containing", "include", "includes", and "including" are not intended to be limiting. It is to be understood that both the foregoing general description and detailed description are exemplary and explanatory only and are not restrictive of the teachings.

[00400] 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).

[00401] The term “or” is used in an inclusive sense, i.e., equivalent to “and/or,” unless the context clearly indicates otherwise.

[00402] The section headings used herein are for organizational purposes only and are not to be construed as limiting the desired subject matter in any way. In the event that any material incorporated by reference contradicts any term defined in this specification or any other express content of this specification, this specification controls. While the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

I. Definitions

[00403] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[00404] “Polynucleotide” and “nucleic acid” are used herein to refer to a multimeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases or base analogs linked together along a backbone, including conventional RNA, DNA, mixed RNA-DNA, and polymers that are analogs thereof. A nucleic acid “backbone” can be made up of a variety of linkages, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds (“peptide nucleic acids” or PNA; PCT No. WO 95/32305), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of a nucleic acid can be ribose, deoxyribose, or similar compounds with substitutions, e.g., 2' methoxy or 2' halide substitutions. Nitrogenous bases can be conventional bases (A, G, C, T, U), analogs thereof (e.g., modified uridines such as 5-methoxyuridine, pseudouridine, or N1-methylpseudouridine, or others); inosine; derivatives of purines or pyrimidines (e.g., 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 bases with 2' methoxy linkages, or polymers containing both conventional bases and one or more base analogs). Nucleic acid includes “locked nucleic acid” (LNA), an analogue containing one or more LNA nucleotide monomers with a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhance hybridization affinity toward complementary RNA and DNA sequences (Vester and Wengel, 2004, *Biochemistry* 43(42):13233-41). RNA and DNA have different sugar moieties and can differ by the presence of uracil or analogs thereof in RNA and thymine or analogs thereof in DNA.

[00405] “Guide RNA”, “gRNA”, and simply “guide” are used herein interchangeably to refer to the guide that directs an RNA-guided DNA binding agent to a target DNA and can be either a crRNA (also known as CRISPR RNA), or the combination of a crRNA and a trRNA (also known as tracrRNA). The crRNA and trRNA may be associated as a single RNA molecule (single guide RNA, sgRNA) or in two separate RNA molecules (dual guide RNA, dgRNA). “Guide RNA” or “gRNA” refers to each type. The trRNA may be a naturally-occurring sequence, or a trRNA sequence with modifications or variations compared to naturally-occurring sequences.

[00406] As used herein, a “guide sequence” refers to a sequence within a guide RNA that is complementary to a target sequence and functions to direct a guide RNA to a target sequence for binding or modification (e.g., cleavage) by an RNA-guided DNA binding agent. A “guide sequence” may also be referred to as a “targeting sequence,” or a “spacer sequence.” A guide sequence can be 20 base pairs in length, e.g., in the case of *Streptococcus pyogenes* (i.e., Spy Cas9) and related Cas9 homologs/orthologs. Shorter or longer sequences can also be used as guides, e.g., 15-, 16-, 17-, 18-, 19-, 21-, 22-, 23-, 24-, or 25-nucleotides in length. For example, in some embodiments, the guide sequence comprises at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149. In some embodiments, the target sequence is in a gene or on a chromosome, for

example, and is complementary to the guide sequence. In some embodiments, the degree of complementarity or identity between a guide sequence and its corresponding target sequence may be about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. For example, in some embodiments, the guide sequence comprises a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-149. In some embodiments, the guide sequence and the target region may be 100% complementary or identical. In other embodiments, the guide sequence and the target region may contain at least one mismatch. For example, the guide sequence and the target sequence may contain 1, 2, 3, or 4 mismatches, where the total length of the target sequence is at least 17, 18, 19, 20 or more base pairs. In some embodiments, the guide sequence and the target region may contain 1-4 mismatches where the guide sequence comprises at least 17, 18, 19, 20 or more nucleotides. In some embodiments, the guide sequence and the target region may contain 1, 2, 3, or 4 mismatches where the guide sequence comprises 20 nucleotides.

[00407] Target sequences for RNA-guided DNA binding agents include both the positive and negative strands of genomic DNA (i.e., the sequence given and the sequence's reverse complement), as a nucleic acid substrate for an RNA-guided DNA binding agent is a double stranded nucleic acid. Accordingly, where a guide sequence is said to be "complementary to a target sequence", it is to be understood that the guide sequence may direct a guide RNA to bind to the reverse complement of a target sequence. Thus, in some embodiments, where the guide sequence binds the reverse complement of a target sequence, the guide sequence is identical to certain nucleotides of the target sequence (e.g., the target sequence not including the PAM) except for the substitution of U for T in the guide sequence.

[00408] As used herein, an "RNA-guided DNA binding agent" means a polypeptide or complex of polypeptides having RNA and DNA binding activity, or a DNA-binding subunit of such a complex, wherein the DNA binding activity is sequence-specific and depends on the sequence of the RNA. Exemplary RNA-guided DNA binding agents include Cas cleavases/nickases and inactivated forms thereof ("dCas DNA binding agents"). "Cas nuclease", also called "Cas protein" as used herein, encompasses Cas cleavases, Cas nickases, and dCas DNA binding agents. Cas cleavases/nickases and dCas DNA binding agents include a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases. As used herein, a "Class 2 Cas nuclease" is a single-chain polypeptide with RNA-guided DNA binding activity. Class 2 Cas nucleases include Class 2

Cas cleavases/nickases (e.g., H840A, D10A, or N863A variants), which further have RNA-guided DNA cleavases or nickase activity, and Class 2 dCas DNA binding agents, in which cleavase/nickase activity is inactivated. Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, C2c3, HF Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9 (e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g., K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants) proteins and modifications thereof. Cpf1 protein, Zetsche et al., *Cell*, 163: 1-13 (2015), is homologous to Cas9, and contains a RuvC-like nuclease domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. *See, e.g.,* Zetsche, Tables S1 and S3. *See, e.g.,* Makarova et al., *Nat Rev Microbiol*, 13(11): 722-36 (2015); Shmakov et al., *Molecular Cell*, 60:385-397 (2015).

[00409] As used herein, “ribonucleoprotein” (RNP) or “RNP complex” refers to a guide RNA together with an RNA-guided DNA binding agent, such as a Cas nuclease, e.g., a Cas cleavase, Cas nickase, or dCas DNA binding agent (e.g., Cas9). In some embodiments, the guide RNA guides the RNA-guided DNA binding agent such as Cas9 to a target sequence, and the guide RNA hybridizes with and the agent binds to the target sequence; in cases where the agent is a cleavase or nickase, binding can be followed by cleaving or nicking.

[00410] As used herein, a first sequence is considered to “comprise a sequence with at least X% identity to” a second sequence if an alignment of the first sequence to the second sequence shows that X% or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAGA comprises a sequence with 100% identity to the sequence AAG because an alignment would give 100% identity in that there are matches to all three positions of the second sequence. The differences between RNA and DNA (generally the exchange of uridine for thymidine or vice versa) and the presence of nucleoside analogs such as modified uridines do not contribute to differences in identity or complementarity among polynucleotides as long as the relevant nucleotides (such as thymidine, uridine, or modified uridine) have the same complement (e.g., adenosine for all of thymidine, uridine, or modified uridine; another example is cytosine and 5-methylcytosine, both of which have guanosine or modified guanosine as a complement). Thus, for example, the sequence 5'-AXG where X is any modified uridine, such as pseudouridine, N1-methyl pseudouridine, or 5-methoxyuridine, is considered 100% identical to AUG in that both are perfectly complementary to the same sequence (5'-CAU). Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the

art. One skilled in the art will understand what choice of algorithm and parameter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server is generally appropriate.

[00411] “mRNA” is used herein to refer to a polynucleotide and comprises an open reading frame that can be translated into a polypeptide (i.e., can serve as a substrate for translation by a ribosome and amino-acylated tRNAs). mRNA can comprise a phosphate-sugar backbone including ribose residues or analogs thereof, e.g., 2'-methoxy ribose residues. In some embodiments, the sugars of an mRNA phosphate-sugar backbone consist essentially of ribose residues, 2'-methoxy ribose residues, or a combination thereof.

[00412] Guide sequences useful in the guide RNA compositions and methods described herein are shown in Table 1 or Table 2 and throughout the application.

[00413] 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.

[00414] As used herein, “knockdown” refers to a decrease in expression of a particular gene product (e.g., protein, mRNA, or both). Knockdown of a protein can be measured by detecting total cellular amount of the protein from a sample, such as a tissue, fluid, or cell population of interest. It can also be measured by measuring a surrogate, marker, or activity for the protein. Methods for measuring knockdown of mRNA are known and include sequencing of mRNA isolated from a sample of interest. In some embodiments, “knockdown” may refer to some loss of expression of a particular gene product, for example a decrease in the amount of mRNA transcribed or a decrease in the amount of protein expressed by a population of cells (including *in vivo* populations such as those found in tissues).

[00415] As used herein, “knockout” refers to a loss of expression from a particular gene or of a particular protein in a cell. Knockout can be measured either by detecting total cellular amount of a protein in a cell, a tissue or a population of cells. In some embodiments, the methods of the invention “knockout” *KLKB1* in one or more samples, e.g., serum, plasma, tissue, or cells (e.g., in a population of cells including *in vivo* populations such as those found in tissues). In some embodiments, a knockout is not the formation of mutant *KLKB1* protein, for example, created by indels, but rather the complete loss of expression of *KLKB1* protein in a cell. As used herein, “*KLKB1*” generally refers to prekallikrein, which is the gene

product of a *KLKB1* gene. Prekallikrein is processed to plasma kallikrein (pKal), and antibodies can detect pKal, prekallikrein, or both. The human wild-type *KLKB1* sequence is available at NCBI Gene ID: 3818; Ensembl: ENSG00000164344. “PKK,” “PPK,” “KLK3,” and “PKKD” are gene synonyms. The human *KLKB1* transcript is available at Ensembl: ENST00000264690, and the cynomolgus wild-type *KLKB1* sequence is available at Ensembl: ENSMFAT00000002355.

[00416] “Hereditary Angioedema” (HAE) is an inflammatory disorder characterized by recurrent episodes of severe swelling (angioedema), due to inactivating mutations of the *SERPING1* gene, which encodes the C1 esterase inhibitor protein (C1-INH). C1-INH blocks the activity of certain proteins that promote inflammation (*e.g.*, in Kinin system). Deficient levels of C1-INH leads to unchecked Factor XII (FXII) and high level of activation of kallikrein (pKal, processed from *KLKB1* protein (prekallikrein)). Kallikrein cleaves high-molecular weight kininogen (HMWK) to release bradykinin, a peptide that impacts vascular permeability. Excessive amount of bradykinin in the blood leads to the fluid leakage through the walls of blood vessels into body tissues, causing swelling seen in individuals with HAE. Thus, in some embodiments, methods for decreasing *KLKB1* activity are provided, wherein once reduced, bradykinin production is decreased and swelling attacks are reduced. Protein levels of prekallikrein/kallikrein, HMWK and its cleavage products, and surrogate labeled substrates of HMWK may be measured to assess efficacy of *KLKB1* knockout.

[00417] As used herein, a “target sequence” refers to a sequence of nucleic acid in a target gene that has complementarity to the guide sequence of the gRNA. The interaction of the target sequence and the guide sequence directs an RNA-guided DNA binding agent to bind, and potentially nick or cleave (depending on the activity of the agent), within the target sequence.

[00418] As used herein, a “YA site” refers to a 5'-pyrimidine-adenine-3' dinucleotide. A “conserved region YA site” is present in the conserved region of an sgRNA. A “guide region YA site” is present in the guide region of an sgRNA. An unmodified YA site in an sgRNA may be susceptible to cleavage by RNase-A like endonucleases, *e.g.*, RNase A. In some embodiments, an sgRNA comprises about 10 YA sites in its conserved region. In some embodiments, an sgRNA comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 YA sites in its conserved region. Exemplary conserved region YA sites are indicated in FIG. 14 (SEQ ID NO: 201), in relation to an sgRNA structure (FIG. 15). Exemplary guide region YA sites are not shown in FIG. 14, as the guide region may be any sequence, including any number of YA sites. In some embodiments, an sgRNA comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the YA sites

indicated in FIG. 14. In some embodiments, an sgRNA comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 YA sites at the following positions or a subset thereof: LS5-LS6; US3-US4; US9-US10; US12-B3; LS7-LS8; LS12-N1; N6-N7; N14-N15; N17-N18; and H2-2 to H2-3. In some embodiments, a YA site comprises a modification, meaning that at least one nucleotide of the YA site is modified. In some embodiments, the pyrimidine (also called the pyrimidine position) of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the pyrimidine). In some embodiments, the adenine (also called the adenine position) of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the adenine). In some embodiments, the pyrimidine position and the adenine position of the YA site comprise modifications.

[00419] As used herein, "treatment" refers to any administration or application of a therapeutic for disease or disorder in a subject, and includes inhibiting the disease, arresting its development, relieving one or more symptoms of the disease, curing the disease, or preventing reoccurrence of one or more symptoms of the disease. For example, treatment of HAE may comprise alleviating symptoms of HAE.

[00420] The term "therapeutically relevant reduction of KLKB1 activity," can mean a greater than about 60% reduction of plasma KLKB1 activity as compared to baseline. See, Banerji et al., *N Engl J Med*, 2017, 376:717-728; Ferrone et al., *Nucleic Acid Therapeutics*, 2019, 82-917. KLKB1 activity is often measured as total kallikrein activity, in which prekallikrein is converted to kallikrein in a sample and total kallikrein activity is measured for the sample. In some instances, a range of KLKB1 activity reduction can mean about 60-80% reduction of plasma KLKB1 activity as compared to baseline. To calculate reduction of an analyte in a subject, a basal value can be obtained by collecting a pretreatment sample from the subject. In some instances, the sample is a serum sample. In certain aspects, the target KLKB1 activity reduction is about a 60% reduction in total kallikrein (prekallikrein and plasma kallikrein) activity as compared to baseline. For example, achieving KLKB1 activity levels within a therapeutic range can mean reducing total kallikrein by about >60% from baseline. In some embodiments, a "normal kallikrein level" or a "normal kallikrein range" is reduced. In some embodiments, a therapeutically relevant reduction of kallikrein activity achieves levels of about 0-60%, 0-50%, 0-40%, 0-30%, 0-25%, 0-20%, 0-15%, 0-10% of a basal value for the subject, or 10-60%, 10-50%, 10-40%, 10-30%, 10-20%, or 20-60%, 20-50%, 20-40%, or 20-30%, of normal kallikrein activity level. KLKB1 activity can be measured by assays known in the field, including assays described herein.

[00421] The term “target KLKB1 protein reduction,” as used herein, means the target level of pKal as compared to baseline. KLKB1 protein levels can be measured by assays known in the field such as ELISA or western blot assays, as described herein. Total KLKB1 protein can be measured with an antibody that detects both prekallikrein and kallikrein and/or after converting prekallikrein to kallikrein in a sample. In some instances, the sample is a serum sample. In certain aspects, the target KLKB1 protein reduction is about a 60% reduction in total kallikrein (prekallikrein and plasma kallikrein) as compared to baseline. In some embodiments, a therapeutically relevant reduction of total kallikrein protein achieves levels of about 0-60%, 0-50%, 0-40%, 0-30%, 0-25%, 0-20%, 0-15%, 0-10% of a basal value for the subject, or 10-60%, 10-50%, 10-40%, 10-30%, 10-20%, or 20-60%, 20-50%, 20-40%, or 20-30%, of normal total kallikrein protein level.

[00422] Circulating plasma cHMWK levels below about 30% total HMWK were associated with decreases in HAE attacks in patients treated with lanadelumab (See Banerji, et al, 2017). In this same study, healthy controls had plasma levels of cHMWK around 8.3% total HMWK. In another study, Suffritti and colleagues found cHMWK plasma levels of an average of about 34.8% in normal controls, about 41.4% in HAE patients in remission and about 58.1% in HAE patients during an attack (Suffritti, et al. Clin Exp Allergy 2014;44:1503-14). Therapeutic treatment can target a ratio of circulating plasma cHMWK to total HMWK of less than about 60%. In some embodiments the ratio of cHMWK to HMWK is less than about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, or more.

[00423] The term “about” or “approximately” means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined.

II. Compositions

A. Compositions Comprising Guide RNA (gRNAs)

[00424] Provided herein are compositions useful for inducing a double-stranded break (DSB), single-strand break, and/or site-specific binding that results in nucleic acid modification within the *KLKB1* gene, e.g., using a guide RNA with an RNA-guided DNA binding agent (e.g., a CRISPR/Cas system). The compositions may be administered to subjects having or suspected of having HAE. The compositions may be administered to subjects having increased serum and/or plasma bradykinin concentration as measured, for example, by a decrease in prekallikrein protein levels in the plasma or serum, by a decrease in total kallikrein (prekallikrein and pKal) protein levels in plasma or serum, by a decrease in

the proportion of circulating cleaved HMWK (cHMWK), or by a decrease in the proportion of cHMWK in citrated plasma. The compositions may be administered to subjects having increased serum and/or plasma prekallikrein and/or kallikrein concentration. The compositions may be administered to subjects having increased serum and/or plasma total kallikrein concentration. The compositions may be administered to subjects having increased serum and/or plasma kallikrein activity. Guide sequences targeting the *KLKB1* gene are shown in Table 1 at SEQ ID NOs: 1-149.

[00425] Each of the guide sequences shown in Table 1 at SEQ ID NOs: 1-149 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: 167) in 5' to 3' orientation. In the case of a sgRNA, the above guide sequences may further comprise additional nucleotides to form a sgRNA, e.g., with the following exemplary nucleotide sequence following the 3' end of the guide sequence:

GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 171) or

GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 172, which is SEQ ID NO: 171 without the four terminal U's) in 5' to 3' orientation. In some embodiments, the four terminal U's of SEQ ID NO: 171 are not present. In some embodiments, only 1, 2, or 3 of the four terminal U's of SEQ ID NO: 171 are present.

[00426] In some embodiments, the sgRNA comprises any one of the guide sequences of SEQ ID Nos: 1-149 and additional nucleotides to form a crRNA, e.g., with the following exemplary nucleotide sequence following the guide sequence at its 3' end:

GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 170) or

GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGGUGC (SEQ ID NO: 173) in 5' to 3' orientation. SEQ ID NO: 173 lacks 8 nucleotides with reference to a wild-type guide RNA conserved sequence:

GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 172).

[00427] In some embodiments, *KLKB1* short-single guide RNAs (*KLKB1* short-sgRNAs) are provided comprising a guide sequence as described herein and a "conserved portion of an sgRNA" comprising a hairpin region, wherein the hairpin region lacks at least

5-10 nucleotides or 6-10 nucleotides. In certain embodiments, a hairpin region of the *KLKB1* short-single guide RNAs lacks 5-10 nucleotides with reference to the conserved portion of an sgRNA, e.g. nucleotides H1-1 to H2-15 in Table 3B and FIG. 15. In certain embodiments, a hairpin 1 region of the *KLKB1* short-single guide RNAs lacks 5-10 nucleotides with reference to the conserved portion of an sgRNA, e.g. nucleotides H1-1 to H1-12 in Table 3B and FIG. 15. See, e.g., WO2019/237069, the contents of which is hereby incorporated by reference in its entirety, for example, at claims 1-15.

[00428] An exemplary “conserved portion of an sgRNA” is shown in Table 3A (see also FIG. 15), which shows a “conserved region” of a *S. pyogenes* Cas9 (“spyCas9” (also referred to as “spCas9”)) sgRNA. The first row shows the numbering of the nucleotides, the second row shows the sequence (SEQ ID NO: 500); and the third row shows “domains.” Briner *et al.*, *Molecular Cell* 56:333–339 (2014) describes functional domains of sgRNAs, referred to herein as “domains”, including the “spacer” domain responsible for targeting, the “lower stem”, the “bulge”, “upper stem” (which may include a tetraloop), the “nexus”, and the “hairpin 1” and “hairpin 2” domains. See, Briner *et al.* at page 334, Figure 1A.

[00429] Table 3B provides a schematic of the domains of an sgRNA as used herein. In Table 3B, the “n” between regions represents a variable number of nucleotides, for example, from 0 to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more. In some embodiments, n equals 0. In some embodiments, n equals 1.

[00430] In some embodiments, the *KLKB1* sgRNA is from *S. pyogenes* Cas9 (“spyCas9”) or a spyCas9 equivalent. In some embodiments, the sgRNA is not from *S. pyogenes* (“non-spyCas9”). In some embodiments, the 5-10 nucleotides or 6-10 nucleotides are consecutive.

[00431] In some embodiments, a *KLKB1* short-sgRNA lacks at least nucleotides 54-58 (AAAAA) of the conserved portion of a *S. pyogenes* Cas9 (“spyCas9”) sgRNA, as shown in Table 3A. In some embodiments, a *KLKB1* short-sgRNA is a non-spyCas9 sgRNA that lacks at least nucleotides corresponding to nucleotides 54-58 (AAAAA) of the conserved portion of a spyCas9 as determined, for example, by pairwise or structural alignment. In some embodiments, the non-spyCas9 sgRNA is *Staphylococcus aureus* Cas9 (“saCas9”) sgRNA.

[00432] In some embodiments, a *KLKB1* short-sgRNA lacks at least nucleotides 54-61 (AAAAAGUG) of the conserved portion of a spyCas9 sgRNA. In some embodiments, a *KLKB1* short-sgRNA lacks at least nucleotides 53-60 (GAAAAAGU) of the conserved portion of a spyCas9 sgRNA. In some embodiments, a *KLKB1* short-sgRNA lacks 4, 5, 6, 7,

or 8 nucleotides of nucleotides 53-60 (GAAAAAGU) or nucleotides 54-61 (AAAAAGUG) of the conserved portion of a spyCas9 sgRNA, or the corresponding nucleotides of the conserved portion of a non-spyCas9 sgRNA as determined, for example, by pairwise or structural alignment.

Table 1: Human *KLKB1* targeted guide sequence, chromosomal coordinates, and human single guide RNAs and dual guide RNAs, and surrogate cynomolgus (cyno) monkey single guides

SEQ ID NO: (human)	Exemplary Genomic Coordinates (hg38)	human guide sequence	human sgRNA	human dgRNA	cyno sgRNA	Cyno guide SEQ ID NO:
1	chr4:186228230-186228252	ACAGGAAACUGUAGCAACA	G012253	CR005916	NA	NA
2	chr4:186228248-186228270	AUAGAUAAUUCACUUAACAC	G012254	CR005917	NA	NA
3	chr4:186232154-186232176	UACAUCCCACCCUCUGAAGA	G012255	CR005918	NA	NA
4	chr4:186251256-186251278	UCUUGAGGAGUAGAGGAACU	G012256	CR005922	NA	NA
5	chr4:186251308-186251330	ACCAAGUAAAAGUUCUUUUGC	G012257	CR005924	NA	NA
6	chr4:186251489-186251511	GGGUAAAUUUAGAAUGGCA	G012258	CR005925	NA	NA
7	chr4:186251504-186251526	AUUUACCGGGAGUUGACUU	G012259	CR005928	NA	NA
8	chr4:186251507-186251529	UACCCGGGAGUUGACUUUGG	G012260	CR005929	NA	NA
9	chr4:186251828-186251850	UCUUUGAGAUUGUUAACAC	G012261	CR005931	NA	NA
10	chr4:186251829-186251851	CUUUGAGAUUGUUAACACU	G012262	CR005932	NA	NA
11	chr4:186251830-186251852	UUUGAGAUUGUUAACACUG	G012263	CR005933	NA	NA
12	chr4:186254748-186254770	UACAUACCAGUUAUUCAA	G012264	CR005943	NA	NA
13	chr4:186251784-186251806	CUCCAACUAGGAUUGCGUUAU	G012265	CR005949	G013933	373
14	chr4:186251792-186251814	AGGAUUGCGUUAUGGGACACA	G012266	CR005951	G013904	344
15	chr4:186251793-186251815	GGAUUGCGUUAUGGGACACAA	G012267	CR005952	G013901	341
16	chr4:186238297-186238319	GUUACUCAGCACCUUUUAUAG	G012268	CR005956	G013945	385
17	chr4:186238263-186238285	UGCCUAAUAAAGUACAGUCC	G012269	CR005959	NA	NA
18	chr4:186251772-186251794	CUAUGGAUGGUUCUCCAAACU	G012270	CR005960	G013922	362
19	chr4:186254601-186254623	GAUUUUUGGCGCAUCUAUAG	G012271	CR005963	G013921	361
20	chr4:186254592-186254614	AUGCGCCAAACAUCUUGCAG	G012272	CR005970	G013885	325
21	chr4:186236785-186236807	CUCCUUUAAAUGUCUCGA	G012273	CR005979	G013905	345
22	chr4:186236863-186236885	UGUUACUGGUGCACCUCUUUU	G012274	CR005982	NA	NA
23	chr4:186254593-186254615	GAUGCGCCAAACAUCUUGCA	G012275	CR005983	G013876	316
24	chr4:186232192-186232214	AUCUGGCAGUAUUGGGCAUU	G012276	CR005992	G013915	355

SEQ ID NO: (human)	Exemplary Genomic Coordinates (hg38)	human guide sequence	human sgRNA	human dgRNA	cyno sgRNA	Cyno guide SEQ ID NO:
25	chr4:186236893-186236915	GCGUGGCAU AUGAAAAAAC	G012277	CR005994	NA	NA
26	chr4:186236798-186236820	UAUAAAGGAGUUAUAGAG	G012278	CR005995	G013913	353
27	chr4:186236938-186236960	ACACCUUAAUUGUACUCAC	G012279	CR005998	NA	NA
28	chr4:186232214-186232236	UGAGGUGCACAUCCACCCA	G012280	NA	NA	NA
29	chr4:186232190-186232212	CUGGCAGUAUUGGGCAUUUG	G012281	NA	NA	NA
30	chr4:186232148-186232170	AAAACGCCUUCUUCAGAGGU	G012282	NA	NA	NA
31	chr4:186232227-186232249	UGAAUAGCAAAACACCUUGGG	G012283	NA	NA	NA
32	chr4:186236821-186236843	AGUCAUUUUAAUGUGCUA	G012284	NA	NA	NA
33	chr4:186236850-186236872	GUGUUGAAGAAUGCCAAAA	G012285	NA	NA	NA
34	chr4:186236910-186236932	UGCCUUGUGAAUUGUUUGCG	G012286	NA	NA	NA
35	chr4:186250265-186250287	GCAUCUUGCGUUCUCAGAU	G012287	NA	G013927	367
36	chr4:186250276-186250298	UCUCAGUUGGGAUUGUUGCC	G012288	NA	NA	NA
37	chr4:186250306-186250328	CUCCAGUUGCUUUUGUGUGU	G012289	NA	NA	NA
38	chr4:186251325-186251347	UAUUUCAAUACAUAUACC	G012290	NA	NA	NA
39	chr4:186251271-186251293	CCAGAUUUGGUGUUUUCUUG	G012291	NA	NA	NA
40	chr4:186251300-186251322	AAGUUCUUUUGCAGGUUAAA	G012292	NA	NA	NA
41	chr4:186251620-186251642	UUUACUCCAGAAAGACUGUA	G012293	NA	NA	NA
42	chr4:186251492-186251514	UGCCAUUCUAAAAUUUACCC	G012294	NA	NA	NA
43	chr4:186251572-186251594	UCAUCUUUGGCAAGUCUCU	G012295	NA	NA	NA
44	chr4:186251510-186251532	UCUCCUCCAAAGUCAAUCC	G012296	NA	NA	NA
45	chr4:186252049-186252071	GGAGGAACAAACUUCUUCUUG	G012297	NA	NA	NA
46	chr4:186252098-186252120	AGGUGAAGCUGACAGCUCAG	G012298	NA	NA	NA
47	chr4:186256046-186256068	CCAUCCGUUACCCAAACAGU	G012299	NA	G013931	371
48	chr4:186256042-186256064	UAUACCAACUUGGGUAAAC	G012300	NA	G012300	NA
49	chr4:186256034-186256056	GCACAAUUUAUACCAACUGU	G012301	NA	NA	NA
50	chr4:186256059-186256081	AACCGGAUGGGGUUCUCGA	G012302	NA	G013932	372

SEQ ID NO: (human)	Exemplary Genomic Coordinates (hg38)	human guide sequence	human sgRNA	human dgRNA	cyno sgRNA	Cyno guide SEQ ID NO:
51	chr4:186256047-186256069	CAACUGUUUGGGUAAACCGGAU	G012303	NA	G013882	322
52	chr4:186256035-186256057	CACAAUUUAUACCAACUGUU	G012304	NA	G012304	NA
53	chr4:186256046-186256068	CCAACUGUUUGGGUAAACCGGA	G012305	NA	G013924	364
54	chr4:186256061-186256083	CUCUUUCGAGAAAGCCCAUC	G012306	NA	NA	NA
55	chr4:186256048-186256070	AACUGUUUGGGUAAACCGGAUG	G012307	NA	G013914	354
56	chr4:186256003-186256025	CCAAUAUGCCUACCUUCCAA	G012308	NA	NA	NA
57	chr4:186256015-186256037	GUGCUUGUGUACCUUUGGA	G012309	NA	G013900	340
58	chr4:186256011-186256033	UUUGUACCUUUGGAAGGU	G012310	NA	NA	NA
59	chr4:186256019-186256041	AAUUGUCUUGUGUACCUU	G012311	NA	NA	NA
60	chr4:186255996-186256018	AAGUAGGCAUUAUUGGUUUU	G012312	NA	NA	NA
61	chr4:186257312-186257334	ACCCAACGGAUUGGUCUGGC	G012313	NA	NA	NA
62	chr4:186257314-186257336	AGCCAGCACAGACCAUCCGU	G012314	NA	NA	NA
63	chr4:186257302-186257324	UUUAAAAUAAACCAACGGA	G012315	NA	G012315	NA
64	chr4:186257326-186257348	CUUGUGGGCUAUAAGAAG	G012316	NA	NA	NA
65	chr4:186257261-186257283	CAUUCUUCAUUUGUUAACCA	G012317	NA	NA	NA
66	chr4:186257284-186257306	UAUAAUCUUGAUAUCCUUUC	G012318	NA	NA	NA
67	chr4:186257313-186257335	GCCAGCACAGACCAUCCGUU	G012319	NA	NA	NA
68	chr4:186257324-186257346	GUUCUGUGGGCUAUAAGA	G012320	NA	G012320	NA
69	chr4:186257325-186257347	UCUGUGUGGGCUAUAAGA	G012321	NA	NA	NA
70	chr4:186258130-186258152	GUCCAUGUACUCAGCGACUU	G012322	NA	G012322	NA
71	chr4:186258128-186258150	CACCAAAGUCGUGAGUACA	G012323	NA	G012323	NA
72	chr4:186258050-186258072	ACACAUGGAUUGUGGCGUU	G012324	NA	G012324	NA
73	chr4:186258068-186258090	UUUGGUGGGCAUACCAAGCU	G012325	NA	G012325	NA
74	chr4:186258204-186258226	CUUGGACUGGUUCUCAUGC	G012326	NA	NA	NA
75	chr4:186258133-186258155	AAGUCGUGAGUACAUAGGAC	G012327	NA	G012327	NA
76	chr4:186258089-186258111	GGGUGAAGGCUUGUGCCCGCA	G012328	NA	G013895	335

SEQ ID NO: (human)	Exemplary Genomic Coordinates (hg38)	human guide sequence	human sgRNA	human dgRNA	cyno sgRNA	Cyno guide SEQ ID NO:
77	chr4:186258054-186258076	AAUGGAAUGUGCGUUUUGGU	G012329	NA	G012329	NA
78	chr4:186258037-186258059	UCCAUUGUGUUUGCAAACUA	G012330	NA	G013942	382
79	chr4:186258067-186258089	GUUUGGUGGGCAUCACACAGC	G012331	NA	NA	NA
80	chr4:186258043-186258065	UUUGCAAACACAAGUGAAUG	G012332	NA	G013916	356
81	chr4:186258103-186258125	GACACCAGGUUGCUCGCCUGC	G012333	NA	NA	NA
82	chr4:186258009-186258031	ACUGUGACUCAGGGAGAUUC	G012334	NA	G013943	383
83	chr4:186258099-186258121	UGUGCCCGCAGGAGCAACC	G012335	NA	NA	NA
84	chr4:186258036-186258058	CCAUUGUGUUUGCAAAACUAA	G012336	NA	G013929	369
85	chr4:186258088-186258110	GGGUGAAGGUGUGCCCGC	G012337	NA	NA	NA
86	chr4:186258117-186258139	GCGACUUUGGUGUAGACACC	G012338	NA	NA	NA
87	chr4:186258036-186258058	CCUUAGUUUGCAAAACAAA	G012339	NA	NA	NA
88	chr4:186258053-186258075	CAUUGGAAUGUGCGUUUUGG	G012340	NA	G012340	NA
89	chr4:186232230-186232252	AACUGAAUAGCAAAACACCUU	NA	CR005919	NA	NA
90	chr4:186238351-186238373	ACAAUUAACAAUUUCUGAAA	NA	CR005920	NA	NA
91	chr4:186238352-186238374	UACAAUUAACAAUUUCUGAA	NA	CR005921	NA	NA
92	chr4:186251263-186251285	GGUGUUUCUUGAGGAGUAG	NA	CR005923	NA	NA
93	chr4:186251490-186251512	CGGGUAAAUUUAGAAUGGC	NA	CR005926	G013884	324
94	chr4:186251494-186251516	CUCCCGGUAAAUUUAGAA	NA	CR005927	G013925	365
95	chr4:186251801-186251823	UAUGGGACACAAAGGAGCUC	NA	CR005930	NA	NA
96	chr4:186252047-186252069	UUGGAGGAACAAACUUCUUCU	NA	CR005934	G013912	352
97	chr4:186252048-186252070	UGGAGGAACAAACUUCUUCU	NA	CR005935	NA	NA
98	chr4:186252056-186252078	CAACUUCUUGGGGAGAG	NA	CR005936	NA	NA
99	chr4:186252123-186252145	CUAUGAGUGACCCUCCACAC	NA	CR005937	G013886	326
100	chr4:186252124-186252146	CUUGUGGAGGGUACUCAU	NA	CR005938	G013938	378
101	chr4:186252134-186252156	GGUCACUCAUAGGACACCAG	NA	CR005939	G013946	386
102	chr4:186252135-186252157	GUCACUCAUAGGACACCAGU	NA	CR005940	G013896	336

SEQ ID NO: (human)	Exemplary Genomic Coordinates (hg38)	human guide sequence	human sgRNA	human dgRNA	cyno sgRNA	Cyno guide SEQ ID NO:
103	chr4:186252163-186252185	ACUGCUGCCACUGCUUUGA	NA	CR005941	NA	NA
104	chr4:186252171-186252193	ACAUUACCCAUCAAAAGCAG	NA	CR005942	G013902	342
105	chr4:186238286-186238308	AGGAACACCUACCGCUAUAA	NA	CR005944	G013871	311
106	chr4:186238265-186238287	CUCGGGACUGACUUUAAU	NA	CR005945	G013889	329
107	chr4:186251786-186251808	GUCCCAUACGCAAUCCUAGU	NA	CR005946	G013890	330
108	chr4:186238293-186238315	CUCAGCACUUUAUAGCGGU	NA	CR005947	G013892	332
109	chr4:186238282-186238304	UAUAGCGGUAGGUUUCUC	NA	CR005948	G013874	314
110	chr4:186238266-186238288	CUAUUAAAGUACAGUCCCGG	NA	CR005950	G013875	315
111	chr4:186238308-186238330	GUGCUGAGUAACGUGGAAUC	NA	CR005953	G013883	323
112	chr4:186238301-186238323	UAUAAAGGUGCUGAGUAACG	NA	CR005954	G013878	318
113	chr4:186251783-186251805	UCUCCAAUAGGAUUGGUA	NA	CR005955	G013908	348
114	chr4:186238281-186238303	AUAGCGGUAGGUUUCUCC	NA	CR005957	G013873	313
115	chr4:186233989-186234011	CUGCCAAAAGUACAUCGAAC	NA	CR005958	G013877	317
116	chr4:186238345-186238367	ACCAUUUCUGAAAAGGCAC	NA	CR005961	NA	NA
117	chr4:186251755-186251777	GUGUUUCUUAAAGAUUAUCUA	NA	CR005962	NA	NA
118	chr4:186238344-186238366	CCAAUUUCUGAAAAGGCACA	NA	CR005964	NA	NA
119	chr4:186251759-186251781	UUCUAAAGAUUAUCUAUGGA	NA	CR005965	G013940	380
120	chr4:186233988-186234010	CUGUUCGAUGUACUUUUGGC	NA	CR005966	NA	NA
121	chr4:186233987-186234009	UGUUCGAUGUACUUUUGGCA	NA	CR005967	G013880	320
122	chr4:186232209-186232231	GGUGGAUUGGACCCUACUC	NA	CR005968	G013939	379
123	chr4:186250308-186250330	GUCCGACACACAAAAGCAUC	NA	CR005969	G013894	334
124	chr4:186236877-186236899	AAACUUGGACGGAUUGUAC	NA	CR005971	G013930	370
125	chr4:186236908-186236930	UGCCACGCAACAUAUUCACA	NA	CR005972	NA	NA
126	chr4:186233992-186234014	GCACCUUUUCGAUGUACUUU	NA	CR005973	G013870	310
127	chr4:186254594-186254616	AGAUCCGCAACAAUCCUGC	NA	CR005974	NA	NA
128	chr4:186232199-186232221	GCACCUCAUCUGGCAGUAUU	NA	CR005975	NA	NA

SEQ ID NO: (human)	Exemplary Genomic Coordinates (hg38)	human guide sequence	human sgRNA	human dgRNA	cyno sgRNA	Cyno guide SEQ ID NO:
129	chr4:186250262-186250284	CAUCUGAGAACGCAAGAUGC	NA	CR005976	G013934	374
130	chr4:186232196-186232218	AUGCCCAAUACUGCCAGAUG	NA	CR005977	NA	NA
131	chr4:186232200-186232222	UGCACCUCAUCUGGCAGUAU	NA	CR005978	G013944	384
132	chr4:186232258-186232280	AUGUCAUUGAUUGAACUUGC	NA	CR005980	G013936	376
133	chr4:186252031-186252053	ACAAGCACACGCAUUGUUGG	NA	CR005981	G013893	333
134	chr4:186254723-186254745	UAUCGCCUUUGAUAAAAUCC	NA	CR005984	G013926	366
135	chr4:186251271-186251293	CCUCAAGAAAAACCAUAUC	NA	CR005985	G013906	346
136	chr4:186232149-186232171	AAACGCCUUUUCAGAGGUG	NA	CR005986	NA	NA
137	chr4:186252028-186252050	AAAACAAGCACACGCAUUGU	NA	CR005987	G013891	331
138	chr4:186234001-186234023	CAUCGAACAGGUGCAGUUUC	NA	CR005988	G013879	319
139	chr4:186254587-186254609	GGCUUCCCCUGCAGGAUGUU	NA	CR005989	G013881	321
140	chr4:186234029-186234051	UUGAUGACCACAUUGCUUCA	NA	CR005990	G013937	377
141	chr4:186254728-186254750	AGGAGCCUGGAGUUUUUAUCA	NA	CR005991	NA	NA
142	chr4:186236783-186236805	UGCCAUUCGAGACAUUUUAUA	NA	CR005993	G013899	339
143	chr4:186232260-186232282	AGCAAGUUCAAUCAAUAGACA	NA	CR005996	G013897	337
144	chr4:186234022-186234044	GGACAUUCCUUGAAAGCAAUG	NA	CR005997	NA	NA
145	chr4:186250330-186250352	GUUGGGGUGAUAGGUGCAGA	NA	CR005999	NA	NA
146	chr4:186232147-186232169	GAAAACGCCUUUCUUCAGAGG	NA	CR006000	NA	NA
147	chr4:186232144-186232166	UAUGAAAACGCCUUUCUUCAG	NA	CR006001	NA	NA
148	chr4:186250277-186250299	CUCAGAUUGGAUGUUGCCA	NA	CR006002	NA	NA
149	chr4:186254579-186254601	CUCUCCUAGGCUUCCCCUGC	NA	CR006003	NA	NA

Table 2. Cyno KLB1 targeted single guide sequences, chromosomal coordinates, and guide sequence homology to human

Cyno sgRNA	Cyno SEQ ID NO	Exemplary Genomic Coordinates (mf5)	cyno guide sequence	Percent homology to human guide
G013870	310	chr5:185648888-185648908	GCACUGGUCGACGUACUUU	90
G013871	311	chr5:185652966-185652986	AGGAACGCCUACCACUAAA	90
G013872	312	chr5:185688465-185688485	UGAUGGAAACGUCGGAUGC	NA
G013873	313	chr5:185652964-185652984	AUAGUGGAGGGGUUCCUCC	90
G013874	314	chr5:185652965-185652985	UAUAGUGGAGGGGUUCCUC	90
G013875	315	chr5:185652946-185652966	CUCUAAAAGCACAGUCCCGG	90
G013876	316	chr5:185684512-185684532	AUAGGCCAAACAUCCGGUA	100
G013877	317	chr5:185648882-185648902	UUGCCAAAAGUACGUCGAGC	85
G013878	318	chr5:185652981-185653001	UAUAAAGGUGUGAAUAAACG	95
G013879	319	chr5:185648894-185648914	CGUCGAGCAGGUGGAAUUUC	85
G013880	320	chr5:185648883-185648903	UGCUCGACGUACUUUUGGCA	90
G013881	321	chr5:185684503-185684523	GGCUUCCUUACCGGAUUGU	85
G013882	322	chr4:186256046-186256066	CAACUUGUGGUAAACUGGAU	100
G013883	323	chr5:185652988-185653008	GUGCUGAAUACGUGGAAUC	95
G013884	324	chr5:185680852-185680872	CGGGUAAUUUUAGAAUUGC	100
G013885	325	chr5:185684511-185684531	AUGGCCCAAACAUCCGGUAA	100
G013886	326	chr5:185681472-185681492	CUAUGAGUGAGCCUCCACAC	100
G013887	327	chr5:185679339-185679359	GGCAACAUCCACAUCCGAGA	NA
G013888	328	chr5:185679426-185679446	UUACGUUCUUAACGAAUUGA	85
G013889	329	chr5:185652948-185652968	CUCGGGACUGUGCUUUAAAG	90
G013890	330	chr5:185681135-185681155	GUCCCAUAGUAAAUCCUAGU	90
G013891	331	chr5:185681374-185681394	AAACAAGCUCACGCAUUGU	95
G013892	332	chr5:185652976-185652996	UUCAGCACCUUUUAUAGUGGU	90
G013893	333	chr5:185681377-185681397	ACAAGCUCACGCAUUGUGG	95
G013894	334	chr5:185679374-185679394	GUUCGACACACAAAGCAUC	95
G013895	335	chr4:186258088-186258108	GGGCGAAGGUGUGCCCGCA	100
G013896	336	chr5:185681481-185681501	GUCACUUAAGGACACACAGU	100
G013897	337	chr5:185647160-185647180	AGCAAGUCCUACAAUAGACA	95
G013898	338	chr5:185679413-185679433	AACGUAAAGAGAGGCAGCU	100
G013899	339	chr5:185651465-185651485	UGCCACGAGACAUUUUAAA	95
G013900	340	chr4:186256017-186256037	GUGUUUGUGUCACCUUUUGGA	100

Cyno sgRNA	Cyno SEQ ID NO	Exemplary Genomic Coordinates (mfs)	cyno guide sequence	Percent homology to human guide
G013901	341	chr4:186251792-186251812	GGAUUACAUAUUGGGACACAA	100
G013902	342	chr5:185681520-185681540	ACAUUACCCAUCAAAAGCAG	100
G013903	343	chr5:185684660-185684680	CAGUGUAUUCAAAAGGAGCC	100
G013904	344	chr5:185681138-185681158	AGGAUUACAUAUUGGGACACA	100
G013905	345	chr5:185651470-185651490	UUCUUUAUAAUUGUCUGG	100
G013906	346	chr5:185680632-185680652	CCUCAAAGAAAAACACCAUC	95
G013907	347	chr5:185688458-185688478	AGAGCAGUGAUGGAAACGCU	NA
G013908	348	chr5:185681129-185681149	UCUCCAAUAAGGAUUAUAUA	90
G013909	349	chr5:185680982-185681002	ACUCCAGAAACUGUAAGG	NA
G013910	350	chr5:185679360-185679380	AGCAUCUGGGGCGAGAACUC	100
G013911	351	chr5:185679372-185679392	UCGACACACAAAAGCAUCUG	NA
G013912	352	chr5:185681393-185681413	UUGGAGGAACAAACUCUUCU	100
G013913	353	chr4:186236797-186236817	UAUAAAGGAUUUAUUGAG	100
G013914	354	chr4:186256047-186256067	AACUUGGGUAAACUGGAUG	100
G013915	355	chr5:185647095-185647115	AUCUGGCAGUGGCGCGUU	100
G013916	356	chr4:186258042-186258062	CUUGCAAAACAAUUGGAUUG	100
G013917	357	chr4:186251628-186251648	UCUCUCCUUAACAGUCUUCU	NA
G013918	358	chr5:185688296-185688316	CUGUGACUCAGGAGAUUCA	100
G013918	358	chr5:185688296-185688316	CUGUGACUCAGGAGAUUCA	100
G013919	359	chr4:186258084-186258104	GGCACAGCCUUGGCCCCAGC	100
G013920	360	chr5:185647084-185647104	CUGGGCGUUCGGGUGUACA	100
G013921	361	chr4:186254600-186254620	GAUGUUUGGCGCAUUUAUAG	100
G013922	362	chr4:186251771-186251791	CUUCGGUUGGUUCUCCAAU	NA
G013923	363	chr5:185684517-185684537	CUAUAUAUUGCGCCAAACAUC	100
G013923	363	chr5:185684517-185684537	CUAUAUAUUGCGCCAAACAUC	100
G013924	364	chr4:186256045-186256065	CCAACUUGUGGUAAACUGGA	100
G013925	365	chr5:185680856-185680876	CUCCCGGUAAAUUUUAGAA	95
G013926	366	chr5:185684639-185684659	UAUCGCCUUAAUAAAACUCC	100
G013926	366	chr4:186254722-186254742	UAUCGCCUUAAUAAAACUCC	100
G013927	367	chr4:186250264-186250284	GCAUCUUGCCUUCUGGAUG	90
G013928	368	chr5:185679421-185679441	UCGUUAUAGAACGUAAAAG	100
G013929	369	chr4:186258038-186258058	CCAUUGUUUUGCAAGCUAA	90
G013930	370	chr5:185651562-185651582	AAAUUGGCAGCGAAUGUUAU	100
G013930	370	chr4:186236879-186236899	AAAUUGGCAGCGAAUGUUAU	100

Cyno sgRNA	Cyno SEQ ID NO	Exemplary Genomic Coordinates (mfs)	cyno guide sequence	Percent homology to human guide
G013931	371	chr4:186256048-186256068	CCAUCCAGUUACCCAAACAGU	100
G013932	372	chr4:186256058-186256078	AACUGGAUUGGGGUUUCGGA	100
G013933	373	chr5:185681130-185681150	CUCCAAUAGGAUUAACAUU	100
G013934	374	chr5:185679328-185679348	CAUCCGAGAAAGGCAAGUUC	90
G013935	375	chr4:186251536-186251556	UUGAAUGAGCUUUCGUUAA	100
G013936	376	chr5:185647161-185647181	AUGUCAUUGAUGGAACUUGC	95
G013937	377	chr5:185648925-185648945	UUGAUGACCACACUGCUUUA	90
G013938	378	chr5:185681470-185681490	CUGUGGAGGAGUACUCAU	100
G013939	379	chr5:185647112-185647132	GGUGAAUUGGACAUCAUC	95
G013940	380	chr5:185681105-185681125	UUCUUAAAGAUUAUCUUCGGA	90
G013941	381	chr5:185685921-185685941	CCUUUGGAAGGUAGGCAUUA	100
G013942	382	chr4:186258039-186258059	UCCAUUGUUGUUGCAAGCUA	100
G013943	383	chr4:186258008-186258028	CCUGAGACUCAGGAGAUUC	100
G013944	384	chr5:185647103-185647123	UGCACAUCAUCUGGCAGUUC	85
G013945	385	chr4:186238299-186238319	GUUAUUCAGCACCUUUAUAG	100
G013946	386	chr5:185681480-185681500	GGUCACUCAUAGGACACCAG	100

[00433] The guide RNAs identified above as “G0XXXXXX” are sgRNAs comprising the identified 20 nucleotide targeting sequence of Table 1 or Table 2, within the guide structure of SEQ ID NO: 300. In some embodiments, the sgRNA comprises any one of the guide RNAs of Tables 1 or 2 and the nucleotides of SEQ ID NO: 300, optionally wherein the sgRNA comprises any one of the modification patterns described in Table 4. In some embodiments, the sgRNA comprises any one of the guide RNAs of Tables 1 or 2 and any of the conserved portion of sgRNAs of Table 4, optionally with any one of the modification patterns described in Table 4.

Table 3A (Conserved Portion of a spyCas9 sgRNA; SEQ ID NO:500)

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													B2-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																														
															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 3B

5' terminus (n)		LS1-6			B1 -2		US1-12		B3-6	
		lower stem	n	bulge	n	upper stem	n	bulge	n	

LS7-12		N1-18		H1-1 thru H1-12	H2-1 thru H2-15	
lower stem	n	nexus	n	hairpin 1	hairpin 2	3' terminus

[00434] In some embodiments, the invention provides a composition comprising one or more guide RNA (gRNA) comprising guide sequences that direct an RNA-guided DNA binding agent, which can be a nuclease (e.g., a Cas nuclease such as Cas9), to a target DNA sequence in *KLKB1*. The gRNA may comprise a crRNA comprising a guide sequence shown in Table 1. The gRNA may comprise a crRNA comprising 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the gRNA comprises a crRNA comprising a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to at least 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the gRNA comprises a crRNA comprising a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a guide sequence shown in Table 1. The gRNA may further comprise a trRNA. In each composition and method embodiment described herein, the crRNA and trRNA may be associated as a single RNA (sgRNA) or may be on separate RNAs (dgRNA). In the context of sgRNAs, the crRNA and trRNA components may be covalently linked, e.g., via a phosphodiester bond or other covalent bond.

[00435] In each of the compositions, use, and method embodiments described herein, the guide RNA may comprise two RNA molecules as a “dual guide RNA” or “dgRNA.” The dgRNA comprises a first RNA molecule comprising a crRNA comprising, e.g., a guide sequence shown in Table 1, and a second RNA molecule comprising a trRNA. The first and second RNA molecules may not be covalently linked, but may form an RNA duplex via the base pairing between portions of the crRNA and the trRNA.

[00436] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise a single RNA molecule as a “single guide RNA” or “sgRNA”. The sgRNA may comprise a crRNA (or a portion thereof) comprising a guide sequence shown in Table 1 covalently linked to a trRNA. The sgRNA may comprise 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the crRNA and the trRNA are covalently linked via a linker. In some embodiments, the sgRNA forms a stem-loop structure via the base pairing between portions of the crRNA and the trRNA. In some embodiments, the crRNA and the trRNA are covalently linked via one or more bonds that are not a phosphodiester bond.

[00437] In some embodiments, the trRNA may comprise all or a portion of a trRNA sequence derived from a naturally-occurring CRISPR/Cas system. In some embodiments, the trRNA comprises a truncated or modified wild type trRNA. The length of the trRNA depends on the CRISPR/Cas system used. In some embodiments, the trRNA comprises or consists of

5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or more than 100 nucleotides. In some embodiments, the trRNA may comprise certain secondary structures, such as, for example, one or more hairpin or stem-loop structures, or one or more bulge structures.

[00438] In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one of SEQ ID NOs: 1-149 is provided. In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one of SEQ ID Nos: 1-149 and any conserved portion of an sgRNA shown in Table 4, optionally having a modification pattern of any of an sgRNA shown in Table 4, optionally wherein the sgRNA comprises a 5' and 3' end modification (if not already shown in the construct of Table 4) is provided. In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one of SEQ ID Nos: 1-149 is provided, wherein the nucleotides of SEQ ID NO: 170, 171, 172, or 173 follow the guide sequence at its 3' end. In some embodiments, the one or more guide RNAs comprising a guide sequence of any one of SEQ ID Nos: 1-149, wherein the nucleotides of SEQ ID NO: 170, 171, 172, or 173 follow the guide sequence at its 3' end, is modified according to the modification pattern of SEQ ID NO: 300.

[00439] In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one of SEQ ID NOs: 1-149 is provided. In one aspect, a composition comprising one or more gRNAs is provided, comprising a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 1-149.

[00440] In other embodiments, a composition is provided that comprises at least one, e.g., at least two gRNA's comprising guide sequences selected from any two or more of the guide sequences of SEQ ID NOs: 1-149. In some embodiments, the composition comprises at least two gRNA's that each comprise a guide sequence at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 1-149.

[00441] The guide RNA compositions of the present invention are designed to recognize (*e.g.*, hybridize to) a target sequence in the *KLKB1* gene. For example, the *KLKB1* target sequence may be recognized and cleaved by a provided Cas cleavase comprising a guide RNA. In some embodiments, an RNA-guided DNA binding agent, such as a Cas cleavase, may be directed by a guide RNA to a target sequence of the *KLKB1* 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.

[00442] In some embodiments, the selection of the one or more guide RNAs is determined based on target sequences within the *KLKB1* gene. In some embodiments, the compositions comprising one or more guide sequences comprise a guide sequence that is complementary to the corresponding genomic region shown in Table 1 below, according to coordinates from human reference genome hg38. Guide sequences of further embodiments may be complementary to sequences in the close vicinity of the genomic coordinate listed in any of the Tables provided herein. For example, guide sequences of further embodiments may be complementary to sequences that comprise 15 consecutive nucleotides ± 10 nucleotides of a genomic coordinate listed in any of the Tables disclosed herein.

[00443] Without being bound by any particular theory, mutations (e.g., frameshift mutations resulting from indels occurring as a result of a nuclease-mediated DSB) in certain regions of the gene may be less tolerable than mutations in other regions of the gene, thus the location of a DSB is an important factor in the amount or type of protein knockdown that may result. In some embodiments, a gRNA complementary or having complementarity to a target sequence within *KLKB1* is used to direct the RNA-guided DNA binding agent to a particular location in the *KLKB1* gene. In some embodiments, gRNAs are designed to have guide sequences that are complementary or have complementarity to target sequences in exon 1, exon 3, exon 4, exon 5, exon 6, exon 8, exon 9, exon 10, exon 11, exon 12, exon 13, exon 14, or exon 15 of *KLKB1*.

[00444] In some embodiments, the guide sequence is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a target sequence present in the human *KLKB1* gene. In some embodiments, the target sequence may be complementary to the guide sequence of the guide RNA. In some embodiments, the degree of complementarity or identity between a guide sequence of a guide RNA and its corresponding target sequence may be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the target sequence and the guide sequence of the gRNA may be 100% complementary or identical. In other embodiments, the target sequence and the guide sequence of the gRNA may contain at least one mismatch. For example, the target sequence and the guide sequence of the gRNA may contain 1, 2, 3, or 4 mismatches, where the total length of the guide sequence is 20. In some embodiments, the target sequence and the guide sequence of the gRNA may contain 1-4 mismatches where the guide sequence is 20 nucleotides.

[00445] In some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an

mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered.

B. Modified gRNAs and mRNAs

[00446] In some embodiments, the gRNA is chemically modified. A gRNA comprising one or more modified nucleosides or nucleotides is called a “modified” gRNA or “chemically modified” gRNA, to describe the presence of one or more non-naturally and/or naturally occurring components or configurations that are used instead of or in addition to the canonical A, G, C, and U residues. In some embodiments, a modified gRNA is synthesized with a non-canonical nucleoside or nucleotide, is here called “modified.” Modified nucleosides and nucleotides can include one or more of: (i) alteration, *e.g.*, replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage (an exemplary backbone modification); (ii) alteration, *e.g.*, replacement, of a constituent of the ribose sugar, *e.g.*, of the 2' hydroxyl on the ribose sugar (an exemplary sugar modification); (iii) wholesale replacement of the phosphate moiety with “dephospho” linkers (an exemplary backbone modification); (iv) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); (v) replacement or modification of the ribose-phosphate backbone (an exemplary backbone modification); (vi) modification of the 3' end or 5' end of the oligonucleotide, *e.g.*, removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, cap or linker (such 3' or 5' cap modifications may comprise a sugar and/or backbone modification); and (vii) modification or replacement of the sugar (an exemplary sugar modification).

[00447] Chemical modifications such as those listed above can be combined to provide modified gRNAs and/or mRNAs comprising nucleosides and nucleotides (collectively “residues”) that can have two, three, four, or more modifications. For example, a modified residue can have a modified sugar and a modified nucleobase. In some embodiments, every base of a gRNA is modified, *e.g.*, all bases have a modified phosphate group, such as a phosphorothioate group. In certain embodiments, all, or substantially all, of the phosphate groups of an gRNA molecule are replaced with phosphorothioate groups. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 5' end of the RNA. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 3' end of the RNA.

[00448] In some embodiments, the gRNA comprises one, two, three or more modified residues. In some embodiments, at least 5% (*e.g.*, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the positions in a modified gRNA are modified nucleosides or nucleotides.

[00449] Unmodified nucleic acids can be prone to degradation by, *e.g.*, intracellular nucleases or those found in serum. For example, nucleases can hydrolyze nucleic acid phosphodiester bonds. Accordingly, in one aspect the gRNAs described herein can contain one or more modified nucleosides or nucleotides, *e.g.*, to introduce stability toward intracellular or serum-based nucleases. In some embodiments, the modified gRNA molecules described herein can exhibit a reduced innate immune response when introduced into a population of cells, both *in vivo* and *ex vivo*. The term “innate immune response” includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, which involves the induction of cytokine expression and release, particularly the interferons, and cell death.

[00450] In some embodiments of a backbone modification, the phosphate group of a modified residue can be modified by replacing one or more of the oxygens with a different substituent. Further, the modified residue, *e.g.*, modified residue present in a modified nucleic acid, can include the wholesale replacement of an unmodified phosphate moiety with a modified phosphate group as described herein. In some embodiments, the backbone modification of the phosphate backbone can include alterations that result in either an uncharged linker or a charged linker with unsymmetrical charge distribution.

[00451] Examples of modified phosphate groups include, phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. The phosphorous atom in an unmodified phosphate group is achiral. However, replacement of one of the non-bridging oxygens with one of the above atoms or groups of atoms can render the phosphorous atom chiral. The stereogenic phosphorous atom can possess either the “R” configuration (herein Rp) or the “S” configuration (herein Sp). The backbone can also be modified by replacement of a bridging oxygen, (*i.e.*, the oxygen that links the phosphate to the nucleoside), with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates)

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

[00452] The phosphate group can be replaced by non-phosphorus containing connectors in certain backbone modifications. In some embodiments, the charged phosphate group can be replaced by a neutral moiety. Examples of moieties which can replace the phosphate group can include, without limitation, *e.g.*, methyl phosphonate, hydroxylamino, siloxane, carbonate, carboxymethyl, carbamate, amide, thioether, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, oxime, methyleneimino, methylenemethylimino, methylenehydrazo, methylenedimethylhydrazo and methyleneoxymethylimino.

[00453] 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.

[00454] 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.

[00455] Examples of 2' hydroxyl group modifications can include alkoxy or aryloxy (OR, wherein “R” can be, *e.g.*, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or a sugar); polyethyleneglycols (PEG), $O(CH_2CH_2O)_nCH_2CH_2OR$ wherein R can be, *e.g.*, H or optionally substituted alkyl, and n can be an integer from 0 to 20 (*e.g.*, from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and from 4 to 20). In some embodiments, the 2' hydroxyl group modification can be 2'-O-Me. In some embodiments, the 2' hydroxyl group modification can be a 2'-fluoro modification, which replaces the 2' hydroxyl group with a fluoride. In some embodiments, the 2' hydroxyl group modification can include “locked” nucleic acids (LNA) in which the 2' hydroxyl can be connected, *e.g.*, by a C₁₋₆ alkylene or C₁₋₆ heteroalkylene

bridge, to the 4' carbon of the same ribose sugar, where exemplary bridges can include methylene, propylene, ether, or amino bridges; O-amino (wherein amino can be, *e.g.*, NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroaryl amino, or diheteroaryl amino, ethylenediamine, or polyamino) and aminoalkoxy, O(CH₂)_n-amino, (wherein amino can be, *e.g.*, NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroaryl amino, or diheteroaryl amino, ethylenediamine, or polyamino). 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).

[00456] "Deoxy" 2' modifications can include hydrogen (*i.e.* deoxyribose sugars, *e.g.*, at the overhang portions of partially dsRNA); halo (*e.g.*, bromo, chloro, fluoro, or iodo); amino (wherein amino can be, *e.g.*, NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroaryl amino, diheteroaryl amino, or amino acid); NH(CH₂CH₂NH)_nCH₂CH₂- amino (wherein amino can be, *e.g.*, as described herein), -NHC(O)R (wherein R can be, *e.g.*, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with *e.g.*, an amino as described herein.

[00457] 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.

[00458] The modified nucleosides and modified nucleotides described herein, which can be incorporated into a modified nucleic acid, can include a modified base, also called a nucleobase. Examples of nucleobases include, but are not limited to, adenine (A), guanine (G), cytosine (C), and uracil (U). These nucleobases can be modified or wholly replaced to provide modified residues that can be incorporated into modified nucleic acids. The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine analog, or pyrimidine analog. In some embodiments, the nucleobase can include, for example, naturally-occurring and synthetic derivatives of a base.

[00459] In embodiments employing a dual guide RNA, each of the crRNA and the tracr RNA can contain modifications. Such modifications may be at one or both ends of the crRNA and/or tracr RNA. In embodiments comprising an sgRNA, one or more residues at one or both ends of the sgRNA may be chemically modified, and/or internal nucleosides may be modified, and/or the entire sgRNA may be chemically modified. Certain embodiments comprise a 5' end modification. Certain embodiments comprise a 3' end modification.

[00460] In some embodiments, the guide RNAs disclosed herein comprise one of the modification patterns disclosed in WO2018/107028 and/or WO2019/237069, the contents of which are hereby incorporated by reference in their entirety. For example, the guide RNAs disclosed herein may comprise the short-guide structure described at claims 1-15 and/or the modification patterns described at claims 16-462 of WO2019/237069. In some embodiments, the guide RNAs disclosed herein comprise one of the structures/modification patterns disclosed in WO 2015/200555, the contents of which are hereby 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 hereby incorporated by reference in their entirety.

C. YA modifications

[00461] A modification at a YA site (also referred to herein as “YA modification”) can be a modification of the internucleoside linkage, a modification of the base (pyrimidine or adenine), e.g. by chemical modification, substitution, or otherwise, and/or a modification of the sugar (e.g. at the 2' position, such as 2'-O-alkyl, 2'-F, 2'-moe, 2'-F arabinose, 2'-H (deoxyribose), and the like). In some embodiments, a “YA modification” is any modification that alters the structure of the dinucleotide motif to reduce RNA endonuclease activity, e.g., by interfering with recognition or cleavage of a YA site by an RNase and/or by stabilizing an RNA structure (e.g., secondary structure) that decreases accessibility of a cleavage site to an RNase. See Peacock et al., *J Org Chem.* 76: 7295–7300 (2011); Behlke, *Oligonucleotides* 18:305-320 (2008); Ku et al., *Adv. Drug Delivery Reviews* 104: 16-28 (2016); Ghidini et al., *Chem. Commun.*, 2013, 49, 9036. Peacock et al., Behlke, Ku, and Ghidini provide exemplary modifications suitable as YA modifications. Modifications known to those of skill in the art to reduce endonucleolytic degradation are encompassed. Exemplary 2' ribose modifications that affect the 2' hydroxyl group involved in RNase cleavage are 2'-H and 2'-O-alkyl, including 2'-O-Me. Modifications such as bicyclic ribose analogs, UNA, and modified internucleoside linkages of the residues at the YA site can be YA modifications. Exemplary

base modifications that can stabilize RNA structures are pseudouridine and 5-methylcytosine. In some embodiments, at least one nucleotide of the YA site is modified. In some embodiments, the pyrimidine (also called “pyrimidine position”) of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the pyrimidine, a modification of the pyrimidine base, and a modification of the ribose, e.g. at its 2' position). In some embodiments, the adenine (also called “adenine position”) of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the pyrimidine, a modification of the pyrimidine base, and a modification of the ribose, e.g. at its 2' position). In some embodiments, the pyrimidine and the adenine of the YA site comprise modifications. In some embodiments, the YA modification reduces RNA endonuclease activity.

[00462] In some embodiments, an sgRNA comprises modifications at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more YA sites. In some embodiments, the pyrimidine of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the pyrimidine). In some embodiments, the adenine of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the adenine). In some embodiments, the pyrimidine and the adenine of the YA site comprise modifications, such as sugar, base, or internucleoside linkage modifications. The YA modifications can be any of the types of modifications set forth herein. In some embodiments, the YA modifications comprise one or more of phosphorothioate, 2'-OMe, or 2'-fluoro. In some embodiments, the YA modifications comprise pyrimidine modifications comprising one or more of phosphorothioate, 2'-OMe, or 2'-fluoro. In some embodiments, the YA modification comprises a bicyclic ribose analog (e.g., an LNA, BNA, or ENA) within an RNA duplex region that contains one or more YA sites. In some embodiments, the YA modification comprises a bicyclic ribose analog (e.g., an LNA, BNA, or ENA) within an RNA duplex region that contains a YA site, wherein the YA modification is distal to the YA site.

[00463] In some embodiments, the sgRNA comprises a guide region YA site modification. In some embodiments, the guide region comprises 1, 2, 3, 4, 5, or more YA sites (“guide region YA sites”) that may comprise YA modifications. In some embodiments, one or more YA sites located at 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus (where “5-end”, etc., refers to position 5 to the 3' end of the guide region, i.e., the most 3' nucleotide in the guide region) comprise YA modifications. In some embodiments, two or more YA sites located at 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end

from the 5' end of the 5' terminus comprise YA modifications. In some embodiments, three or more YA sites located at 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus comprise YA modifications. In some embodiments, four or more YA sites located at 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus comprise YA modifications. In some embodiments, five or more YA sites located at 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus comprise YA modifications. A modified guide region YA site comprises a YA modification.

[00464] In some embodiments, a modified guide region YA site is within 17, 16, 15, 14, 13, 12, 11, 10, or 9 nucleotides of the 3' terminal nucleotide of the guide region. For example, if a modified guide region YA site is within 10 nucleotides of the 3' terminal nucleotide of the guide region and the guide region is 20 nucleotides long, then the modified nucleotide of the modified guide region YA site is located at any of positions 11-20. In some embodiments, a YA modification is located within a YA site 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleotides from the 3' terminal nucleotide of the guide region. In some embodiments, a YA modification is located 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleotides from the 3' terminal nucleotide of the guide region.

[00465] In some embodiments, a modified guide region YA site is at or after nucleotide 4, 5, 6, 7, 8, 9, 10, or 11 from the 5' end of the 5' terminus.

[00466] In some embodiments, a modified guide region YA site is other than a 5' end modification. For example, an sgRNA can comprise a 5' end modification as described herein and further comprise a modified guide region YA site. Alternatively, an sgRNA can comprise an unmodified 5' end and a modified guide region YA site. Alternatively, an sgRNA can comprise a modified 5' end and an unmodified guide region YA site.

[00467] In some embodiments, a modified guide region YA site comprises a modification that at least one nucleotide located 5' of the guide region YA site does not comprise. For example, if nucleotides 1-3 comprise phosphorothioates, nucleotide 4 comprises only a 2'-OMe modification, and nucleotide 5 is the pyrimidine of a YA site and comprises a phosphorothioate, then the modified guide region YA site comprises a modification (phosphorothioate) that at least one nucleotide located 5' of the guide region YA site (nucleotide 4) does not comprise. In another example, if nucleotides 1-3 comprise phosphorothioates, and nucleotide 4 is the pyrimidine of a YA site and comprises a 2'-OMe, then the modified guide region YA site comprises a modification (2'-OMe) that at least one nucleotide located 5' of the guide region YA site (any of nucleotides 1-3) does not comprise.

This condition is also always satisfied if an unmodified nucleotide is located 5' of the modified guide region YA site.

[00468] In some embodiments, the modified guide region YA sites comprise modifications as described for YA sites above.

[00469] Additional embodiments of guide region YA site modifications are set forth in the summary above. Any embodiments set forth elsewhere in this disclosure may be combined to the extent feasible with any of the foregoing embodiments.

[00470] In some embodiments, the sgRNA comprises a conserved region YA site modification. Conserved region YA sites 1-10 are illustrated in FIG. 14. In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conserved region YA sites comprise modifications.

[00471] In some embodiments, conserved region YA sites 1, 8, or 1 and 8 comprise YA modifications. In some embodiments, conserved region YA sites 1, 2, 3, 4, and 10 comprise YA modifications. In some embodiments, YA sites 2, 3, 4, 8, and 10 comprise YA modifications. In some embodiments, conserved region YA sites 1, 2, 3, and 10 comprise YA modifications. In some embodiments, YA sites 2, 3, 8, and 10 comprise YA modifications. In some embodiments, YA sites 1, 2, 3, 4, 8, and 10 comprise YA modifications. In some embodiments, 1, 2, 3, 4, 5, 6, 7, or 8 additional conserved region YA sites comprise YA modifications.

[00472] In some embodiments, 1, 2, 3, or 4 of conserved region YA sites 2, 3, 4, and 10 comprise YA modifications. In some embodiments, 1, 2, 3, 4, 5, 6, 7, or 8 additional conserved region YA sites comprise YA modifications.

[00473] In some embodiments, the modified conserved region YA sites comprise modifications as described for YA sites above.

[00474] Additional embodiments of conserved region YA site modifications are set forth in the summary above. Any embodiments set forth elsewhere in this disclosure may be combined to the extent feasible with any of the foregoing embodiments.

[00475] In some embodiments, an sgRNA comprising the guide sequence of any one of SEQ ID NOs: 1-149 and any conserved portion of an sgRNA shown in Table 4, optionally having a modification pattern of any of an sgRNA shown in Table 4, optionally wherein the sgRNA comprises a 5' and 3' end modification (if not already shown in the construct of Table 4) is provided.

[00476] In some embodiments, the sgRNA comprises any of the modification patterns shown below in Table 4, where N is any natural or non-natural nucleotide, and wherein the

totality of the N's comprise a *KLKB1* guide sequence as described herein in Table 1. Table 4 does not depict the guide sequence portion of the sgRNA. The modifications remain as shown in Table 4 despite the substitution of N's for the nucleotides of a guide. That is, although the nucleotides of the guide replace the "N's", the nucleotides are modified as shown in Table 4.

Table 4: sgRNA modification patterns and conserved portions of an sgRNA. The guide sequence is not shown and will append the shown sequence at its 5' end.

SEQ ID NO	Name	Sequence
171	Exemplary conserved portion	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
172	Exemplary conserved portion	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUGGCACCGAGUCGGUGC
173	Exemplary conserved portion	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGGUGC
170	Exemplary conserved portion	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGGUGCUUUU
168	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U
169	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGGUGCmU*mU*mU*U
400	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU
401	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmU mCmGmGmUmGmCmU*mU*mU*mU
402	Exemplary – mod only	GUUUUAGAGCUAmGmAmAmAUAGCAAGUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U
403	Exemplary – mod only	GUUUUAGAmGmCmUmAGAAAmUmAmGmCAAGUAAAAUAAGGCUAG UCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U
404	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU* mU*U
405	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmA mCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
406	Exemplary – mod only	mGUUUUmAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAG GGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmC mAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
407	Exemplary – mod only	fGfUfUfUfUfAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUA AGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
408	Exemplary – mod only	mGfUfUfUfUfAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUA AAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmG mCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
409	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUmUmAAAmAmU AAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmG mCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

SEQ ID NO	Name	Sequence
410	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAfAfAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
411	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUfUmAfAmAfAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
412	Exemplary – mod only	mGUUUUmAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAAAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
413	Exemplary – mod only	mGUUUUmAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAfAfAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
414	Exemplary – mod only	mGUUUUmAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUfUmAfAmAfAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
415	Exemplary – mod only	fGfUfUfUfAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAAAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
416	Exemplary – mod only	fGfUfUfUfAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAfAfAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
417	Exemplary – mod only	fGfUfUfUfAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUfUmAfAmAfAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
418	Exemplary – mod only	GUUUUAmGmAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAUUAAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
419	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCmAmAmGmUUAUUAAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
420	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAUUAAAGGCUAGUCCGUUAfAfUfCfAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
421	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAUUAAAGGCUAGUCCGUUAUmUmCmAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
422	Exemplary – mod only	fGfUfUfUfAmGmAmGmCmUmAmGmAmAmAmUmAmGmCmAmAmGmUmUmAfAfAmAmUAAGGCUAGUCCGUUAUmUmCmAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU
423	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAUUAAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmA mCmCmGmAmGmUmCmGmGmUmGmCmUmUmUmU
424	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAUUAAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmA mCmCmGmAmGmUmCmGmGmUmGmCmUmU*mU*mU
425	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAUUAAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmA mCmCmGmAmGfUfCfGfGfUfGfCfU*fU*fU*mU
426	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAUUAAAGGCUAGUCCGUUAUCAfAmCfUmUfGmAfAmAfAmAfGmUfGmGfCmAfCmCfGmAfGmUfCmGfGmUfGmCfU*mU*fU*mU
427	Exemplary – mod only	mGUUUUmAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAUUAAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

SEQ ID NO	Name	Sequence
428	Exemplary – mod only	fGfUfUfUfAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
429	Exemplary – mod only	mGfUfUfUfUmAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
430	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAAAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
431	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAfAfAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
432	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUfUmAfAfAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
433	Exemplary – mod only	mGUUUUmAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAAAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
434	Exemplary – mod only	mGUUUUmAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAfAfAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
435	Exemplary – mod only	mGUUUUmAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUfUmAfAfAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
436	Exemplary – mod only	fGfUfUfUfAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAAAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
437	Exemplary – mod only	fGfUfUfUfAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUmUmAfAfAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
438	Exemplary – mod only	fGfUfUfUfAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUfUmAfAfAmAmUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
439	Exemplary – mod only	GUUUUAmGmAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
440	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCmAmAmGmUUA AAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
441	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUfAfUfCfAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
442	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAmUmCmAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
443	Exemplary – mod only	fGfUfUfUfAmGmAmGmCmUmAmGmAmAmAmUmAmGmCmAmAmGmUmUmAfAfAmAmUAAGGCUAGUCCGUUAmUmCmAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU
444	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmUmUmUmU
445	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmUmU*mU*mU

SEQ ID NO	Name	Sequence
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447	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAmCfUmUfGmAfAmAfAmAfGmUfGmGfCmAfCmCfGmAfGmUfCmGfGmUfGmCfU*mU*fU*mU
448	Exemplary – guide region mod only	mN*mN*mN*mNnn*N*fN*fN*fN*fNnnfNnnfNnnfNnn
449	Exemplary – guide region mod only	mN*mN*mN*mNnn*N*fN*fN*fN*fNnnfNnnfNnnfNnn
450	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
174	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUAAGCACCGAGUCGG*mU*mG*mC
175	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUCAGCACCGAGUCGG*mU*mG*mC
176	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
177	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
178	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGAGCUGGCACCGAGUCGG*mU*mG*mC
179	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGAAAUUGGCACCGAGUCGG*mU*mG*mC
180	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGAAAGGGCACCGAGUCGG*mU*mG*mC
181	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAAUUGGCACCGAGUCGG*mU*mG*mC
182	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAAGUGGCACCGAGUCGG*mU*mG*mC
183	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACAGUGGCACCGAGUCGG*mU*mG*mC
184	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACAAGGGCACCGAGUCGG*mU*mG*mC
185	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAUGGCACCGAGUCGG*mU*mG*mC
186	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAGGCACCGAGUCGG*mU*mG*mC
187	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGGGCACCGAGUCGG*mU*mG*mC
188	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGGCACCGAGUCGG*mU*mG*mC
189	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
190	Exemplary – mod only	GUUUUAGAGCGCAAGCGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
191	Exemplary – mod only	GUUUUAGAGCGCAAGCGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
192	Exemplary – mod only	GUUUUAGAGCGGAAACGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
193	Exemplary – mod only	GUUUUAGAGCGGAAACGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
194	Exemplary – mod only	GUUUUAGAGCGGAAAGGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC

SEQ ID NO	Name	Sequence
195	Exemplary – mod only	GUUUUAGAGCUGAAAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAA CUUGGCACCGAGUCGG*mU*mG*mC
196	Exemplary – mod only	GUUUUAGAGCGAAAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACU UGGCACCGAGUCGG*mU*mG*mC
197	Exemplary – mod only	GUUUUAGAGCGAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GGCACCGAGUCGG*mU*mG*mC
198	Exemplary – mod only	GUUUUAGAGCAAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GGCACCGAGUCGG*mU*mG*mC
199	Exemplary – mod only	GUUUUAGAGGAAACAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG GCACCGAGUCGG*mU*mG*mC
202	Exemplary – mod only	GUUUUAGAGCAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG GCACCGAGUCGG*mU*mG*mC
203	Exemplary – mod only	GUUUUAGAGCGAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG GCACCGAGUCGG*mU*mG*mC
204	Exemplary – mod only	GUUUUAGAGCGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGC ACCGAGUCGG*mU*mG*mC
205	Exemplary – mod only	GUUUUAGAGAACAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGC ACCGAGUCGG*mU*mG*mC
206	Exemplary – mod only	GUUUUAGAGACAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCA CCGAGUCGG*mU*mG*mC
207	Exemplary – mod only	GUUUUAGAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCAC CGAGUCGG*mU*mG*mC
208	Exemplary – mod only	GUUUUAGAAAAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCAC CGAGUCGG*mU*mG*mC
209	Exemplary – mod only	GUUUUAGAAAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACC GAGUCGG*mU*mG*mC
210	Exemplary – mod only	GUUUUAGAGCUAGAAAUAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCG*mG*mU*mG
211	Exemplary – mod only	GUUUUAGAGCUAGAAAUAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUC*mG*mG*mU
212	Exemplary – mod only	GUUUUAGAGCUAGAAAUAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAGU*mC*mG*mG
213	Exemplary – mod only	GUUUUAGAGCUAGAAAUAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAG*mU*mC*mG
214	Exemplary – mod only	GUUUUAGAGCUAGAAAUAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGA*mG*mU*mC
215	Exemplary – mod only	GUUUUAGAGCUAGAAAUAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCG*mA*mG*mU
216	Exemplary – mod only	GUUUUAGAGCGAAAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACUG GCACCGAGUCGG*mU*mG*mC
217	Exemplary – mod only	GUUUUAGAGCGAAAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAGGC ACCGAGUCGG*mU*mG*mC
218	Exemplary – mod only	GUUUUAGAGAAAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGC ACCGAGUCGG*mU*mG*mC
219	Exemplary – mod only	GUUUUAGAGGAAACAAGUUAAAAUAAGGCUAGUCCGUUAUCAAUUGGC ACCGAGUCGG*mU*mG*mC
220	Exemplary – mod only	GUUUUAGAGAAAAGUUAAAAUAAGGCUAGUCCGUUAUCAAUUGGCAC CGAGUCGG*mU*mG*mC
221	Exemplary – mod only	GUUUUAGAGGAAACAAGUUAAAAUAAGGCUAGUCCGUUAUCACUGGC ACCGAGUCGG*mU*mG*mC
222	Exemplary – mod only	GUUUUAGAGAAAAGUUAAAAUAAGGCUAGUCCGUUAUCAGGCACCG AGUCGG*mU*mG*mC
223	Exemplary – mod only	GUUUUAGAGCGAAAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGC UAUGGCACCGAGUCGG*mU*mG*mC
224	Exemplary – mod only	GUUUUAGAGCUAGAAAUAAGCAAGUUAAAAUAAGGCAGUCCGUUAUCA ACUUGGCACCGAGUCGG*mU*mG*mC

SEQ ID NO	Name	Sequence
225	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUGUCCGUUAUCA ACUUGGCACCGAGUCGG*mU*mG*mC
226	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUGUCCGUUAUCAA CUUGGCACCGAGUCGG*mU*mG*mC
227	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGUUCCGUUAUCAA CUUGGCACCGAGUCGG*mU*mG*mC
228	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGUUCCGUUAUCAAC UUGGCACCGAGUCGG*mU*mG*mC
229	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGAUCCGUUAUCAAC UUGGCACCGAGUCGG*mU*mG*mC
230	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUGAAAUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
231	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
232	Exemplary – mod only	GUUUUAGAGCGAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
233	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCGAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
234	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCCGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
235	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
236	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
237	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
238	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
239	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUUGUC AACUUGGCACCGAGUCGG*mU*mG*mC
240	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUCUC AACUUGGCACCGAGUCGG*mU*mG*mC
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242	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUG AACUUGGCACCGAGUCGG*mU*mG*mC
243	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mC*mC
244	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGAACCGAGUCGG*mU*mU*mC
245	Exemplary – mod only	GUUUUAGAGCGAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUGUG AACUUGGAACCGAGUCGG*mU*mU*mC
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247	Exemplary – mod only	GUUUUAGAGCGAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUGUG GCUUGGAACCGAGUCGG*mU*mU*mC
248	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
249	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
250	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
251	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
252	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC

SEQ ID NO	Name	Sequence
253	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
254	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
255	Exemplary – mod only	GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUC AACUUGGCACCGAGUCGG*mU*mG*mC
256	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC
257	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCAAAAUGGCACCGAGUCGG*mU*mG*mC
258	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCACAAGGGCACCGAGUCGG*mU*mG*mC
259	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCAAAAUGGCACCGAGUCGG*mU*mG*mC
260	Exemplary – mod only	GUUUUAGAGCGCGAAGCGCAAGUAAAAUAAGGCUAGUCCGUUAUCA AAAUGGCACCGAGUCGG*mU*mG*mC
261	Exemplary – mod only	GUUUUAGAGCUGAAAAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAA AAUGGCACCGAGUCGG*mU*mG*mC
262	Exemplary – mod only	GUUUUAGAGCGAAAAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAAAA UGGCACCGAGUCGG*mU*mG*mC
263	Exemplary – mod only	GUUUUAGAGCAAAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAAAAU GGCACCGAGUCGG*mU*mG*mC
264	Exemplary – mod only	GUUUUAGAmGmCmGmAmAmAmGmCAAGUAAAAUAAGGCUAGUCCGU UAUCAACUUGGCACCGAGUCGG*mU*mG*mC
265	Exemplary – mod only	GUUUUAGAmGmCmGmAmAmAmGmCAAGUAAAAUAAGGCUAGUCCGU UAUCAAGAAUUGGCACCGAGUCGG*mU*mG*mC
266	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCAmCmGmAmAmAmGmGmCmAmCmCmGmAmGmU mCmGmG*mU*mG*mC
267	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCAmAmAmAmUmGmGmCmAmCmCmGmAmGmUmCmG mG*mU*mG*mC
268	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCAmGmAmAmAmGmGmCmAmCmCmGmAmGmUmCmG mCmGmG*mU*mG*mC
269	Exemplary – mod only	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGG CUAGUCCGUUAUCAAmAmAmUmGmGmCmAmCmCmGmAmGmUmCmGm G*mU*mG*mC

[00477] In some embodiments, the modified sgRNA comprises the following sequence:

mN*mN*mN*NNNNNNNNNNNNNNNGUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU (SEQ ID NO: 300), where “N” may be any natural or non-natural nucleotide, and wherein the totality of N’s comprise a *KLKB1* guide sequence as described in Table 1. For example, encompassed herein is SEQ ID NO: 300, where the N’s are replaced with any of the guide sequences disclosed herein in Table 1 (SEQ ID Nos: 1-149). Also encompassed herein are

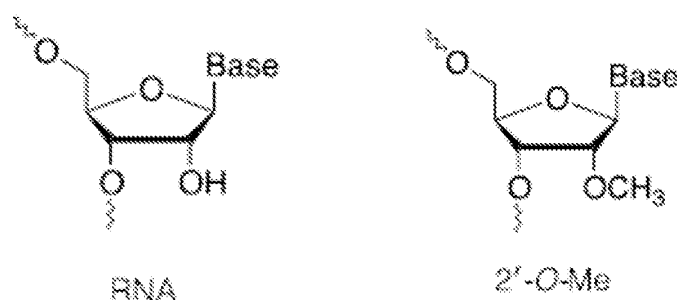
guide RNAs combining any of the guide sequences of Table 1 (SEQ ID Nos: 1-149)

combined with a conserved portion of an sgRNA, e.g. a sequence of Table 4.

[00478] Any of the modifications described below may be present in the gRNAs and mRNAs described herein.

[00479] The terms “mA,” “mC,” “mU,” or “mG” may be used to denote a nucleotide that has been modified with 2'-O-Me.

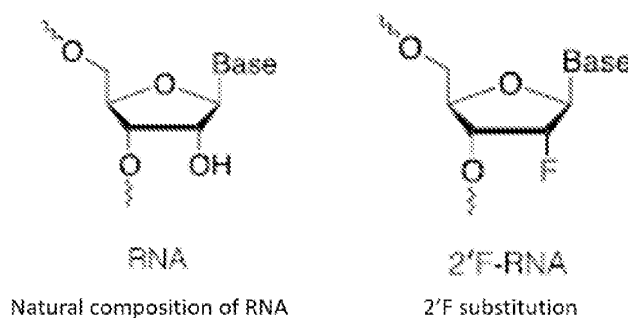
[00480] Modification of 2'-O-methyl can be depicted as follows:



[00481] 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.

[00482] In this application, the terms “fA,” “fC,” “fU,” or “fG” may be used to denote a nucleotide that has been substituted with 2'-F.

[00483] Substitution of 2'-F can be depicted as follows:

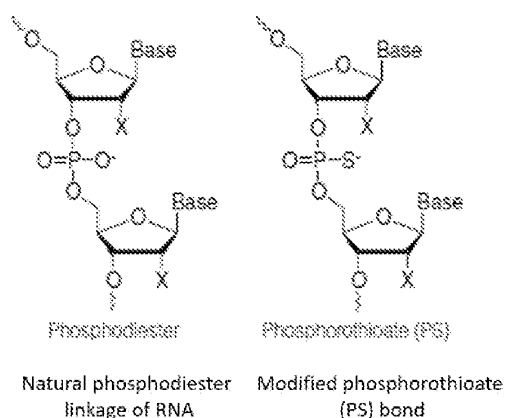


[00484] Phosphorothioate (PS) linkage or bond refers to a bond where a sulfur is substituted for one nonbridging phosphate oxygen in a phosphodiester linkage, for example in the bonds between nucleotides bases. When phosphorothioates are used to generate oligonucleotides, the modified oligonucleotides may also be referred to as S-oligos.

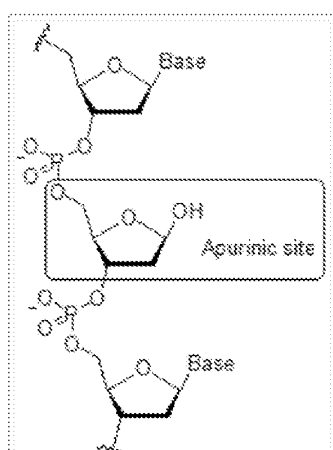
[00485] A “*” may be used to depict a PS modification. In this application, the terms A*, C*, U*, or G* may be used to denote a nucleotide that is linked to the next (e.g., 3') nucleotide with a PS bond.

[00486] In this application, the terms “mA*,” “mC*,” “mU*,” or “mG*” may be used to denote a nucleotide that has been substituted with 2'-O-Me and that is linked to the next (e.g., 3') nucleotide with a PS bond.

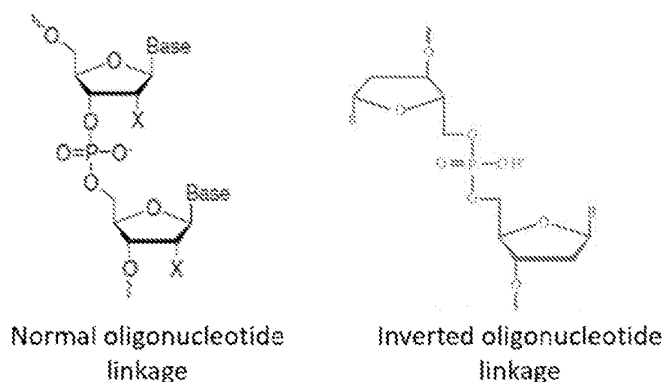
[00487] The diagram below shows the substitution of S- into a nonbridging phosphate oxygen, generating a PS bond in lieu of a phosphodiester bond:



[00488] 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:



[00489] Inverted bases refer to those with linkages that are inverted from the normal 5' to 3' linkage (i.e., either a 5' to 5' linkage or a 3' to 3' linkage). For example:



[00490] 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.

[00491] In some embodiments, one or more of the first three, four, or five nucleotides at the 5' terminus, and one or more of the last three, four, or five nucleotides at the 3' terminus are modified. In some embodiments, the modification is a 2'-O-Me, 2'-F, inverted abasic nucleotide, PS bond, or other nucleotide modification well known in the art to increase stability and/or performance.

[00492] 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.

[00493] 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.

[00494] In some embodiments, the guide RNA comprises a modified sgRNA. In some embodiments, the guide RNA comprises any conserved portion of an sgRNA shown in Table 4, optionally having a modification pattern of any of an sgRNA shown in Table 4, optionally wherein the sgRNA comprises a 5' and 3' end modification (if not already shown in the

construct of Table 4) is provided. In some embodiments, the sgRNA comprises the modification pattern of any of an sgRNA shown in Table 4, where N is any natural or non-natural nucleotide, and where the totality of the N's comprise a guide sequence that directs a nuclease to a target sequence in *KLKB1*, e.g., as shown in Table 1.

[00495] In some embodiments, the guide RNA comprises a sgRNA comprising any one of the guide sequences of SEQ ID No: 1-149 and any conserved portion of an sgRNA shown in Table 4, optionally having a modification pattern of any of an sgRNA shown in Table 4, optionally wherein the sgRNA comprises a 5' and 3' end modification (if not already shown in the construct of Table 4). In some embodiments, the guide RNA comprises a sgRNA comprising any one of the guide sequences of SEQ ID No: 1-149 and the nucleotides of SEQ ID No: 170, 171, 172, or 173, wherein the nucleotides of SEQ ID No: 170, 171, 172, or 173 are on the 3' end of the guide sequence, and wherein the sgRNA may be modified as shown in Table 4 or SEQ ID NO: 300.

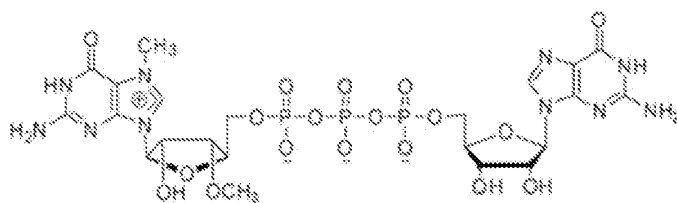
[00496] As noted above, in some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered. In some embodiments, the ORF encoding an RNA-guided DNA nuclease is a "modified RNA-guided DNA binding agent ORF" or simply a "modified ORF," which is used as shorthand to indicate that the ORF is modified.

[00497] In some embodiments, the modified ORF may comprise a modified uridine at least at one, a plurality of, or all uridine positions. In some embodiments, the modified uridine is a uridine modified at the 5 position, e.g., with a halogen, methyl, or ethyl. In some embodiments, the modified uridine is a pseudouridine modified at the 1 position, e.g., with a halogen, methyl, or ethyl. The modified uridine can be, for example, pseudouridine, N1-methyl-pseudouridine, 5-methoxyuridine, 5-iodouridine, or a combination thereof. In some embodiments, the modified uridine is 5-methoxyuridine. In some embodiments, the modified uridine is 5-iodouridine. In some embodiments, the modified uridine is pseudouridine. In some embodiments, the modified uridine is N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a combination of N1-methyl pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a

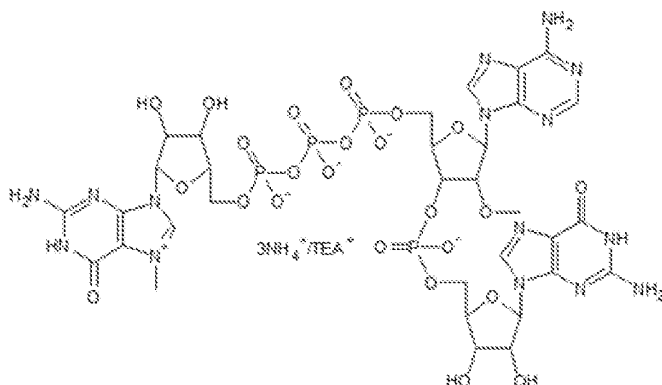
combination of 5-iodouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and 5-iodouridine. In some embodiments, the modified uridine is a combination of 5-iodouridine and 5-methoxyuridine.

[00498] In some embodiments, an mRNA disclosed herein comprises a 5' cap, such as a Cap0, Cap1, or Cap2. A 5' cap is generally a 7-methylguanine ribonucleotide (which may be further modified, as discussed below e.g. with respect to ARCA) linked through a 5'-triphosphate to the 5' position of the first nucleotide of the 5'-to-3' chain of the mRNA, i.e., the first cap-proximal nucleotide. In Cap0, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-hydroxyl. In Cap1, the riboses of the first and second transcribed nucleotides of the mRNA comprise a 2'-methoxy and a 2'-hydroxyl, respectively. In Cap2, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-methoxy. See, e.g., Katibah et al. (2014) *Proc Natl Acad Sci USA* 111(33):12025-30; Abbas et al. (2017) *Proc Natl Acad Sci USA* 114(11):E2106-E2115. Most endogenous higher eukaryotic mRNAs, including mammalian mRNAs such as human mRNAs, comprise Cap1 or Cap2. Cap0 and other cap structures differing from Cap1 and Cap2 may be immunogenic in mammals, such as humans, due to recognition as "non-self" by components of the innate immune system such as IFIT-1 and IFIT-5, which can result in elevated cytokine levels including type I interferon. Components of the innate immune system such as IFIT-1 and IFIT-5 may also compete with eIF4E for binding of an mRNA with a cap other than Cap1 or Cap2, potentially inhibiting translation of the mRNA.

[00499] 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.



[00500] CleanCap™ AG (m7G(5')ppp(5')(2'OMeA)pG; TriLink Biotechnologies Cat. No. N-7113) or CleanCap™ GG (m7G(5')ppp(5')(2'OMeG)pG; TriLink Biotechnologies Cat. No. N-7133) can be used to provide a Cap1 structure co-transcriptionally. 3'-O-methylated versions of CleanCap™ AG and CleanCap™ GG are also available from TriLink Biotechnologies as Cat. Nos. N-7413 and N-7433, respectively. The CleanCap™ AG structure is shown below.



[00501] Alternatively, a cap can be added to an RNA post-transcriptionally. For example, Vaccinia capping enzyme is commercially available (New England Biolabs Cat. No. M2080S) and has RNA triphosphatase and guanylyltransferase activities, provided by its D1 subunit, and guanine methyltransferase, provided by its D12 subunit. As such, it can add a 7-methylguanine to an RNA, so as to give Cap0, in the presence of S-adenosyl methionine and GTP. See, e.g., Guo, P. and Moss, B. (1990) *Proc. Natl. Acad. Sci. USA* 87, 4023-4027; Mao, X. and Shuman, S. (1994) *J. Biol. Chem.* 269, 24472-24479.

[00502] 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.

D. Ribonucleoprotein complex

[00503] In some embodiments, the disclosure provides compositions comprising one or more gRNAs comprising one or more guide sequences from Table 1 or 2 and an RNA-guided DNA binding agent, e.g., a nuclease, such as a Cas nuclease, such as Cas9. In some embodiments, the RNA-guided DNA-binding agent has cleavase activity, which can also be referred to as double-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nuclease. Examples of Cas9 nucleases include those of

the type II CRISPR systems of *S. pyogenes*, *S. aureus*, and other prokaryotes (see, e.g., the list in the next paragraph), and modified (e.g., engineered or mutant) versions thereof. See, e.g., US2016/0312198 A1; US 2016/0312199 A1. Other examples of Cas nucleases include a Csm or Cmr complex of a type III CRISPR system or the Cas10, Csm1, or Cmr2 subunit thereof; and a Cascade complex of a type I CRISPR system, or the Cas3 subunit thereof. In some embodiments, the Cas nuclease may be from a Type-IIA, Type-IIB, or Type-IIC system. For discussion of various CRISPR systems and Cas nucleases see, e.g., Makarova et al., NAT. REV. MICROBIOL. 9:467-477 (2011); Makarova et al., NAT. REV. MICROBIOL. 13: 722-36 (2015); Shmakov et al., MOLECULAR CELL, 60:385-397 (2015).

[00504] Non-limiting exemplary species that the Cas nuclease can be derived from include *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus sp.*, *Staphylococcus aureus*, *Listeria innocua*, *Lactobacillus gasseri*, *Francisella novicida*, *Wolinella succinogenes*, *Sutterella wadsworthensis*, *Gammaproteobacterium*, *Neisseria meningitidis*, *Campylobacter jejuni*, *Pasteurella multocida*, *Fibrobacter succinogenes*, *Rhodospirillum rubrum*, *Nocardiopsis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *Alicyclobacillus acidocaldarius*, *Bacillus pseudomyoides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Lactobacillus buchneri*, *Treponema denticola*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas sp.*, *Crocospaera watsonii*, *Cyanothece sp.*, *Microcystis aeruginosa*, *Synechococcus sp.*, *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor beccsii*, *Candidatus Desulforudis*, *Clostridium botulinum*, *Clostridium difficile*, *Fingoldia magna*, *Natronaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochroamatium vinosum*, *Marinobacter sp.*, *Nitrosococcus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc sp.*, *Arthrospira maxima*, *Arthrospira platensis*, *Arthrospira sp.*, *Lyngbya sp.*, *Microcoleus chthonoplastes*, *Oscillatoria sp.*, *Petrotoga mobilis*, *Thermosipho africanus*, *Streptococcus pasteurianus*, *Neisseria cinerea*, *Campylobacter lari*, *Parvibaculum lavamentivorans*, *Corynebacterium diphtheria*, *Acidaminococcus sp.*, *Lachnospiraceae bacterium ND2006*, and *Acaryochloris marina*.

[00505] In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nuclease is the Cas9 nuclease from

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

[00506] In some embodiments, the gRNA together with an RNA-guided DNA binding agent is called a ribonucleoprotein complex (RNP). In some embodiments, the RNA-guided DNA binding agent is a Cas nuclease. In some embodiments, the gRNA together with a Cas nuclease is called a Cas RNP. In some embodiments, the RNP comprises Type-I, Type-II, or Type-III components. In some embodiments, the Cas nuclease is the Cas9 protein from the Type-II CRISPR/Cas system. In some embodiment, the gRNA together with Cas9 is called a Cas9 RNP.

[00507] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 protein comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 protein is a wild type Cas9. In each of the composition, use, and method embodiments, the Cas induces a double strand break in target DNA.

[00508] In some embodiments, chimeric Cas nucleases are used, where one domain or region of the protein is replaced by a portion of a different protein. In some embodiments, a Cas nuclease domain may be replaced with a domain from a different nuclease such as FokI. In some embodiments, a Cas nuclease may be a modified nuclease.

[00509] In other embodiments, the Cas nuclease may be from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a component of the Cascade complex of a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a Cas3 protein. In some embodiments, the Cas nuclease may be from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease may have an RNA cleavage activity.

[00510] In some embodiments, the RNA-guided DNA-binding agent has single-strand nickase activity, i.e., can cut one DNA strand to produce a single-strand break, also known as a “nick.” In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. A nickase is an enzyme that creates a nick in dsDNA, i.e., cuts one strand but not the other of the DNA double helix. In some embodiments, a Cas nickase is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which an endonucleolytic active site is inactivated, e.g., by one or more alterations (e.g., point mutations) in a catalytic domain. See, e.g., US Pat. No. 8,889,356 for discussion of Cas nickases and exemplary catalytic domain alterations. In some embodiments, a Cas nickase such as a Cas9 nickase has an inactivated RuvC or HNH domain.

[00511] In some embodiments, the RNA-guided DNA-binding agent is modified to contain only one functional nuclease domain. For example, the agent protein may be modified such that one of the nuclease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, a nickase is used having a RuvC domain with reduced activity. In some embodiments, a nickase is used having an inactive RuvC domain. In some embodiments, a nickase is used having an HNH domain with reduced activity. In some embodiments, a nickase is used having an inactive HNH domain.

[00512] In some embodiments, a conserved amino acid within a Cas protein nuclease domain is substituted to reduce or alter nuclease activity. In some embodiments, a Cas nuclease may comprise an amino acid substitution in the RuvC or RuvC-like nuclease domain. Exemplary amino acid substitutions in the RuvC or RuvC-like nuclease domain include D10A (based on the *S. pyogenes* Cas9 protein). See, e.g., Zetsche et al. (2015) *Cell* Oct 22;163(3): 759-771. In some embodiments, the Cas nuclease may comprise an amino acid substitution in the HNH or HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the *S. pyogenes* Cas9 protein). See, e.g., Zetsche et al. (2015). Further exemplary amino acid substitutions include D917A, E1006A, and D1255A (based on the *Francisella novicida* U112 Cpf1 (FnCpf1) sequence (UniProtKB - A0Q7Q2 (CPF1_FRATN))).

[00513] In some embodiments, an mRNA encoding a nickase is provided in combination with a pair of guide RNAs that are complementary to the sense and antisense strands of the target sequence, respectively. In this embodiment, the guide RNAs direct the nickase to a target sequence and introduce a DSB by generating a nick on opposite strands of the target sequence (i.e., double nicking). In some embodiments, use of double nicking may

improve specificity and reduce off-target effects. In some embodiments, a nickase is used together with two separate guide RNAs targeting opposite strands of DNA to produce a double nick in the target DNA. In some embodiments, a nickase is used together with two separate guide RNAs that are selected to be in close proximity to produce a double nick in the target DNA.

[00514] In some embodiments, the RNA-guided DNA-binding agent lacks cleavase and nickase activity. In some embodiments, the RNA-guided DNA-binding agent comprises a dCas DNA-binding polypeptide. A dCas polypeptide has DNA-binding activity while essentially lacking catalytic (cleavase/nickase) activity. In some embodiments, the dCas polypeptide is a dCas9 polypeptide. In some embodiments, the RNA-guided DNA-binding agent lacking cleavase and nickase activity or the dCas DNA-binding polypeptide is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which its endonucleolytic active sites are inactivated, e.g., by one or more alterations (e.g., point mutations) in its catalytic domains. See, e.g., US 2014/0186958 A1; US 2015/0166980 A1.

[00515] In some embodiments, the RNA-guided DNA-binding agent comprises one or more heterologous functional domains (e.g., is or comprises a fusion polypeptide).

[00516] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent into the nucleus of a cell. For example, the heterologous functional domain may be a nuclear localization signal (NLS). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-10 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with one NLS. Where one NLS is used, the NLS may be linked at the N-terminus or the C-terminus of the RNA-guided DNA-binding agent sequence. It may also be inserted within the RNA-guided DNA binding agent sequence. In other embodiments, the RNA-guided DNA-binding agent may be fused with more than one NLS. In some embodiments, the RNA-guided DNA-binding agent may be fused with 2, 3, 4, or 5 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (e.g., two SV40 NLSs) or different. In some embodiments, the RNA-guided DNA-binding agent is fused to two SV40 NLS sequences linked at the carboxy terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs, one linked at the N-terminus and one at the C-terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with 3 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with no NLS. In some embodiments, the NLS may be a

monopartite sequence, such as, *e.g.*, the SV40 NLS, PKKKRKV (SEQ ID NO: 600) or PKKKRRV (SEQ ID NO: 601). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 602). In a specific embodiment, a single PKKKRKV (SEQ ID NO: 600) NLS may be linked at the C-terminus of the RNA-guided DNA-binding agent. One or more linkers are optionally included at the fusion site.

[00517] 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).

[00518] In some embodiments, the heterologous functional domain may be a marker domain. Non-limiting examples of marker domains include fluorescent proteins, purification tags, epitope tags, and reporter gene sequences. In some embodiments, the marker domain may be a fluorescent protein. Non-limiting examples of suitable fluorescent proteins include green fluorescent proteins (*e.g.*, GFP, GFP-2, tagGFP, turboGFP, sfGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreen1), yellow fluorescent proteins (*e.g.*, YFP, EYFP, Citrine, Venus, YPet, PhiYFP, ZsYellow1), blue fluorescent proteins (*e.g.*, EBFP, EBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire,), cyan

fluorescent proteins (*e.g.*, ECFP, Cerulean, CyPet, AmCyan1, Midoriishi-Cyan), red fluorescent proteins (*e.g.*, mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRed1, AsRed2, eqFP611, mRaspberry, mStrawberry, Jred), and orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato) or any other suitable fluorescent protein. In other embodiments, the marker domain may be a purification tag and/or an epitope tag. Non-limiting exemplary tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein (MBP), thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1, AU5, E, ECS, E2, FLAG, HA, nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, 6xHis, 8xHis, biotin carboxyl carrier protein (BCCP), poly-His, and calmodulin. Non-limiting exemplary reporter genes include glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), beta-galactosidase, beta-glucuronidase, luciferase, or fluorescent proteins.

[00519] In additional embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to a specific organelle, cell type, tissue, or organ. In some embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to mitochondria.

[00520] In further embodiments, the heterologous functional domain may be an effector domain. When the RNA-guided DNA-binding agent is directed to its target sequence, *e.g.*, when a Cas nuclease is directed to a target sequence by a gRNA, the effector domain may modify or affect the target sequence. In some embodiments, the effector domain may be chosen from a nucleic acid binding domain, a nuclease domain (*e.g.*, a non-Cas nuclease domain), an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. In some embodiments, the heterologous functional domain is a nuclease, such as a FokI nuclease. See, *e.g.*, US Pat. No. 9,023,649. In some embodiments, the heterologous functional domain is a transcriptional activator or repressor. See, *e.g.*, Qi et al., "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression," *Cell* 152:1173-83 (2013); Perez-Pinera et al., "RNA-guided gene activation by CRISPR-Cas9-based transcription factors," *Nat. Methods* 10:973-6 (2013); Mali et al., "CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," *Nat. Biotechnol.* 31:833-8 (2013); Gilbert et al., "CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes," *Cell*

154:442-51 (2013). As such, the RNA-guided DNA-binding agent essentially becomes a transcription factor that can be directed to bind a desired target sequence using a guide RNA.

E. Determination of efficacy of gRNAs

[00521] In some embodiments, the efficacy of a gRNA is determined when delivered or expressed together with other components forming an RNP. In some embodiments, the gRNA is expressed together with an RNA-guided DNA binding agent, such as a Cas protein, *e.g.*, Cas9. In some embodiments, the gRNA is delivered to or expressed in a cell line that already stably expresses an RNA-guided DNA nuclease, such as a Cas nuclease or nickase, *e.g.*, Cas9 nuclease or nickase. 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 a mRNA encoding an RNA-guided DNA nuclease, such as a Cas nuclease or nickase, *e.g.*, Cas9 nuclease or nickase.

[00522] As described herein, use of an RNA-guided DNA nuclease and a guide RNA disclosed herein can lead to double-stranded breaks (DSB), single-strand break, and/or site-specific binding that results in nucleic acid modification 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.

[00523] In some embodiments, the efficacy of particular gRNAs is determined based on *in vitro* models. In some embodiments, the *in vitro* model is HEK293 cells stably expressing Cas9 (HEK293_Cas9). In some embodiments, the *in vitro* model is HUH7 human hepatocarcinoma cells. In some embodiments, the *in vitro* model is HepG2 cells. In some embodiments, the *in vitro* model is primary human hepatocytes. In some embodiments, the *in vitro* model is primary cynomolgus hepatocytes. With respect to using primary human hepatocytes, commercially available primary human hepatocytes can be used to provide greater consistency between experiments. In some embodiments, the number of off-target sites at which a deletion or insertion occurs in an *in vitro* model (*e.g.*, in primary human hepatocytes) is determined, *e.g.*, by analyzing genomic DNA from primary human hepatocytes transfected *in vitro* with Cas9 mRNA and the guide RNA. In some embodiments, such a determination comprises analyzing genomic DNA from primary human hepatocytes transfected *in vitro* with Cas9 mRNA, the guide RNA, and a donor oligonucleotide. Exemplary procedures for such determinations are provided in the working examples below.

[00524] 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. In some embodiments, the efficacy of particular gRNAs is determined in PHH or PCH for a gRNA selection process.

[00525] In some embodiments, the efficacy of particular gRNAs is determined based on *in vivo* models. In some embodiments, the *in vivo* model is a rodent model. In some embodiments, the rodent model is a mouse which expresses a *KLKB1* gene. In some embodiments, the rodent model is a mouse which expresses a human *KLKB1* gene. In some embodiments, the *in vivo* model is a non-human primate, for example cynomolgus monkey.

[00526] In some embodiments, the efficacy of a guide RNA is measured by percent editing of *KLKB1*. Indel percentage can be calculated from NGS sequencing. In some embodiments, the percent editing of *KLKB1* is compared to the percent editing necessary to achieve knockdown of prekallikrein and/or kallikrein protein, e.g., from cell culture media or cell lysates in the case of an *in vitro* model or plasma containing circulating levels in the case of an *in vivo* model.

[00527] In some embodiments, the efficacy of a guide RNA is measured by the number and/or frequency of indels at off-target sequences within the genome of the target cell type. In some embodiments, efficacious guide RNAs are provided which produce indels at off-target sites at very low frequencies (e.g., <5%) in a cell population and/or relative to the frequency of indel creation at the target site. Thus, the disclosure provides for guide RNAs which do not exhibit off-target indel formation in the target cell type (e.g., a hepatocyte such as PHH), or which produce a frequency of off-target indel formation of <5% in a cell population and/or relative to the frequency of indel creation at the target site. In some embodiments, the disclosure provides guide RNAs which do not exhibit any off-target indel formation in the target cell type (e.g., hepatocyte). In some embodiments, guide RNAs are provided which produce indels at less than 5 off-target sites, e.g., as evaluated by one or more methods described herein. In some embodiments, guide RNAs are provided which produce indels at less than or equal to 4, 3, 2, or 1 off-target site(s) e.g., as evaluated by one or more methods described herein. In some embodiments, the off-target site(s) does not occur in a protein coding region in the target cell (e.g., hepatocyte) genome.

[00528] In some embodiments, linear amplification is used to detect gene editing events, such as the formation of insertion/deletion (“indel”) mutations, translocations, and homology directed repair (HDR) events in target DNA. For example, linear amplification

with a unique sequence-tagged primer and isolating the tagged amplification products (herein after referred to as “UnIT,” or “Unique Identifier Tagmentation” method) may be used.

[00529] In some embodiments, the efficacy of a guide RNA is determined by measuring levels of KLKB1, pKal, total KLKB1 (prekallikrein + pKal), KLKB1 activity, HMWK, HMWK activity, and/or bradykinin, in a sample such as a body fluid, *e.g.*, serum, plasma, or blood.

[00530] In some embodiments, the efficacy of a guide RNA is determined by measuring KLKB1 mRNA levels. A decrease in KLKB1 mRNA levels is indicative of an effective guide RNA.

[00531] In some embodiments, the efficacy of a guide RNA is determined by measuring levels of bradykinin in a sample such as a body fluid, *e.g.*, serum, plasma, or blood.

[00532] In some embodiments, the efficacy of a guide RNA is determined by measuring levels of bradykinin and/or its degradation products in a sample. In some embodiments, the efficacy of a guide RNA is determined by measuring levels of bradykinin and/or its degradation products in the serum or plasma. A decrease in the levels of bradykinin and/or its degradation products in the serum or plasma is indicative of an effective guide RNA.

[00533] One method to detect bradykinin in circulating blood is provided in Ferreira, et al., Br. J. Pharmac. Chemother. (1967), 29, 367-377. Bradykinin may also be detected by an enzyme-linked immunosorbent assay (ELISA) assay with cell culture media or serum or plasma. (See, *e.g.*, Abcam Cat. No. ab136936; Markit-M Bradykinin (Gentaur)). In some embodiments, levels of bradykinin are measured in the same *in vitro* or *in vivo* systems or models used to measure editing. In some embodiments, levels of bradykinin are measured in cells, *e.g.*, primary human hepatocytes. In some embodiments, levels of bradykinin are measured in a fluid such as serum or plasma. In some embodiments circulating levels of bradykinin are measured.

[00534] In some embodiments, the efficacy of a guide RNA is determined by measuring levels of total kallikrein (prekallikrein and plasma kallikrein (pKal)) in a sample. In some embodiments, the efficacy of a guide RNA is determined by measuring levels of total kallikrein in a sample such as a body fluid, *e.g.*, serum, plasma, or blood. In some embodiments, the efficacy of a guide RNA is determined by measuring levels of total kallikrein in the serum or plasma. A decrease in the levels of total kallikrein in the serum or plasma is indicative of an effective guide RNA. In some embodiments, serum and/or plasma

total kallikrein is decreased below 40% of basal levels. In some embodiments, levels of total kallikrein are measured using an enzyme-linked immunosorbent assay (ELISA) assay with cell culture media or serum or plasma. In some embodiments, levels of total kallikrein are measured in the same *in vitro* or *in vivo* systems or models used to measure editing. In some embodiments, levels of total kallikrein are measured in cells, e.g., primary human hepatocytes. In some embodiments, levels of total kallikrein are measured in PHH and PCH cells.

[00535] In some embodiments, the efficacy of a guide RNA is determined by measuring levels of prekallikrein and/or kallikrein in a sample such as a body fluid, *e.g.*, serum, plasma, or blood. In some embodiments, the efficacy of a guide RNA is determined by measuring levels of prekallikrein and/or kallikrein in the serum or plasma. A decrease in the levels of prekallikrein and/or kallikrein in the serum or plasma is indicative of an effective guide RNA. In some embodiments, levels of prekallikrein and/or kallikrein are measured using an enzyme-linked immunosorbent assay (ELISA) assay with cell culture media or serum or plasma. In some embodiments, levels of prekallikrein and/or kallikrein are measured in the *in vitro* or *in vivo* systems or models used to measure editing. In some embodiments, levels of prekallikrein and/or kallikrein are measured in cells, *e.g.*, primary human hepatocytes, in plasma, or in cell culture media. In some embodiments, levels of prekallikrein and/or kallikrein are measured from a plasma sample. In some embodiments, levels of prekallikrein and/or kallikrein are measured from a serum sample. Prekallikrein and/or pKal protein levels are optionally measured by ELISA after an activation step to convert prekallikrein to its active form, pKal.

[00536] In some embodiments, the efficacy of a guide RNA is determined by measuring levels of prekallikrein in a sample. In some embodiments, the efficacy of a guide RNA is determined by measuring levels of prekallikrein in a sample such as a body fluid, *e.g.*, serum, plasma, or blood. In some embodiments, the efficacy of a guide RNA is determined by measuring levels of prekallikrein in the serum or plasma. A decrease in the levels of prekallikrein in the serum or plasma is indicative of an effective guide RNA. In some embodiments, serum and/or plasma prekallikrein is reduced at least 60%, 70%, 80%, 85%, 90%, 95% or more. In some embodiments, serum and/or plasma total kallikrein, prekallikrein and/or kallikrein is decreased by about 60-80%, 60-90%, 60-95%, 60-100%, 85-95%, or 85-100%. In some embodiments, levels of prekallikrein are measured using an enzyme-linked immunosorbent assay (ELISA) assay with cell culture media or serum or plasma. In some embodiments, levels of prekallikrein are measured in the *in vitro* or *in vivo*

systems or models used to measure editing. In some embodiments, levels of prekallikrein are measured in cells, *e.g.*, primary human hepatocytes, in plasma, or in cell culture media. In some embodiments, levels of prekallikrein are measured from a plasma sample. In some embodiments, levels of prekallikrein are measured from a serum sample.

[00537] In some embodiments, the efficacy of a guide RNA is determined by measuring levels of pKal in a sample. In some embodiments, the efficacy of a guide RNA is determined by measuring levels of pKal in the serum or plasma. A decrease in the level of pKal in the serum or plasma is indicative of an effective guide RNA. In some embodiments, level of pKal is reduced at least 60%, 70%, 80%, 85%, 90%, 95% or more. In some embodiments, serum and/or plasma pKal is decreased by about 60-80%, 60-90%, 60-95%, 60-100%, 85-95%, or 85-100%. In some embodiments, levels of pKal are measured using an enzyme-linked immunosorbent assay (ELISA) assay with cell culture media or serum or plasma. In some embodiments, levels of pKal are measured in the *in vitro* or *in vivo* systems or models used to measure editing. In some embodiments, levels of pKal are measured in cells, *e.g.*, primary human hepatocytes, in plasma, or in cell culture media. In some embodiments, levels of pKal are measured from a plasma sample. In some embodiments, levels of pKal are measured from a serum sample.

[00538] In some embodiments, the efficacy of a guide RNA is determined by measuring levels of circulating cleaved HMWK (cHMWK) and total HMWK in citrated serum or citrated plasma. In some embodiments, the efficacy of a guide RNA is determined by measuring levels of circulating cleaved HMWK (cHMWK) and total HMWK in the serum or plasma. A decrease in the proportion of cleaved HMWK compared to total HMWK is indicative of an effective guide RNA. In some embodiments, the proportion of cleaved HMWK compared to total HMWK can target a ratio of circulating plasma cHMWK to total HMWK of less than about 60%. In some embodiments the ratio of cHMWK to HMWK is less than about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, or more. In some embodiments, levels of prekallikrein are measured using western Blotting assay with cell culture media or serum or plasma. In some embodiments, levels of cHMWK and total HMWK are measured in the *in vitro* or *in vivo* systems or models used to measure editing. In some embodiments, levels of cHMWK and total HMWK are measured in cells, *e.g.*, primary human hepatocytes, in plasma, or in cell culture media. In some embodiments, levels of cHMWK and total HMWK are measured from a plasma sample. In some embodiments, levels of cHMWK and total HMWK are measured from a serum sample.

[00539] In some embodiments, the efficacy of a guide RNA is determined by measuring pKal activity in a sample. A decrease in the pKal activity is indicative of an effective guide RNA. In some embodiments, the efficacy of a guide RNA is determined by measuring pKal activity in the serum or plasma.

[00540] In some embodiments, the pKal activity is measured as the capacity of a citrated serum sample or citrated plasma sample to convert HMWK to cHMWK (See Banerji et al., N Engl J Med 2017;376:717-28.). A decrease in the final proportion of cHMWK to total HMWK indicates a decrease in pKal activity. The levels of cHMWK and full length HMWK can be measured by western blotting. In other embodiments, pKal activity is measured as the capacity of a citrated serum sample or citrated plasma sample to enzymatically cleave a HWMK-like peptide substrate, in which case a decrease in substrate cleavage indicates a decrease in pKal activity.

[00541] In some embodiments, the pKal activity is reduced by at least 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% or more. In some embodiments, the pKal activity is decreased by about 60-80%, 60-90%, 60-95%, 60-100%, 85-95%, or 85-100%. In some embodiments, pKal activity is reduced to less than about 40% of basal levels. In some embodiments, pKal activity is reduced to about 40-50% of basal levels. In some embodiments, pKal activity is reduced to 20-40 or 20-50% of basal levels. In some embodiments, levels of pKal activity are measured in the *in vitro* or *in vivo* systems or models used to measure editing. In some embodiments, levels of pKal activity are measured in cells, e.g., primary human hepatocytes, in plasma, or in cell culture media. In some embodiments, levels of pKal activity are measured from a plasma sample. In some embodiments, levels of pKal are measured from a serum sample.

III. Therapeutic Methods

[00542] The gRNAs and associated methods and compositions disclosed herein are useful in treating and preventing HAE and preventing symptoms of HAE. In some embodiments, the gRNAs and associated methods and compositions are useful for reducing the frequency of HAE attacks. In some embodiments, the gRNAs and associated methods and compositions are useful for preventing HAE attacks. In some embodiments, the gRNAs disclosed herein are useful in treating and preventing bradykinin production and accumulation, bradykinin-induced swelling, angioedema obstruction of the airway, or asphyxiation. In some embodiments, the gRNAs disclosed herein are useful in treating or preventing angioedema and attacks caused by HAE. In some embodiments, the gRNAs

disclosed herein are useful for reducing the frequency of angioedema attacks, such as HAE attacks. In some embodiments, the gRNAs disclosed herein are useful for reducing the severity of angioedema attacks. In some embodiments, the gRNAs disclosed herein are useful for reducing the frequency and/or severity of attacks, such as HAE attacks. In some embodiments, the gRNAs disclosed herein are useful for achieving remission of angioedema attacks, such as HAE attacks. In some embodiments, the gRNAs disclosed herein are useful for achieving durable remission, e.g. maintained for at least 1 month, 2 months, 4 months, 6 months, 1 year, 2 years, 5 years, 10 years or more.

[00543] The gRNAs and associated methods and compositions disclosed herein are useful to decrease KLKB1 mRNA production. Therefore, in one aspect, effectiveness of treatment/prevention can be assessed by measuring KLKB1 mRNA levels, wherein a decrease in KLKB1 mRNA levels indicates effectiveness.

[00544] The gRNAs and associated methods and compositions disclosed herein are useful to decrease prekallikrein protein levels in plasma or serum. Therefore, in one aspect, effectiveness of treatment/prevention can be assessed by measuring prekallikrein protein levels or total kallikrein protein levels, wherein a decrease in prekallikrein and/or kallikrein protein indicates effectiveness. In some embodiments, effectiveness of treatment/prevention can be assessed by measuring prekallikrein protein in a sample, such as serum or plasma, wherein a decrease in prekallikrein indicates effectiveness. For example, plasma or serum prekallikrein can be measured by ELISA as described in Ferrone JD, Bhattacharjee G, Revenko AS, et al. IONIS-PKK_{Rx} a Novel Antisense Inhibitor of Prekallikrein and Bradykinin Production. *Nucleic Acid Ther.* 2019;29(2):82–91. Similarly, kallikrein can be measured by ELISA as described herein, and administration of the gRNAs disclosed herein can decrease kallikrein protein levels in plasma or serum.

[00545] The gRNAs and associated methods and compositions disclosed herein are useful to decrease total kallikrein (prekallikrein and pKal) protein levels in plasma or serum. Therefore, in one aspect, effectiveness of treatment/prevention can be assessed by measuring total kallikrein (prekallikrein and pKal) protein levels, wherein a decrease in total kallikrein protein indicates effectiveness. Total kallikrein, prekallikrein, and/or kallikrein may be measured before or after activation to release plasma kallikrein. In some embodiments, effectiveness of treatment/prevention can be assessed by measuring prekallikrein and/or pKal protein in a sample, such as serum or plasma, wherein a decrease in prekallikrein protein indicates effectiveness. In some embodiments, effectiveness of treatment/prevention can be assessed by measuring pKal protein in a sample, such as serum or plasma, wherein a decrease

in pKal protein indicates effectiveness. For example, levels of prekallikrein and pKal protein can be measured by ELISA, for example by using the Prekallikrein and Kallikrein Human ELISA Kit (Abcam, Eugene, OR). Prekallikrein and/or pKal protein levels are optionally measured by ELISA after an activation step to convert prekallikrein to its active form, pKal.

[00546] The gRNAs and associated methods and compositions disclosed herein are useful to decrease the proportion of circulating cleaved HMWK (cHMWK) compared to total HMWK in citrated serum or citrated plasma. Therefore, in one aspect, effectiveness of treatment/prevention can be assessed by measuring total HMWK and cHMWK protein levels, wherein a decrease in the proportion of cleaved HMWK indicates effectiveness. In some embodiments, effectiveness of treatment/prevention can be assessed by measuring total HMWK and cHMWK protein levels in a sample, such as serum or plasma, wherein a decrease in the proportion of cHMWK indicates effectiveness. For example, the proportion of cHMWK compared to total HMWK in citrated serum or citrated plasma samples can be measured by western blotting as described in Suffritti C, Zanichelli A, Maggioni L, Bonanni E, Cugno M, Cicardi M. High-molecular weight kininogen cleavage correlates with disease states in the bradykinin-mediated angioedema due to hereditary C1-inhibitor deficiency. *Clin Exp Allergy* 2014;44:1503-14 and in Banerji A, Busse P, Shennak M, et al. Inhibiting plasma kallikrein for hereditary angioedema prophylaxis. *N Engl J Med* 2017;376:717-28.

[00547] Circulating plasma cHMWK levels below about 30% total HMWK were associated with decreases in HAE attacks in patients treated with lanadelumab (See Banerji, et al, 2017). In this same study, healthy controls had plasma levels of cHMWK around 8.3% total HMWK. In another study, Suffritti and colleagues found cHMWK plasma levels of an average of about 34.8% in normal controls, about 41.4% in HAE patients in remission and about 58.1% in HAE patients during an attack (Suffritti, et al. *Clin Exp Allergy* 2014;44:1503-14). Accordingly, in some embodiments, the gRNAs and associated methods and compositions disclosed herein are useful for reducing circulating cHMWK levels such that a subject exhibits reduced number of HAE attacks. In some embodiments, the gRNAs and associated methods and compositions disclosed herein are useful to reduce a subject's proportion of cHMWK in citrated plasma to below 30%. In some embodiments, the gRNAs and associated methods and compositions disclosed herein are useful to reduce a subject's proportion of cHMWK in citrated plasma to below 30%, 20%, and/or 10%. In some embodiments, the gRNAs and associated methods and compositions disclosed herein are useful to reduce a subject's proportion of cHMWK in citrated plasma to about those of healthy controls.

[00548] The gRNAs and associated methods and compositions disclosed herein can be useful to decrease the spontaneous pKal activity in serum or plasma. Therefore, in one aspect, effectiveness of treatment/prevention can be assessed by measuring spontaneous pKal activity, wherein a decrease in spontaneous pKal activity indicates effectiveness. In some embodiments, effectiveness of treatment/prevention can be assessed by measuring spontaneous pKal activity in a sample, such as serum or plasma, wherein a decrease in spontaneous pKal activity indicates effectiveness. In certain embodiments, the gRNAs and associated methods and compositions disclosed herein are useful to decrease the basal level of circulating pKal and circulating pKal activity.

[00549] The gRNAs and associated methods and compositions disclosed herein can be useful to decrease the inducible pKal activity in serum or plasma. Therefore, in one aspect, effectiveness of treatment/prevention can be assessed by measuring inducible pKal activity, wherein a decrease in inducible pKal activity indicates effectiveness. In some embodiments, effectiveness of treatment/prevention can be assessed by measuring inducible pKal activity in a sample, such as serum or plasma, wherein a decrease in inducible pKal activity indicates effectiveness. In some examples, pKal activity can be induced by exposure of a sample to FXIIa (See Banerji et al., *N Engl J Med* 2017;376:717-28.) In some examples, pKal activity can be induced by incubation of a sample with dextran sulfate (See Ferrone, et al. *Nucleic Acid Ther.* 2019;29(2):82-91.) In some examples pKal activity can be induced by addition of ellagic acid to a sample (Aygören-Pürsün, et al. *J Allergy Clin Immunol* 2016; 138: 934-936.)

[00550] In some examples, pKal activity is measured as the capacity of a citrated serum sample or citrated plasma sample to convert HMWK to cHMWK (See Banerji et al., *N Engl J Med* 2017;376:717-28.) wherein a decrease in the final proportion of cHMWK to total HMWK indicates a decrease in pKal activity. The proportion of cHMWK and full length HMWK can be measured by Western blotting, for instance as described by Suffritti, et al. *Clin Exp Allergy* 2014;44:1503-14. In other examples, pKal activity is measured as the capacity of a citrated serum sample or citrated plasma sample to enzymatically cleave a HMWK-like peptide substrate, in which case a decrease in substrate cleavage indicates a decrease in pKal activity. In one example, the substrate peptide can be the chromogenic substrate H-D-Pro-Phe-Arg- p-nitroanilide peptide (Bachem, Cat. L-2120) and cleavage can be measured as change in A405 (See Defendi et al, *PLoS One* 2013;8:e70140). In another example the substrate peptide can be the fluorogenic substrate H-Pro-Phe-Arg-AMC (Sigma, Cat No. P9273) and cleavage can be measured as fluorescence changes as excitation and

emission wavelengths at 360 and 480 nm, respectively (See Banerji, et al., *N Engl J Med* 2017;376:717-28).

[00551] In one study, reduction of induced pKal activity by more than 40% was associated with a reduction in HAE attacks (Banerji, et al., *N Engl J Med* 2017;376:717-28). Reduction of induced pKal activity by at least 50% was associated with a reduction in HAE attacks with treatment by BCX7353 (Aygören-Pürsün, et al., *N Engl J Med* 2018; 379:352-362). Reductions of induced pKal activity by 60% have been associated with reduction in attacks in treatment with lanadelumab (Banerji, et al., *N Engl J Med* 2017;376:717-28). Accordingly, in some embodiments, administration of the gRNAs and compositions disclosed herein are useful for reducing kallikrein activity, e.g. total kallikrein, prekallikrein, and/or pKal activity) such that a subject exhibits fewer HAE attacks.

[00552] In some embodiments, administration of the gRNAs and compositions disclosed herein reduces a subject's pKal activity to less than about 40% of basal levels. In some embodiments, administration of the gRNAs and compositions disclosed herein reduces a subject's pKal activity to about 40-50% of basal levels. In some embodiments, administration of the gRNAs and compositions disclosed herein reduces a subject's pKal activity to 20-40% or 20-50% of basal levels.

[00553] In some embodiments, any one or more of the gRNAs, compositions, or pharmaceutical formulations described herein is for use in preparing a medicament for treating or preventing a disease or disorder in a subject. In some embodiments, treatment and/or prevention is accomplished with a single dose, e.g., one-time treatment, of medicament/composition. In some embodiments, the disease or disorder is HAE.

[00554] In some embodiments, the invention comprises a method of treating or preventing a disease or disorder in subject comprising administering any one or more of the gRNAs, compositions, or pharmaceutical formulations described herein. In some embodiments, the disease or disorder is HAE. In some embodiments, the gRNAs, compositions, or pharmaceutical formulations described herein are administered as a single dose, e.g., at one time. In some embodiments, the single dose achieves durable treatment and/or prevention. In some embodiments, the method achieves durable treatment and/or prevention. Durable treatment and/or prevention, as used herein, includes treatment and/or prevention that extends at least i) 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks; ii) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 30, or 36 months; or iii) 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years. In some embodiments, a single dose of the gRNAs, compositions, or pharmaceutical

formulations described herein is sufficient to treat and/or prevent any of the indications described herein for the duration of the subject's life.

[00555] In some embodiments, the invention comprises a method or use of modifying (e.g., creating a double strand break) a target DNA comprising, administering or delivering any one or more of the gRNAs, compositions, or pharmaceutical formulations described herein. In some embodiments, the target DNA is the *KLKB1* gene. In some embodiments, the target DNA is in an exon of the *KLKB1* gene. In some embodiments, the target DNA is in exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 of the *KLKB1* gene.

[00556] In some embodiments, the invention comprises a method or use for modulation of a target gene comprising, administering or delivering any one or more of the gRNAs, compositions, or pharmaceutical formulations described herein. In some embodiments, the modulation is editing of the *KLKB1* target gene. In some embodiments, the modulation is a change in expression of the protein encoded by the *KLKB1* target gene.

[00557] In some embodiments, the method or use results in gene editing. In some embodiments, the method or use results in a double-stranded break within the target *KLKB1* gene. In some embodiments, the method or use results in formation of indel mutations during non-homologous end joining of the DSB. In some embodiments, the method or use results in an insertion or deletion of nucleotides in a target *KLKB1* gene. In some embodiments, the insertion or deletion of nucleotides in a target *KLKB1* gene leads to a frameshift mutation or premature stop codon that results in a non-functional protein. In some embodiments, the insertion or deletion of nucleotides in a target *KLKB1* gene leads to a knockdown or elimination of target gene expression.

[00558] In some embodiments, the method or use results in *KLKB1* gene modulation. In some embodiments, the *KLKB1* gene modulation is a decrease in gene expression. In some embodiments, the method or use results in decreased expression of the protein encoded by the target gene in a population of cells or *in vivo*.

[00559] In some embodiments, a method of inducing a double-stranded break (DSB) within the *KLKB1* gene is provided comprising administering a composition comprising a guide RNA comprising any one or more guide sequences of SEQ ID NOs: 1-149. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 1-149 are administered to induce a DSB in the *KLKB1* gene. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[00560] In some embodiments, a method of modifying the *KLKB1* gene is provided comprising administering a composition comprising a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 1-149. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 1-149 are administered to modify the *KLKB1* gene. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[00561] In some embodiments, a method of treating or preventing hereditary angioedema (HAE) is provided comprising administering a composition comprising a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 1-149. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 1-149 are administered to treat or prevent HAE. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[00562] In some embodiments, a method of decreasing or eliminating bradykinin production and accumulation is provided comprising administering a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 1-149. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[00563] In some embodiments, a method of treating or preventing bradykinin-induced swelling is provided comprising administering a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 1-149. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[00564] In some embodiments, a method of treating or preventing bradykinin-induced angioedema is provided comprising administering a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 1-149. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[00565] In some embodiments, a method of treating or preventing obstruction of the airway and/or asphyxiation is provided comprising administering a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 1-149. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

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

[00566] In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 1-149 are administered to reduce bradykinin levels in the plasma, serum, or blood. The gRNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[00567] In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 1-149 are administered to decrease bradykinin in the serum or plasma. The gRNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[00568] In some embodiments, the gRNAs comprising the guide sequences of Table 1 together with an RNA-guided DNA nuclease such as a Cas nuclease induce DSBs, and non-homologous ending joining (NHEJ) during repair leads to a mutation in the *KLKB1* gene. In some embodiments, NHEJ leads to a deletion or insertion of a nucleotide(s), which induces a frame shift or nonsense mutation in the *KLKB1* gene.

[00569] In some embodiments, administering the guide RNAs of the invention (e.g., in a composition provided herein) decrease levels (e.g., serum or plasma levels) of total kallikrein, prekallikrein, and/or kallikrein in the subject, and therefore prevents bradykinin overproduction and accumulation. In some embodiments, administering the guide RNAs of the invention (e.g., in a composition provided herein) decrease kallikrein activity levels (e.g., serum or plasma levels) in the subject, and therefore prevents bradykinin overproduction and accumulation.

[00570] In some embodiments, the methods provided herein result in fewer attacks that include fluid leakage through blood cells to tissues. In some embodiments, the methods provided herein reduce the frequency of attacks that increase swelling in organ tissues. In some embodiments, administering the guide RNAs of the invention (e.g., in a composition provided herein) decrease the frequency or severity of angioedema attacks.

[00571] In some embodiments, the subject is mammalian. In some embodiments, the subject is a primate, e.g. human.

[00572] In some embodiments, the use of a guide RNAs comprising any one or more of the guide sequences in Table 1 or Table 2 (e.g., in a composition provided herein) is provided for the preparation of a medicament for treating a human subject having HAE.

[00573] In some embodiments, the guide RNAs, compositions, and formulations are administered intravenously. In some embodiments, the guide RNAs, compositions, and formulations are administered by infusion. In some embodiments, the guide RNAs, compositions, and formulations are administered into the hepatic circulation.

[00574] In some embodiments, a single administration of a composition comprising a guide RNA provided herein is sufficient to knock down expression of the protein. In other embodiments, more than one administration of a composition comprising a guide RNA provided herein may be beneficial to maximize therapeutic effects.

[00575] In some embodiments, treatment slows or halts HAE disease progression.

[00576] In some embodiments, treatment slows or halts progression of angioedema. In some embodiments, treatment results in improvement, stabilization, or slowing of change in symptoms of HAE.

A. Delivery of gRNA Compositions

[00577] Lipid nanoparticles (LNPs) are a well-known means for delivery of nucleotide and protein cargo and may be used for delivery of the guide RNAs, compositions, or pharmaceutical formulations disclosed herein. In some embodiments, the LNPs deliver nucleic acid, protein, or nucleic acid together with protein.

[00578] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein to a subject, wherein the gRNA is associated with an LNP. In some embodiments, the gRNA/LNP is also associated with a Cas9 or an mRNA encoding Cas9.

[00579] In some embodiments, the invention comprises a composition comprising any one of the gRNAs disclosed and an LNP. In some embodiments, the composition further comprises a Cas9 or an mRNA encoding Cas9.

[00580] In some embodiments, the LNPs comprise cationic lipids. In some embodiments, the LNPs comprise (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) or another ionizable lipid. See, e.g., lipids of WO/2017/173054 and references described therein. In some embodiments, the LNPs comprise molar ratios of a cationic lipid amine to RNA phosphate (N:P) of about 4.5, 5.0, 5.5, 6.0, or 6.5. In some embodiments, the term cationic and ionizable in the context of LNP lipids is interchangeable, e.g., wherein ionizable lipids are cationic depending on the pH.

[00581] In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in preparing a medicament for treating a disease or disorder.

[00582] Electroporation is a well-known means for delivery of cargo, and any electroporation methodology may be used for delivery of any one of the gRNAs disclosed herein. In some embodiments, electroporation may be used to deliver any one of the gRNAs disclosed herein and Cas9 or an mRNA encoding Cas9.

[00583] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein to an *ex vivo* cell, wherein the gRNA is associated with an LNP or not associated with an LNP. In some embodiments, the gRNA/LNP or gRNA is also associated with a Cas9 or an mRNA encoding Cas9.

[00584] In some embodiments, the guide RNA compositions described herein, alone or encoded on one or more vectors, are formulated in or administered via a lipid nanoparticle; see e.g., WO/2017/173054 and WO 2019/067992, the contents of which are hereby incorporated by reference in their entirety.

[00585] In certain embodiments, the invention comprises DNA or RNA vectors encoding any of the guide RNAs comprising any one or more of the guide sequences described herein. In some embodiments, in addition to guide RNA sequences, the vectors further comprise nucleic acids that do not encode guide RNAs. Nucleic acids that do not encode guide RNA include, but are not limited to, promoters, enhancers, regulatory sequences, and nucleic acids encoding an RNA-guided DNA nuclease, which can be a nuclease such as Cas9. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, or a crRNA and trRNA. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a sgRNA and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas nuclease, such as Cas9 or Cpf1. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas protein, such as, Cas9. In one embodiment, the Cas9 is from *Streptococcus pyogenes* (i.e., Spy Cas9). In some embodiments, the nucleotide sequence encoding the crRNA, trRNA, or crRNA and trRNA (which may be a sgRNA) comprises or consists of a guide sequence flanked by all or a portion of a repeat sequence from a naturally-occurring CRISPR/Cas system. The nucleic acid comprising or consisting of the crRNA, trRNA, or crRNA and trRNA may further comprise a vector sequence wherein the vector sequence comprises or

consists of nucleic acids that are not naturally found together with the crRNA, trRNA, or crRNA and trRNA.

[00586] This description and exemplary embodiments should not be taken as limiting. For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities, percentages, or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about,” to the extent they are not already so modified. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[00587] It is noted that, as used in this specification and the appended claims, the singular forms “a,” “an,” and “the,” and any singular use of any word, include plural referents unless expressly and unequivocally limited to one referent. As used herein, the term “include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

EXAMPLES

[00588] 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

[00589] Capped and polyadenylated *Streptococcus pyogenes* (“Spy”) Cas9 mRNA containing N1-methyl pseudo-U was generated by *in vitro* transcription using a linearized plasmid DNA template and T7 RNA polymerase. Plasmid DNA containing a T7 promoter and a sequence for transcription (for producing mRNA comprising an mRNA described herein (see SEQ ID NOs: 501-516 in Table 5 below for Cas9 ORFs) was linearized by incubating at 37°C to complete digestion with XbaI with the following conditions: 200 ng/μL plasmid, 2 U/μL XbaI (NEB), and 1x reaction buffer. The XbaI was inactivated by heating the reaction at 65°C for 20 min. The linearized plasmid was purified from enzyme and buffer

salts using a silica maxi spin column (Epoch Life Sciences) and analyzed by agarose gel to confirm linearization. The IVT reaction to generate Cas9 mRNA was incubated at 37°C for 4 hours in the following conditions: 50 ng/μL linearized plasmid; 2 mM each of GTP, ATP, CTP, and N1-methyl pseudo-UTP (Trilink); 10 mM ARCA (Trilink); 5 U/μL T7 RNA polymerase (NEB); 1 U/μL Murine RNase inhibitor (NEB); 0.004 U/μL Inorganic *E. coli* pyrophosphatase (NEB); and 1x reaction buffer. After the 4-hour incubation, TURBO DNase (ThermoFisher) was added to a final concentration of 0.01 U/μL, and the reaction was incubated for an additional 30 minutes to remove the DNA template. The Cas9 mRNA was purified from enzyme and nucleotides using a MegaClear Transcription Clean-up kit according to the manufacturer's protocol (ThermoFisher). Alternatively, the Cas9 mRNA was purified with a LiCl precipitation method, which in some cases was followed by further purification by tangential flow filtration. The transcript concentration was determined by measuring the light absorbance at 260 nm (Nanodrop), and the transcript was analyzed by capillary electrophoresis by Bioanalyzer (Agilent).

[00590] Table 5: Exemplary Cas9 mRNA Sequences

SEQ ID NO	Sequence
501	GGGTCCCGCAGTGGCGTCCAGCGGCTCTGCTTGTGTTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGAC AAGAAAGTACAGCATCGAGCTGGACATCGGAACAAACAGCTCGGATCGGACGTATCACAGACGAATACAAAGTCCCGAG CAAGAAAGTTCAAGGTCCTGGGAAACACAGACAGACACACATCAAGAAAGCTGATCGGAGCACTGCTGTTCTCGACAGCG GAGAAACAGCAGCAAGCAAGACTGAAGAGAACAGCAAGAAAGATACACAGAAAGAAAGAACAGAACTCTGCTACCT GCAGGAAATCTTCAGCAACGAAATGGCAAGGTGACGACACGCTTCTCCACAGACTGGAAAGAAAGCTTCTGCTCGAAG AAGACAAGAGCAGCAAGACACCCGATCTTCGGAAACATCTCGACGAAAGTGCATACCCAGAAAGTACCCGACAATC TACCACCTGAGAAAGAAAGCTGGTCGACAGCAGACAGCAAGGACAGCTTGAGACTGATCTACCTGGCACTGGCACACATGAT CAAGTTTCAGAGGACATTCCTGATCGAAGGAGACCTGAAACCCGGACACACGACGTCGACAAAGCTGTTTCATCCAGCTGG TCCAGACATACAAACAGCTGTTTCGAAAGAAACCCGATCAACGCAAGCGGAGTGCACGCAAGGCAATCCTGAGCGCAAGA CTGAGCAAGAGCAGAGACTGGAAACCTGATCGCACAGCTGCCGGAGAAAGAAAGACGACTGTTCCGGAAACCTTGA TCGCACTGAGCTGGGACTGACACCGAACTTCAAGAGCACTTGACCTGGCAGGAGCAAGCAGCTGCAGCTGAGCAAG GACACATACGACGACGACCTGGACAACTTCTGGCACAGATCGGAGACCAAGTACGACAGACCTGTTCTGGCAGCAAGAA CCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCAAAAGGCCACCGCTGAGCGCAAGCATGA TCAAGAGATACGACGAACACCCAGGACCTGACACTGCTGAAGGCACTGTGTCAGACAGCAGCTGCCGAAAGTACAAAG GAAATCTTTCGACCAAGCAAGAACGGATACGCAAGGATACATCGACGAGGAGCAAGCAGGAAAGAAATTTACAAAGTT CATCAAGCCGATCTTGGAAGAGATGGACGGAAACAGAAAGAACTGCTGGTCAAGCTGAACAGAGAAAGACCTGCTGAGAAAG CAGAGAACATTCGACAACGGAAAGCATCCCGCACAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAAGACAGGAAG ACTTCTACCCGTTCTGAAAGGACAAACAGAGAAAGATCGAAAGATCCTGACATTCAGAAATCCCGTACTACGTCGGACCCGC TGGCAAGAGGAAACAGCAGATTCGATGGATGACAAAGAAAGAGCGGAGAAACAAATCACACCTGGAACTTCGAAAGAAAGT CGTCGACAAAGGAGCAAGCGCAGAGCTTCATCGAAAGAAATGACAACTTCGACAGAAACCTGCCGAAACGAAAGGTTCC TGCCGAAAGCAGCAGCTGCTGTACGAATCTTCACAGTCTAACACGAACTGACAAAGGTCAAAGTACGTACAGAGGAATG AGAAAGCCGCAATTCCTGAGCGGAGAACAGAAAGAGCAATCTGTCGACCTGCTGTTCAGACAAACAGAAAGGTACACAGT CAAGCAGCTGAAGGAAGACTACTTCAAGAAAGATCGAATGCTTCGACAGCTCGAAATCAGCGGAGTCAAGACAGATTCA ACGCAAGCTTGGGAAACATACCGACCTGCTGAAGATCATCAAGGACAAAGGACTTCTGGACAAACGAAAGAAACCGAAAGAC ATCCTGGAAAGACATCGTCTGACACTGACACTGTTTCGAAAGACAGAGAAATGATCGAAAGAAAGTGAAGACATACGCACA CCTGTTTCGACGACAAGGTATGAAGCAGCTGAAGAGAAAGATACACAGGATGGGAAAGACTGAGCAGAAAGCTGATC AACGGAATCAGAGACAAAGCAGCGGAAAGACAATCCTGGACTTCTGAAGAGCAGCAGGATTCGCAACAGCAAACTTCAT GCAAGTATCCAGACGACAGCTGACATTCAAGGAAGACATCCAGAGGACAGGTCAGCGGACAGGAGACAGCCTG CACGAACACATCGCAACCTTGGCAGGAAGCCCGGCAATCAAGAAAGGAAATCCTGCAGACAGTCAAGGTCGTCGACGAACT GGTCAAGGTCTATGGGAAGACACAAAGCGGAAACATCGTATCGAAATGGCAAGAGAAACACAGACACAGAAAGGA CAGAAAGAACAGCAGAGAAAGAAATGAAGAGAAATCGAAAGGAAATCAAGGAACTGGGAAAGCCAGATCCTGAAAGGAACAC CCGGTCGAAACACACAGCTGCAGAAAGGAAAGCTGTACCTGTACTACCTGCAGAACGGAAAGAGACATGTAGTCGACCA

	GGAACTGGACATCAACAGACTGAGCGACTACGACGCTCGACCACATCGTCCCGAGAGCTTCTCTGAAAGGACGACAGCATCG ACAAAGGTCTTGACAAAGAACGACAAAGAACAGAGAAAGAGCGACAACTGTCGAGCGAAGAAAGTCTCAAGAAAGAT GAAGAACTACTGGAGACAGCTGTGAACGCAAGCTGATCACACAGAGAAAGTTTCGACAACTGACAAAGGCAGAGAGA GGAGGACTGAGCGAACTGGACAAAGCGAGGATTCTAAGAGACAGCTGGTGAACAAAGACAGATCACAAGCACAGCTCG CACAGATCTGGACAGCAGAATGAACACAAAGTACGACGAAACGACAACTGATCAGAGAAAGTCAAGGTCTATCACACTG AAGAGCAAGCTGGTCAAGCGACTTCAGAAAGGACTTCAGAGTTCAGAGAAATCAACAACCTACCAACGACGACA CGAGCATACCTGACGCGACTGTCGGAACAGCATGATCAAGAAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAG ACTCAAAGGTCTACGACGCTCAGAAAGATGATCGCAAGAGCGCAACAGGAAATCGGAAAGGCAACAGCAAGTACTTCTTC TACAGCAACATCATGAACCTTCTCAAGACAGAAATCACACTGGCAACCGGAGAAATCAGAAAGAGACCCGCTGATCGAAAC AAACGGAGAACAGGAAATCTGTCTGGACAAAGGAAAGAGACTTCGCAACAGTCAAGAAAGTCTGAGCATGCCGACAG GTCAACATCTGTCAAAGAACAGAGATCCAGACAGGAGGATTCAGCAAGGAAAGCATCTCTCCGAAGAGAAACAGCGACA AGCTGATCGCAAGAAAGAGACTGGGACCCGAAAGATACGGAGGATTTCGACAGCCGACAGCTCGCATACAGCGTCTCTG GTCGTCGCAAGGTCGAAAGGGAAGAGCAAGAAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAAATCATGGAAG GAAGCAGCTTCTGAAAGAACCCGATCGACTTCTGGAAGCAAGGGATACAAAGGAAGTCAAGAAAGGACCTGATCATCAAG CTGCCGAAGTACAGCCTGTTCGAACTGGAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAACTTCGAAAGGGAA ACGAACCTGGCACTGCCGAGCAAGTACGTCAACTTCTGTACTTGGCAAGCCACTACGAAAGAGTGAAGGGAAAGCCCGAA GACAAACGAAACAGAGCAGCTGTCTGTCGAACAGCAACAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAG CAAGAGATCATCTGGCAGACGCAACCTGGACAAGGTCTGAGCGCATACAAACAGCACAGAGACAAGCCGATCAGA GAACAGCGAGAAACATCATCTCCACTGTTCACACTGACAAACCTGGGAGCACCCGCGAGCATTCAAATTCGACACAAC AATCGACAGAAAGAGATACAAAGCACAAAGGAAGTCTTGACGCAACACTGATCCACAGAGCATCACAGGACTGTACG AAACAAAGATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAAGCCCGAAAGAAAGAGAAAGGTCTAGCTAGCCAT CACATTTAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAATGAAGATCAATAGCTTATTCATCTCTTTCTTTT TCGTTGGTGTAAAGCCAACACCCCTGTCTAAAAAACATAAATTTCTTTAATCAATTTTGCCTCTTTTCTCTGTGCTTCAATTAAT AAAAATGGAAAGAACCTCGAG
502	AUGGACAAGAAAGUACAGCAUCGGACUGGACAUCGGAAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAG AGGUCCUGGGAACACAGACAGACACAGCAUCAAGAAAGAACCCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG GAGAACAGCAAGAAAGAUACAAAGAAAGAAAGAACAGAAUUCUGUACCCGAGGAAAUUCUACGCAACGAAAUUGGC UUCACAGACUGGAAAGAAAGUUCUGGUCGAAAGAGACAAAGAACGACAAAGACACCCGAAUUCUGGAAACAUCGUC AAAAGUACCCGACAUCUACCCUGAGAAAGAGUUGGUCAGACAGACAGAAAGGACAGCCUGAGACUGAUCUACCU AAGUUCAGAGGACACUUCUUGAUCGAAAGGAGACCUGAAACCCGGACACAGCGACGUCGACAAGCUGUUCUACUCCAGCUGG GUUCGAAAGAAACCCGAUACGCAAGCGGAGUCGACGCAAGGCAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAG CAGCUGCCGGGAGAAAGAAAGACGAGACUUGUUGGAAACCUUGAUCGACUGAGCCUGGGACUGACACCGAACUUCAAAG AAGACGCAAGACUGCAGCAGCAAGGACACAUACGACAGCAGCCUGGACACCUUGGUGGACAGACUCCGAGACCCAGUA GCAAGAACCUUGAGCGACGCAUCCUGUGAGCGACAUCCUGAGAGUCAACACAGAAUACAAAGGACCCGUGAGCG CGACGAAACACCAACAGGACCUAGACACUGCUGAAGGCAUCUGGUCAGACAGCAGCUGGCCGGAAGAAAGUACAAAGGAAUUCU

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	GAACGCAGUCGUGGAAACAGCACUGAUCAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGU CUACGACGUCAGAAAGAUUAUCGCAAGAGCGGAACAGGAAUUCGAAAGGCAACAGAAAGUACUUCUUCUACAGCAA CAUCAUGAACUUCUACAAGACAGAAUACACUUGGCAACCGGAAUUCAGAAAGAGACCGCUGAUCGAAACAACGG AGAAACAGGAGAAUUCGUGGGACAAAGGAAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUCCCGCAGGUCAA CAUCGUCAGAAGACAGAGUCCAGACAGGAGAUUCAGCAAGGAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCU GAUCGCAAGAAAGAGGACUGGGACCCGAAGUACGGAAGAUUCGACGCCGACAGUCGCAUACAGCGUCCUGGU CGUCGCAAGGUCGAAAGGGAAAGAGCAAGAGUAGAGGUAAGGAACUGUGGGAUACAAUUAUGGAAAG AAGCAGUUCGAAAGAAACCCGUAUCUCCUGGAGCAAGGGAUACAAAGGAUACAAGAGGACCUAUAUCA GUCGCCGAAGUACAGCCUGUUCGACUUGAAACCGAAAGAGAAUUCUGGCAAGCGCAGGAGACUGCAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUAACUUCUGUACCGGCAAGCCACUACGAAAGCUGAAGGGAGCC GGAAACAACGAACAGAGCAGUUGUUCGUAACAGCAAGCAACUACUGGACGAAAUUAUCGAACAUAACGGA AUUCAGCAAGAGAUUCCUGGCAGACGAACCUUGGACAAAGUCCUGAGCGCAUACAACAAGCACAGACAAGCC GAUCAGAACAGGCAAGAAACAUAUCCACCUUUCACACUGACAACCUUGGAGCACCGGCAGCAUUAAGUACUU CGACACAACAUAUCGACAGAAAGAGUAACACAAGCAAAAGGAAGUCCUGGACGCAACACUGAUCCACCAGAGCAUCAC AGGACUGUACGAACAAGAAUUCGACCUUGAGCCAGCUGGGAGGAGAC
504	AUGGACAAGAGUACGCUCCGACUGGACUUGGAAACAACAGCGUCCGGAUGGGCAGUACACAGACGAAUACAAG GUCCCGAGCAAGAGUUAAGGUCCUGGAAACACAGACAGACACAGCAUCAAGAAGAACCUUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAAGAGCAACAGACUGAAGAGAACAGCAAGAGAAUACACAAGAAAGAAACAGA AUCUGCUACCUGCAGGAAAUUCUACGCAACGAAAUUGCAAGAGGUCGACGACAGCUUCUUCACAGACUGGAAAGC UUCUUGGUCGAAAGACAAAGAGCAGCAAGACACCCGUAUUCUGGAAACAUCGUCGACGAAAGUCGCAUCCACGAA AAGUACCCGACAUCUACCCUGAGAAAGAGCUGGUCGACAGACAGCAAGGACAGCCUGAGACUUAUCCUG GCACUGGCACACAUGAUCAAUUCAGAGGACACUUCUUGAUCGAAAGGAGACCUUACCCGACAAACAGCGACGUCGAC AAGCUUUCAUCCAGCUGGUCCAGACAUACAACAGCAGUUCGGAAGAAACCCGAUACAACGCAAGCGAGUCGACGCA AAGGCAUCCUGAGCGCAAGACUGAGCAAGAGAGCAAGAGACUUGGAAACCUUGAUCGCAAGCUGCCGGGAGAAAGAA AACGGACUUGUCCGAAACCUGAUCGCACUGAGCCUGGACUGACACCGAAACUUAAGAGCAACUUCGACCUGGCAGAA GACGCAAGCUGCAGCAGGACACAUACGACGACGACCUUGGACAAACCUUGGACAGAUCCGAGACAGAUCCGAGCCAGUAC GCAGACCUUUCCUGGCAGCAAGAAACCUUGAGCGCAAUCCUGCUGAGCGAUCCUGAGAGUACAACACAGAAAU ACAAGGCACCCUGAGCGCAAGCAUGAUCAGAGAUACGACGAACACCAAGGACCUUGACACUUGGAGGACU GUACAGACAGCUGCCGGAAAGUACAAGGAAAUUCUUCUUCGACAGCAAGCAAGGAGUACCGGAGUACAUCGAC GGAGGACAAAGCCAGGAAAUUCUACAAGUUAUCAAGCCGUAUCCUGGAAAGAUUGGACGGAACAGAAACUCCUG GUCAAGCUGAACAGAGAAACCUUGAGAAAGCAGAGACAUUCGACAACGGAAAGCAUCCGACCAAGUCCACCU GGAGAACUGCAGCAUCCUGAGAGAGAGGAGAGCUUCUACCCGUAUCCUGAGGACAAACAGAAAGAUCCGAAAG AUCCUGACAUUCGAAUCCCGUAUCUACGUCGACCGCUGGCAAGAGGAAACAGCAGAUUCGCAUGGAGACAGAAAG AGCGAAAGAAACAACCGUGGAACUUCGAAAGAGUUCGUCGACAAAGGAGCAAGCGCACAGAGCUUCACGAAAGA AUGACAAACUUCGACAGAAACCUGCCGAAACGAAAGGUGUCCGCCGAAAGCAGCCUGCUGUACGAAUACUUCACAGUC

<p>UACAAACGAAACUGACAAAAGGUC AAGUACGUACACAGAAAGGAUUGAGAAAAGCCGGCAUUCUUGAGCGGAGAACACAGAAAG GCAAUCGUCGACCUUGUGUUAAGACAAACACAGAAAGGUCACAGUCACAGUAGCGUAGAGGAAGACUACUUAAGAAAGAU GAAUGCUUCGACAGCGUCGAAUUCAGCGGAGUCGAGAGACAGAUUAACGCAAGCCUGGGAACAUACACGACCUUGCUG AAGAUCAUCAAGGACAAAGGACUUCUUGGACAAACGAAAGAAACGAAAGACAUCCUGGAAAGACAUCCUGCCUGACACUGACA CUGUUCGAAGACAGAGAAAUUGAUCGAAAGAAAGACUGAAGACAUACGCACACCUUGUUCGACGACAAAGGUCAUGAAGCAG CUGAAGAGAAAGAUACACAGGAUUGGGAAGACUGAGAGCAGAAAGCGUAUACCGAAUUCAGAGACAAAGCAGAGCGG AAAGACAUCCUGGACUUCUGAAGAGCGACGGUACUUCGCAACAGAAACUUAUGCAGCGUACUACACGACGACAGCCU GACAUUCAAGGAAGACAUCCAGAGGCACAGGUCAGCGGACAGGAGACGCCUGCAACACACUCCGCAACACUCCGCAACCCUGGC AGGAAGCCCGCAAUCAAGAAAGGGAUCCUGCAGACAGUCAAAGGUCGUCGACGAACUGGUCAAGGUCAUGGGAAGACA CAAGCCGGAACAUCGUAUUGGAAAGAGAAAGAAACAGACAACAGAAAGGACACAGAAAGCAGAGAGAAAG AAUGAAGAGAAUCGAAAGAAAGGAUCAAAGGAACUGGGAAGCCAGAUCCUGAAAGAAACACCCGGUAGAAACACACAGCU GCAGAACGAAAGCUUACCUAGUACCUAGACAGAACCGGAAGAGACAUAGUACGUCGACCGAGGAACUUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCAUUCGUCCCGCAGAGCUCCUGAAGGACGACAGCAUCGACACAAAGGUCCUGAC AAGAAAGCAGACAAAGACAGGAAAGAGCGACAAACGUCCCGAGCGAAAGUUCUCAAAGAAAGAAACUACUGGAG ACAGCUGUGAACGCAAGAGUACACAGAGAAAGUUCGACAAACUUGACAAAGGCAAGAGGAGAGAGAGGAGGAGCAG ACUGGACAAGGCAGGAUUAUCAAGAGACAGCUGGUCGAAACAAAGACAGAUCAACAGACUCCUGGCAAGAGUCCUGGA CAGCAGAAUGAACACAAAGUACGACGAAACGACAAAGCUGAUCAGAGAAAGUCAAAGUACACACUAGAGAAAGCAAGCU GGUCAGCGACUUCAGAAAGGACUUCGACUACAAAGGUCAGAGAAAUCAAACUACCCACACGACACGACGCAUA CCUGAACGACGUCGGAACAGCACUGAUCAGAAAGUACCCGAAAGCUGGAAAGCGAAUUCGUUACGGAGACUACAA GGUUAACGACGUCAGAAAGAUUAUCGCAAGAGCGCAACAGGAAUUCGGAAAGGCAACAGCAAAGUACUUCUACAG CAACAUCAUGAACUUCUCAAAGACAGAAUACACUGGCAACCGGAGAAUUCAGAAAGAGCCGUGAUCGAAACAA CGGAGAAACAGGAGAAUUCGUCUGGACAAAGGAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUCCGCGCAGGU CAACUUCGUAAGAGACAGAAAGUCCAGACAGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAGAGAAACAGCGACAA GCUUACGCAAGAAAGAGGACUGGGACCCGAGAAAGUACGGAGUUCGACAGCCGACAGUCGCAUACAGCGUCCU GGUCGUCGCAAGGUCGAAAGGGAAGAGCAAGAGCUGAAAGAGCGUCAAAGGAACUGCUGGGAUUCACAAUUAUGGA AAGAAAGCAGUUCGAAAGAACCCGACUUCGACUUCUUGGAAAGCAAGGGAUCAAAGGAAAGUCAAAGGACCCUGAUCAU CAAAGCUGCCGAAAGUACAGCCUUGUUCGAAACUGGAAACCGGAAGAAAGAAUUCUGGCAAGCGCAGGAGAACUGCAGAA GGGAAACGAAACUGGACUUGCCGAGCAAGUACGUCAAACUUCUUGUACCUUGGCAAGCCACUACGAAAGCUGAAGGGAAG CCCGGAAGACAAACGAAAGCAGCUUUCGUGAACAGCAGCAACAGCUCUACCUUGGACGAAUUCAGCAAGAUACAG CGAAUUCAGCAAGAGAGUACUCCUGGACAGCAACACCUUGGACAGGUCCUGAGCGCAUACAAACAGCACAGAGACAA GCCGUAUCAGAGAACAGGCAAGAAACAUUCCACCUUACACAGACAAACUUGGGAGCACCCGCAUUAAGUA CUUCGACACAAACAUUCGACAGAAAGAGAUACACAGCACAAGGAUUCUUGGACGCAACACUAGUCCACGAGCAU CACAGGACUGUACGAAACAAAGAAUCGACCUAGGCCAGCUGGGGAGAGACGGAAGCGGAAAGCCCGAAGAAAGAGAAA GGUCGACGGAAGCCCGAAGAAAGAGAGAAAGGUCGACAGCGGAUAG</p>	
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505	GACAAAGAAUACAGCAUCCGGACUCCGGACAUCCGGAACAAACAGCGUCCGGAUUGGGCAGUCAUACACAGACGAAUACAAAGGUC CCGAGCAAGAAAGUUCAAAGGUCCUGGGAACACACAGACAGACACAGCAUCAAAGAAACCUAGAUCCGGACACUCUGUUC GACAGCGGAGAAACAGCAGAAAGCAACAGACUAGAGAGAAACAGCAAGAAAGAAUACACAAAGAAAGAAACAGAAUUC UGCACCUCCAGGAAUUCUACGCAACGAAUUGGCAAGGUCGACGACGCUUCUCCACAGACUGGAAAGAAAGCUUC CUGGUCGAAAGACAAAGACACGAAAGACACCCGAUUCUGGAAACAUUCGUCGACGAAUGCGCAUCCACGAAAG UACCCGACAUUCUACCCACCUAGAGAAAGAGGUGGUGGACGACACAGACAAAGCAGACCUGAGACUGAUCCACCUUGGCA CUGGCACAUUAUUAAGUUCAGAGGACAUUCUUGAUCCGAGGAGACCUAGACCCGGAACACAGCGACUCCGACAAAG CUGUUAUCCAGCUGGUCCAGCAUACACACGUGUUGAAAGAAACCCGAUACACGCAAGCGGAGUCGACGCAAG GCAUCCUGAGCGCAAGAGCAAGAGCAAGACUGGAAACCUAGUCCGACAGCUGCCGGGAGAAAGAAAGAAC GGACUUGUCGGAACCUGAUCGACUGAGCCUGGGACUGACACCGAACUUC AAGAGCAACUUCGACCUGGCAAGAC GCAAGCUGCAGCAGCAAGGACACAUACGACGACGACCCUGGACACCUUGGCAACAGAUCCGAGACCAAGUACGCA GACCUUUCUGGACGCAAGAACCUAGCGACGCAUCCUGCUGAGCGCAUCCUGAGAGUACACACAGAAAUACACA AAGCACCCGUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCAAGACCUAGAGCAGCUUUAAGGCAACUGGUC AGACAGCAGCUGCCGGAAGAAUACAAAGGAAAUUCUUCGACCAGAGCAAGAAACGGAUACGCAAGAUACAUCCGACGGA GGAGCAAGCCAGGAAAGAUUCACAGUUCUACAGCCGAUCCUGGAAGAAAGAUUGGACGGAACAGAAAGAUCCUGGUC AAGCUGAACAGAGAACCUUGCCGAACGAAAGGUCUCCGCGAAGCAGACGCUUGUACGAAUACUUCACAGUCUAC AACGAAUCGACAAAGGUCAAAGUACGUCACAGAAAGAAUAGAAAGCCCGCAUUCUGAGCGGAGAACAGAAAGGCA AUCGUCGACCUUGUUCAAAGACAAACAGAAAGGUCACAGUCAAGCAGUGAAGGAAAGACUACUUCACAAAGAAUCGAA UGCUCGACAGCUGCGAAAUUCAGCGGAGUCGAAAGACAGAUUUCACAGGAGCAAGCGCAACAGCUUCACUUCGAAAGAU AUCAUACAGGACAGGACUUCUGGACACGAAAGAAACGAAAGACAUCCUGGAGACAUCCUGGAGACACUGACACUG UUCGAAACAGAGAAUUAUGAUCGAAAGAAAGACUAGAGACAUACGACACCUUGUUCGACGACAAAGGUCUAGAACGUCG AAGAGAAAGAUACACAGGAUUGGGAAGACUGAGCAGAAAGCUGAUACGGAUACGGAACAGACAGACGAGCGGAAA GACAAUCCUGGACUUCUAGAGCAGCGGAGUUCGCAACAGAAACUUCUAGCGUAGUCCACGACGACAGCCUGAC AUUCAGGAAGACAUCCAGAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAAACAUCCGCAACCCUGGACG AAGCCCGCAUUCAGAAAGGAAUCCUGCAGACAGUCAAAGGUCGACGAAAGGUGUACAGGUCUAGGGAAGACACAA GCCGAAACAUUCGUAUCGAAUUGGCAAGAGAAACAGACAAACAGAGGACAGAAAGACAGCAGAGAAAGAAU GAAAGAUUCGAGAAAGAAUACAGGAUUGGGAAGCCAGAUUCUAGGAAACACCCGUGGAAACACACACAGCUGCA GAACGAAAGCUGUACCUAGUACUACCUUGCAGAAAGGAAAGAGACUAGUACGUCACCGAGACUCCGACUACAGACU GAGCGACUACGACGUCACCAUCCUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCCGACAAACAGGUCUAGACAA AAGCGACAAAGAACAGAGGAAAGAGCGACAAACGUCGCCGAGGAAAGUUCGUAAGAAAGUAGAAACUACUGGAGACA GCUUCGUAACGCAAGCUGAUACACACAGAGAAAGUUCGACAAACCUGACAAAGGACAGAGAGAGGAGGACUCUGAGGAAACU
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	GGACAAAGGCAGGAUUCAUCAAAGAGACAGCUGGUCGAAACACAGACAGAUACAAAGCACGUCGACAGAUCCUGGACAG CAGAAUAGAACACAAAGUACGACGAAACGACAAAGCUGAUACAGAGAAAGUCAGAGUACAUCACACUGAAGAGCAAGCUGGU CAGCGAUUCAGAAAGGACUUCACAGUUCACAAAGUACAGAGAAUACAACUACACACACGACACGACGCGCAUACCU GAACGAGUCGCGAAACAGCACUGAUCAAGAAUACCCGAAAGCUGGAAAGCGAAUUCGUUACGCGAGACUACAAGGU CUACGACGUCAGAAAGAUCAUCGCAAGAGCGAACAGGAAUUCGGAAGGCAACAGCAAAAGUACUUCUUCUACAGCAA CAUCAUGAACUUCUCAAAGACAGAAUUCACACUAGGAAACGGAACAGAAAGAGACCGCUGAUCCGAAACAAACCGG AGAAACAGGAGAAUUCGUUCGGGACAAAGGAGAGAGAUUCGCAACAGUCAGAAAGGUCCUGAGCAUCCGCGAGUCAA CAUCGUCAAAGAGAGAUUCCAGAGGAGAUUCAGGAAGCAUCCUGCCGAAAGAGAAACAGCAACGCGACAAAGCU GAUCGCAAGAAAGAGACUGGGACCCGAAAGAAUACGGAGGAUUCGACAGCCGACAGUCGCAUACAGCGUCCUGGU CGUCGCAAGGGCGAAAGGAAAGAGCAAGAGCUGAAGAGCGUCAAGGAACUGUGGAAUCACAAUCAUGGAAAG AAGCAGUUCGAAAGAAACCGAUUCGACUUCUGGAAAGCAAGGGAUACAAGGAAGUACAAGAGGACCUAGAUCAA GCUCCGAAAGUACAGCCUUGUUCGAAUCUGGAAACCGGAAGAAAGAGAAUUCUGGCAAGCGCAGGAGAACUCGAAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUCACUUCUGUACCGGCAAGCCACUACGAAAGCUAGAAAGCGAAAGCCC GGAAGACAACGACAGAAAGCAGCUGUUCGCAACAGCACAAAGCACUACCGGACGAAUUCGAAACAGACAGUACAGGA AUUCAGCAAGAGAGAUUCUGGACAGCACAACCGUGGACAAAGGUCCUGAGCGCUACAACAAGACACAGACAAAGCC GAUCAGAGAACAGGCAGAAACAUCUCCACCUUUCACACUGACAACCUUGGAGCACCAGGACGCAUACAAGUACUU CGACACAACAUCGACAGAAAGAGAUACACAAGCAAAAGGAAGUCCUGGACGCAACACUGAUCCACAGAGCAUAC AGGACUGUACGAAACAAGAUUCGACCUAGCCAGCUGGGAGGAGACGGAAGCGGAAAGCCGGAAGAAAGAGAAAGGU CGACGGAAGCCCCGAAAGAAAGAGAAAGGUACAGCGGA
506	GGGTCCCGAGTCCGCGTCCAGCGGCTCTGCTTGTGTCGTGTCGTTGCAGGCTTATTCGGATCCATGGACAAAGAAAG TACAGCATCGGACTGGACATCGGAACAACAGCGTCGGATGGGAGTCATCACAGACGAATACAAGGTCCCAGCAAGAA GTTCAAGGTCTCTGGGAAACACAGACAGACACAGCATCAAGAAAGAACCTGATCGGAGCACTGTCTTCGACAGCGGAGAA CAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAAGATACACAAGAAAGAAAGACAATCTGCTACCTGCAGGA AATCTTCAGCAACGAAATGGCAAGGTCTGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCTGTCGAAAGAGACA AGAAACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAGTACCCGACAATCTACCAC CTGAGAAAGAAAGCTGGTCGACAGCACAGACAAGGACAGCCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTT CAGAGGACACTTCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTTCATCCAGCTGGTCCAGA CATACAACAGCTGTTGGAAGAAACCCGATCAACGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCGCAAGACTGAGC AAGAGCAGAAAGACTGGAAACCTGATCGCACAGCTGCCGGGAGAAAGAAAGACCGGACTGTTCCGGAACCTGATCGCAC TGAGCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAGACGCAAGCTGCAAGTGAAGCAAGGACACA TACGACGACAGCCTGGACACCTGCTGGCACAGATCGGAGACCAGTACGACAGCAAGCTGTTCTTGGCAGCAAGAACTTGAG CGACGCAATCTGCTGAGCGCATCTGAGAGTCAACAGAGAAATCAAAAAGGACCGCTGAGCGCAAGCATGATCAAGA GATACGACGAACACCCAGGACCTGACATGCTGAAGGCACTGGTCAGACGACGCTGCCGGAAGAAAGTACAGAGGAAATC TTCTTCGACCAGAGCAAGAACGGATACGCAAGGATATCGACGAGGAGCAAGCCAGGAAGAAATTCACAAAGTTTCATCAA GCCGATCCTTGAAAGATGGACGGAAACAGAAAGAACTGCTGGTCAAGCTGAACAGAGAAAGAACCTGCTGAGAAAGCAGAGA

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	<p>GAAAAGATACACAAGCACAAAGGAAGTCTCTGGACGCAACACTGATCCACAGAGCATCACAGGACTGTACGAAACAAG AATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCGGAAGAAAGAGAAAGAAAGAGTCTAGCTAGCCATCACATTT AAAAGCATCTCAGCCTACCATGAGAAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTCTTTTCTGTTGG TGTAAGCCCAACACCCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTCTCTGTGCTTCAATTAATAAAAAAT GGAAAGAACCTCGAG</p> <p>ATGGACAAAGAGTACAGCATCGGACTGGACATCGGAACAAAACAGCGTCGGATGGCAGTCTATCACAGACGAATACAAAGGT CCCAGACAAGAGTTCAAGGTCTCTGGGAAACACAGACAGACACAGCATCAAGAAAGAACTCTGATCGGAGCAGCTGCTGTTCTG ACAGCGGAGAAAACAGCAGAACCAAGACTGAAGAGAGACAGCAAGAAAGAAAGATACACAAAGAAAGAAACAGAAATCT GCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTGACGACAGCTTCTTCCAC^{gg}CTGGAAAGAAAGCTTCTCTGGT CGAAGAAGACAAGAGCACGAAAGACACCCGATCTTCGGAACACATCTGTCAGCAAGTCGATACACAGGAAAAGTACCCG ACAACTACCACTGAGAAAGAGTGGTCGACAGCACAGACAAAGGACCTGAGACTGATCTACCTGGCACTGGCACA CATGATCAAGTTCAAGGACACTTCTGATCGAAGGAGACCTGAACCCGGACAAACGCGACGTGCGACAAAGCTGTTCTATCC AGCTGGTCCAGACATACAACCACTGTTTCGAAGAAACCCGATCAACGCAAGCGGAGTCGACGCAAGGCAATCCTGAGC GCAAGACTGAGCAAGAGCAGAAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAAGACGGACTGTTCTGGAA ACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTTCGACCTGGCAGAAAGCACAAGCTGCAAGCTG AGCAAGGACACATACGACGACACTTGGACAACTCTGGCAGACAGTCGGAGACCACTGCGCAGACCTGTTCTCTGGCAGC AAAGAACCTGAGCGACGCAATCTTCTGAGCGACATCTGAGAGTCAACAGAAATCAAAAGGACCCGCTGAGCGCA GCATGATCAAGAGATACGACGAACACCAAGGACCTGACACTGCTGAAGGCCTGAGTCAAGACGAGCAGCTGCCGGAAAAG TACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAAGGATACATCGACGGAGGAGCAAGCCAGGAAGAAATCTA CAAGTTTCATCAAGCCGATCTCTGGAAAAGATGGACGGAAACAGAAAGAACTGCTGGTCAAGCTGAACAGAGAACCTGCTGA GAAAGCAGAGAACATTCGACAAACGGAAGCATCCCGCACAGATCCACCTGGGAGAACTGCACGCAATCTTGAGAAGACA GGAAAGCTTCTACCCGTTCTCTGAAAGGACACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAAATCCCGTACTACGTCG GACCGTGGCAAGAGGAAACAGCAGATTTCGATGGATGACAAAGAGCGGAAAGAAACAATCACACCCGTGGAACTTCGA AGAACTGCTCGACAAGGAGCAAGCGCACAGAGCTTCAGAAAGAAATGACAAACTTCGACAAAGAACCTGCCGAAACGAA AAGTCTCTGCCGAAAGCACAGCTGCTGTACGAATACCTTCACAGTCAACGAACTGACAAAGGTCAAGTACGTACACAGA AGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAAGGCAATCGTCGACCTGCTGTTCACAGACAAACAGAAAG GTCACAGTCAAGCAGCTGAAGGAAAGACTACTTCAAGAAAGATCGAATGCTTCGACAGCTCGAAATCAGCGGAGTCTGAAGA CAGATTCAACGCAAGCCTGGGAACATACCAAGCCTGCTGAGATCATCAAGGACAAGGACTTCTCTGGACAACGAAAGAA ACGAAGACATCTCTGGAAGACATCGTCTGACACTGACACTGTTCTGGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACA TACGCACACCTGTTCTGACGACAAGGTCTATGAAGCAGTGAAGAGAAAGATACACAGGATGGGAAAGACTGAGCAGAA AGCTGATCAACGGAATCAGAGCAAGCAGCGGAAAGACAATCTGGACTTCTGAAAGCAGCGAGTTCGCAACACAG AAACTTCATGCAGCTGATCCACGACAGACGCTGACATTCAGGAAGACATCCAGAACGCAAGGTCAAGCGGACAGGGAG ACAGCTGCACGAACACATCGCAACCTGGCAAGAGCCCGGCAATCAAGAAAGGAAATCTCTGACAGACATCAAGGTCGTC GACGAACTGGTCAAGGTCTATGGGAAAGACACAAGCCGGAAACATCGTTCATCGAAATGGCAAGAGAAACCCAGACAACAC AGAAAGGACAGAGAAACAGCAGAGAGAAAGAAATGAAGAGAGAAATCGAAAGGAAATCAAGGAACTGGGAAGCCAGATCTCTGA</p>
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	AGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAAGCTGTACCTGTACTACCTGCAaAACGGAAGAGACATGTAC GTCGACCAGGAACCTGGACATCAACAGACTGAGCGACTACGACGTGACCCACATCTGTCCCGCAGAGCTTCTCTGAAGGACGA CAGCATCGACAAACAAGGTCTTGACAAAGAACGCGACAAGAACAGAGAAAGAGCGACAAACGTCCCGAGCGAAAGAGTCGTC AAGAAATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAAGCTGATCACACAGAGAAAGTTTGACAACTGACAAAGG CAGAGAGGAGGACTGAGCGAACTGGACAAGCAGGATTCTATCAAGAGACAGCTGGTGAAACAAAGACAGATCACAAA GCACGTGCAACAGATCTGGACAGCAGAAATGAACACAAAGTACGACGAAAACGCAAGCTGATCAGAGAAAGTCAAGGTC ATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAAGAAATCAACAACTACCA CCACGCACACGACGCATACCTGAACGCAAGCTGTCGGAACAGCACTGATCAAGAAAGTACCCCGAAGCTGGAAAGCGAAATTCG TCTACGGAGACTACAAGGTCTACGACGTCAAGAAAGATGATCGCAAGAGCGAAACAGGAATCGGAAAGGCAACAGCAAA GTACTTCTTCTACAGCAACATCATGAATCTTCAAGACAGAAATCACACTGGCAAAACGGAGAAATCAGAAAAGAGACCCGCT GATCGAAACAAACGGAGAAACAAGGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAAGAAAGTCTCTGAGC ATGCCGAGGTCAACATCGTCAAGAAAGACAGAAAGTCCAGACAGGAGATTTCAGCAAGGAAAGCATCTTGCCGAAGAGAA ACAGCGACAAGCTGATCGCAAGAAAGAGGACTGGACCCGAAAGAGTACGGAAGATTTCGACAGCCCGACAGTCCGATA CAGCGTCTGCTGTCGCAAGGTGCAAGAAAGGAAAGAGCAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACA ATCATGGAAGAAGAGCTTCGAAAGAACCCGATCGACTTCTTGAAAGCAAAAGGATACAAGGAAGTCAAGAAAGGAC TGATCATCAAGCTGCCGAAGTACAGCTGTTCGAACTGGAAACCGGAAGAAAGAGAAATGCTGGCAAGCGAGGAGAACTG CAGAAAGGAAACGAACCTGGCAGCAAGTACGTCAACTTCTGTACCTGGCAAGCCACTACGAAAGCTGAAGGG AAGCCGGAAAGACAACGAACAGAGCAGCTGTCGCAACAGCAAGCACTACCTGGACGAAATCATCGAACAGATCA GCGAATTCAAGCAAGAGATCATCTTGGCAGACGCAAACTGGACAAAGTCTGAGCGCATACAACAGCAAGAGACAAAG CCGATCAGAGAAACAGGCAAGAAACATCATCCACCTGTTACACTGACAAACCTGGAGCACCAGGAGCATTCAAAGTACTT CGACACAACAATCGACAGAAAGAGATACACAAGCAAAAGGAAGTCTCTGGACGCAACACTGATCCACAGAGCATCACA GGACTGTACGAAACAAGAAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGAAAGCCCGAAGAAAGAGAAAGGTCT AG
508	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGATACAAGGT GCCCAGCAAGAAAGTTCAAGGTCTGGCAACACCCGACAGACACAGCATCAAGAAGAACCTGTATCGGCGCCCTGCTGTTCG ACAGCGCGAGACCCGCGAGGCCACAGACTGAAGAGAAACCGCCAGAAGAAAGATACACCAGAAGAAAGAACAGAATCTG CTACCTGCAGGAGATCTTCAAGCAACGAGATGGCCAAAGTGGACGACAGCTTCTCCACAGACTGGAGGAGAGCTTCTGCTGGT GGAGGAGGACAAGAAAGCAGAGAGACACCCCATCTTCGGCAACAATCGTGGACGAGGTGGCTACACGAGAGAAAGTACCCC ACCATCTACCACCTGAGAAAGAGCTGGTGGACAGCACCCGACAAGGCCGACCTGAGACTGATCTACCTGGCCCTGGCCCA CATGATCAAGTTCAAGGGCCACTTCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTTCATCCA GCTGGTGCAAGACTACAACCAAGCTGTTGCGAGGAGAACCCCATCAACGCCAGCGGCTGGACGCCAAGGCCATCTCTGAGCG CCAGACTGAGCAAGAGCAAGACTGGAGAACCTGATCGCCCAAGTGGCCGCGCAAGAAAGAACGGCCTGTTTCGGCAA CCTGATCGCCCTGAGCCTGGGCTGACCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGAGCCAAAGCTGCGAGCTGAG CAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCAGATCGGCGACCAAGTACGCCGACCTGTTCTTGGCCGCCA AGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGGAGATCAACCAAGGCCCCCTCTGAGCGCCAGC

ATGATCAAGAGATACGACGAGACACCAACAGGACCTGACCCCTGCTGAAGGCCCTGTTGAGACAGCAGCTGCCCCGAGAAAGTA CAAGGAGATCTTCTCGACAGAGCAAGAAACGGCTACGCCGGCTACATCGACGGCGCCAGCCAGGAGAGTTCTACA AGTTCAACAGCCCATCTCTGGAGAAAGATGGACGGCACCCAGGAGGCTGCTGGTGAAGCTGAACAGAGAGGACCTGCTGAGA AAGCAGAGAACCTTCGACAAACGGCAGCATCCCCACCAGATCCACCTGGCGGAGTGCACGCCATCCTGAGAAACAGAGGA GGACTTCTACCCCTTCTGAAAGGACAAACAGAGAGAAAGATCGAGAAAGATCCTGACCTTCAAGATCCCTACTACGTGGGCC CCTGGCCAGAGGCAACAGCAGATTCGCTGACAGAGAAAGAGCGAGGACCATCACCCCTTGAACTTCGAGGAGG TGGTGGACAAAGGGGCCAGGCCACAGCTTTCATCGAGAGAAATGACCAACTTCGACAAAGAACTGCCCCACGAGAAAGGTG CTGCCCCAGCAGACGCTGTACGAGTACTTCACCGTGTAAACGAGCTGACCAAGGTGAAGTACGTGACCCGAGGGCAT GAGAAAGCCCGCTTCTGAGCGCGGAGCAGAAAGAGGCCATCTGGACCTGCTGTTCAAGACCAACAGAAAGGTGACCCG TGAAAGCAGCTGAAGGAGGACTACTTCAAGAAAGATCGAGTGTCTCGACAGCTGGAGATCAGCGCTGGAGACAGATTTC AACGCCAGCTGGGCACTACACGACCTGCTGAAGATATCAAGGACAAAGACTTCTTGGACAAACGAGAGAAACGAGGA CATCTGGAGGACATCTGCTGACCCCTGACCCCTGTTTCGAGGACAGAGAGATGATCGAGGAGAGACTGAAGACCTACGCC ACCTGTTGACGACAAGGTGATGAAGCAGCTGAAGAGAAAGATACACCGGCTGGGGCAGACTGAGCAGAAAGCTGAT CAACGGCATCAGAGACAAAGCAGAGCGCAAGACCATCTTGACTTCTGAAAGAGCGACGGCTTCGCCAACAGAAACTTCA TGACGTGATCCACGACGACGCTGACCTTCAAGGAGGACATCCAGAGGCCAGAGTGAAGGCCAGGGCGACAGCCTG CACGACACATCGCCAACTGGCCGGCAGCCCGCATCAAGAAAGGGCATCTCGACACCTGAAAGGTGAAGGTGGTGAACGAGCT GGTGAAGGTGATGGCAGACACAGCCCGAGAACATCTGTGATCGATGGCCAGAGAGAACAGACCCAGAACAAAGGC CAGAAAGAACAGCAGAGAGAAATGAAGAGAAATCGAGGAGGACATCAAGGAGCTGGCAGCCAGATCTGAAAGGAGCACCC CCGTGGAGAACACCCAGCTGCAGAACGAGAAAGCTGTACTCTGTACTACCTGCAGAACGGCAGAGACATGTACGTGGACACG GAGCTGGACATCAACAGACTGAGCGACTACGACGTGGACCCACATCTGCCCCAGAGCTTCTGAAAGGACGACAGCATCGA CAACAAAGTGTGACCAAGACGACAAAGACAGAGGCAACGCGCACAACGTGCCAGCGAGGAGGTGCTGAAGAAAGATG AAGAACTACTGGAGACAGCTGCTGAACGCCAAGCTGATCACCCAGAGAAAGTTTCGACAACTGACCAAGGCCAGAGAGG CGCCTGAGCGAGCTGGACAAAGGCCGGCTTCAATCAAGAGACAGCTGGTGGAGACCAAGCAGAGATCAACAAAGCAGCTGGCCC AGATCTGGACAGCAGAAATGAACACCAAGTACGACGAGAACGACAAAGTGTATCAGAGAGGTGAAGGTGATCACCCCTGAA GAGCAAGCTGGTGAGCGACTTCAGAAAGGACTTCCAGTTCTACAGGTGAGAGATCAACAATCCACCCAGCCACG ACGCTACCTGAACGCCGTGGTGGCACCCCTGTATCAAGAAAGTACCCCAAGCTGGAGAGCGAGTTCTGTACGGCGAC TACAAAGTGTACGACGTGAGAAAGATGATCGCCAAGAGCGAGACAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTCTA CAGCAACATCATGAACCTTCTTCAGACCGAGATCACCTGGCCAACGGCGAGATCAGAAAGAGACCCCTGATCGAGACCA ACGGCGAGACCGCGAGATCGTGTGGGACAAGGGCAGAGACTTCGCCACCTGAGAAAGGTGTGAGCATGCCCCAGGTG AACATCGTGAAGAAAGACCGAGGTGCAGACCGCGGCTTCAGCAAGGAGAGCATCTGCCCCAAGAGAAACAGCGACAAGC TGATCGCCAGAAAGAAAGGACTGGGACCCCAAGAAAGTACGGCGCTTCGACAGCCCCACCTGGCCTACAGCTGTGTTG GTGGCCAAAGGTGGAGAAAGGCAAGAGCAAGAAAGTGAAGAGCGTGAAGGAGTGTGGGCTACCATCATGGAGAGAA GCAGCTTCGAGAAAGACCCCATCGACTTCTGGAGGCCAAGGGCTCAAGGAGGTGAAGAGGACCTGATCATCAAGCTG CCCAAGTACAGCCTGTTGAGCTGGAGAACGGCAGAAAGAGAAATGTGGCCAGCGCCGGCGAGCTGCAGAAAGGGCAACG AGCTGGCCCTGCCCAGCAAGTACGTGAACCTTCTGTACCTGGCCAGCCACTACGAGAAAGCTGAAGGGCAGCCCCGAGGAC

	<p>AACGAGCAGAAGCAGCTGTTCTGTGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTTCAGCAA GAGAGTGATCTTGCCGACGCCAACCTGGACAAGGTGCTGAGCGCTACAAACAAGCAGAGACAAAGCCATCAGAGAGC AGCCGAGAACATCATCCACCTGTTACCTGACCAACCTGGGCGCCCGCCCTTCAAAGTACTTCGACACCAACCATCG ACAGAAAGAGATACACAGCACCAAGGAGGTGCTGGACGCCACCTGATCCACCAAGCATCACCGCCCTGTACGAGACC AGAATGACCTGAGCCAGCTGGCGGCGACGGCGGCGAGCCCAAGAAAGAGAAAGGTGTGA</p> <p>GGGTCCCGCAGTCGGCTCCAGCGGCTCTGTTGTTGTTGTTGCGGCTTATTCGGATCCGCCACCATGGAC AAGAAATACAGCATCGGCTGGACATCGGCACCAACAGCTGGCTGGCGGTGATCATCCGACGAGTACAAGTGCCAG CAAGAAATTCAAGGTGCTGGGCAACACCGACAGACACAGCATCAAGAAACCTGATCGGCGCCCTGCTTCGACAGCG GCGAGACCGCCGAGGCCACAGACTGAAGAGAACCGCCAGAAAGATACACCAAGAAAGAAACAAGATCTGCTACCT GCAGGAGATCTTACGAACGAGATGGCAAGGTGGACGACAGCTTCTCCACAGACTGGAGGAGAGCTTCTTGTGTGAGG AGGACAAGAACGACGAGAGACACCCATCTTCGGCAACATCTGTGGACAGGTGGCTACCCAGAGAAAGTACCCACCATC TACCACCTGAGAAAGAGCTGGTGGACAGCACCGACCAAGGCCGACCTGAGACTGATCTACCTGGCCCTGGCCACATGAT CAAGTTGAGAGGCCACTTCTGATCGAGGGGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTTCATCCAGCTGGT GCAGACCTACAACAGCTGTTGAGGAGAAACCCATCAACGCCAGCGGCTGGAGGCCAAGGCCATCCTGAGCGCCAGAC TGAGCAAGAGCAGAGACTGGAGAACCTGATCGCCAGCTGCCCGGCGAGAAAGAAACGGCTGTTTCGGCAACCTGATC GCCCTGAGCTGGGCTGACCCCCAACCTTCAAGACAACCTTCGACCTGGCCGAGGACGCCAAGCTGCAAGTGAAGGA CACCTACGACGACGACCTGGACAACCTGCTGGCCAGATCGGCGACCACTACGCCGACCTGTTCTGGCCGCCAAGAACCT GAGCGAGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCAACAGGCCCTGAGCGCCAGCATGATCA AGAGATACGACGACACCAAGGACCTGACCTGCTGAAGGCCCTGTTGAGACAGCAGCTGCCCGAGAAAGTACAAGGA GATCTTCTCGACCAAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCCAGCCAGGAGGAGTTCTACAAGTTTCA CAAGCCCATCTTGAGAAAGATGGACGGCACCCGAGGAGCTGTTGTTGAAGCTGAACAGAGAGGACCTGCTGAGAAAGCAG AGAACCTTCGACAACGGCAGCATCCCCACCAAGATCCACCTGGCGGAGCTGCACGCCATCCTGAGAAAGACAGGAGACTT CTACCCCTTCTGAAAGGACAACAGAGAGAAAGATCGAGAAAGATCTTGACCTTCAGAAATCCCTACTACGTGGGCCCTGGC CAGAGGCAACAGCAGATTGCTGATGACCAAGAAAGAGCGAGGAGACCATCACCCCTGGAATTCGAGGAGGTGGTGG ACAAGGCGCCAGCGCCAGAGCTTCATCGAGAGATGACCAACTTCGACAAGAACCTGCCAACGAGAAGGTGCTGCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGAGAA GCCCCCTTCTTGAGCGGCGAGCAGAAAGGCCATCTGGACCTGCTGTTCAGAACCAACAGAAAGGTGACCCGTGAAGC AGCTGAAGGAGGACTACTTCAAGAAAGATCGAGTGTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACAGATTCAACGCC AGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCTTGACAACGAGGAGAACGAGGACATCCT GGAGGACATCTGCTGACCTGACCTGTTTCGAGGACAGAGAGATGATCGAGGAGAGACTGAAGACCTACGCCACCTGT TCGACGACAAGGTGATGAAGCAGCTGAAGAGAAAGATACACCGCTGGGCGAGACTGAGCAAGAAAGTGTATCAACGG CATCAGAGACAAAGCAGCGGCAAGACCATCTCTGGAAGCTTCCTGAAGAGCGGCTTCGCCAACAGAAACTTCATGCAGC TGATCCAGCAGACAGCCTGACCTTCAAGGAGGACATCCAGAAAGGCCAGGTGAGCGGCCAGGGCGACAGCCTGCACCGAG CACATCGCCAACTGGCCGCGAGCCCCGCCATCAAGAAAGGCACTCCTGCAGACCTGTAAGGTGGTGGACGAGCTGGTGAA GGTGATGGGCGAGACACAAGCCCCGAGAAACATCTGATCGAGATGGCCAGAGAGAACCAAGACCAACCCAGAAAGGGCCAGAAAG</p>
509	

	AACAGCAGAGAGAGAAATGAAGAAATCGAGGAGGGCATCAAGGAGCTGGCAGCCAGATCTCTGAAGGAGCACCCCGTGG AGAACACCCAGCTGCAGAACGAGAAAGCTGTACCTGTACTCTGCAGAACGGCAGAGACATGTAGTGGACCCAGAGAGCTG GACATCAACAGACTGACGACTACGACGTGGACCATCATCTGTCGCCACAGAGCTTCTTGAAGGACGACAGCATCGACAAACA GGTGTGACCAAGAGCGACAAGAACAGAGGCAAGAGGACAACGTGCCACGCGAGGAGGTGGTGAAGAAAGATGAAGAAC TACTGGAGACAGCTGCTGAACGCCAAGCTGATACCCAGAGAAAGTTGACAACTGACCAGGCCGAGAGAGCGGCCCT GAGCGAGCTGGACAAGGCCGCTTCATCAAGAGACAGCTGTGTGAGACAGACAGATCACCAAGCACGTGGCCAGATCC TGGACAGCAGAAATGAACACCAAGTACGACGAGAACGACAAGCTGTGATCAGAGAGGTGAAGGTGATCACCTGGAAGACA GCTGGTGAAGGACTTCAGAGTTCAGAGTTCACAAAGGTGAGAGATCAACAATACCACCCACGCCACGACGCCCT ACCTGAACGCCGTGGTGGCACCCGCTGATCAAGAAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAG GTGTACGACGTGAGAAAGATGATGCCAAGAGCGAGCAGAGATCGCAAGGCCACCCCAAGTACTTCTTACAGCAA CATATGAATTTTCAAGACCGAGATCACCTGGCCACGGGAGATCAGAAAGACACCCCTGTATCGAGACCAACGGCG AGCCGGCGAGATCGTGTGGACAAGGGCAGAGACTTCGCCACCGTGAAGAAAGGTGCTGAGCATGCCCAAGGTGAACATC GTGAAGAAAGACCGAGGTGACACCGGGCTTCAGCAAGGAGAGCATCTGCCAAGAGAAACAGCGACAAGCTGATCG CCAGAAAGAAAGGACTGGGACCCCAAGAAAGTACGGCGCTTCGACAGCCCCACCGTGGCTACAGCGTCTGTGGTGGCC AAGGTGGAGAGGGCAAGACAAGAAAGCTGAAGAGCTGAAGAGCTGTGGCATCACCATCATCGAGAGAAAGCAGCT TCGAGAAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAGGACCTGATCATCAAGCTGCCCAAG TACAGCTGTTCGAGCTGGAGAACGGCAGAAAGAAATGCTGGCCAGCGCGGAGCTGCAGAAAGGCAACGAGCTGG CCCTGCCACGAAAGTACGTGAATTCCTGTACCTGGCCAGCCACTACGAGAAAGTGAAGGGCAGCCCCGAGGACCAACGAG CAGAAAGCAGCTGTTCGTGGAGCAGCAACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGAGAGT GATCTGGCCGACGCCAACCTGGACAAGGTGTGAGCGCCTACAACAAGCAGACAGAGACAAGCCCATCAGAGCAGGCCG AGAACATCATCCACCTGTACCCCTGACCAACCTGGCGCCCCGCCCTTCAAGTACTTCGACACCAACCATCGACAGAA AGAGATACACCAAGCACCAGGAGGTGCTGGACGCCACCCCTGATCCACAGAGCATCACCGGCTGTACGAGACCAAGAAATC GACCTGAGCCAGCTGGCGGCGACGGCGGCGGCGAGCCCCAAGAAAGAGAAAGGTGTGACTAGCCATCACATTTAAAA GCATCTCAGCCTACCATGAGAAATAGAGAAAGAAAAATGAAGATCAATAGCTTATTCATCTCTTTTCTTTTCTGTTGGTGT AAGCCAAACACCCCTGTCTAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTCTCTGTGCTTCAATTAATAAAAAATGGA AAGAACCTCGAG
510	ATGGACAAGAAAGTACTCTATCGGTTTGGACATCGGTACCAACTCTGTGCGTTGGCGCTCATCCGACGAATACAAAGTGC CCATCTAAGAAAGTTCAAGGTCTTGGGTAAACCCGACAGACACTCTATCAAGAAGAACTTGATCGGTGCTTGTGTTCTGAC TCTGGTGAACCCGCCAAGCCACAGATTGAAGAGAACCCGCCAGAAAGATACCCAGAAAGAAAGAACAGAAATCTGCT ACTTGCAAGAAATCTTCTAAACGAAATGGCCAAAGTGCAGACTCTTCTCCACAGATTGGAAGAAATCTTCTTGGTCTGA AGAAAGACAAGAGCACGAAAGACACCAATCTTCGGTAACTCTGTCGAGGAAGTGCCTACCACGAAAGATACCCAAACCA TCTACCACTTGAGAAAGAAAGTTGGTGCAGCTCTACCGACAAGGCCGACTTGAGATTGATCTACTTGGCTTGGCCACATGA TCAAGTTCAGAGGTCACTTGTGATCGAAGGTGACTTGAACCCAGACAACCTGACGTCGACAAGTGTTCATCCAAATGGT CCAAACCTACAACCAATTTGTTCTGAAGAAACCCCAATCAACGCCCTCTGGTGTGACGCCAAGGCCATCTTGTCTGCCAGATT GTCTAAGAGCAGAAAGATTGGAAAACTTGATCGGCCAATTGGCCAGGTGAAAAAGAAAGAACCGGTTTGTTCGGTAACTTGATCGC

<p> CTTGTCTTTGGGTTTGACCCCAAACTTCAAGTCTAACTTCGACTTGGCCGAAGACGCCAAAGTTGCAATTGTCTAAGGACACC TACGACGACACTTGGACAACTTGTGGCCCAAATCGGTGACCAATACGCCGACTTGTCTTGGCCGCCAAGAACTTGTCT GACGCCATCTTGTGTGACTCTTGAGAGTCAACACCGAAATCAACAAAGGCCCAATTGTCTGCTCTATGATCAAGAGAT ACGACGAACACCAAGACTTGACCTTGTGAAGCCTTGGTCAGACAACAATTGCCAGAAAAGTACAAGGAAATCTTCT TCGACCAATCTAAGAACGGTTACGCCGGTACATCGACGGTGGTGGCTCTCAAGAGAAATCTCAAGTTCTCAAGGCCAA TCTTGGAAAAGATGGACGGTACCGAAAGAAATGTGGTCAAGTTGAACAGAGAAAGTGTGGAGAAAGCAAGAACCTTC GACAAACGGTTCTATCCCAACACCAATCCACTTGGTGAATTCAGCCATCTTGAGAAAGACAAGAAAGCTTACCCATTC TTGAAGGACAACAGAGAAAAGATCGGAAAAGATCTTGACCTTCAGAAATCCCATCTACCTGGTCCATTGGCCAGAGGTAA CAGCAGATTGCGCTGGATGACCAAGAAAGTCTGAAGAACCAATCACCCCATGGAACCTCGAAAGAAAGTCTCGACAAAGGTG CCTCTGCCCAATCTTTCATCGAAAAGATGACCAACTTCGACAAGAACTTGCCAAACGAAAAGGTCTTGCCAAAAGCACCTT TGTGTACGAATCTTACCGTCTACAAGAAATTGACCAAGGTCAAGTACGTACCGAAAGGTATGAGAAAGCCAGCCTTCT TGTCTGGTGAACAAAAGAAAGGCCATCGTCGACTTGTGTTCAGACCAACAGAAAGGTACCGTCAAGCAATTGAAGGAA GACTACTTCAAGAAAGATCGAATGCTTCGACTCTGTGCAAAATCTTGGTGTGAAAGACAGATTCAACGCCCTCTTTGGGTACCT ACCACGACTTGTGAAGATCATCAAGGACAAGGACTTCTTGGACAACGAAGAAAACGAAAGACATCTTGGAAAGACATCGTTC TTGACCTTGACCTTGTTCGAAGACAGAGAAATGATCGAAGAAAGATTGAAGACCTACGCCCACTTGTTCGAGACAAAGGTCT ATGAAGCAATTGAAGAGAAAGATACACCGTTGGGTAGATTGAGCAGAAAGTTGATCAACGGTATCAAGAGACAAGC AATCTGGTAAAGACCATCTTGGACTTCTTGAAGTCTGACGGTTTCGCCAACAGAAATCTCATGCAATTGATCCACGACGACTC TTTGACCTTCAAGGAAGACATCCAAAAGGCCAAAGTCTCTGGTCAAGGTGACTCTTTCACGAACACATCGCCAACTTGGC CGGTTCTCCAGCCATCAAGAAAGGTATCTTGCAAAACCGTCAAGGTCTGACGAAATTTGGTCAAGGTCAATGGGTAGACAAA GCCAGAAAACATCGTCAATCGAAATGGCCAGAGAAAACCAACCCACCCAAAAGGTCAAAAAGAACAGCAGAGAAAAGAATG AAGAGAAATCGAAGAAAGGTATCAAGGAATTGGGTCTCAATCTTGAAGAACACCCAGTCGAAAACACCCAAATTGCAAAA CGAAAAGTTGTACTTGTACTTGTGCAAAAACGGTAGAGACATGTACGTGACCAAGAAATGGACATCAACAGATTGTCTGA CTACGACGTCGACCAATCGTCCCAATCTTCTTGAAGGACGACTCTATCGACAACAAGGTCTTGGACAGATCTGACAA GAACAGAGGTAAAGTCTGACACGTCCCATCTGAAGAAAGTCTCAAGAAAGATGAAGAACTACTGGAAGACAATTGTGAAACG CCAAGTTGATCACCCAAAGAAAGTTCGACAATCTGACCAAGGCCGAAAGAGGTGGTTGTCTGAATTGGACAAGGCCGGT TTCATCAAGAGACAATTGGTCAACACGACAAAATCAACCAAGCACGTGCCCCAAATCTTGGACAGCAGAAATGAACACCAA GTACGACGAAAACGACAAGTTGATCAGAGAAGTCAAGGTCTACCTTGAAGTCTAAGTTGGTCTCTGACTTCAGAAAAGG ACTTCCAATTCTACAAGGTCAAGAGAAATCAACAACTACCAACACGCCCAACGCGCTACTTGAACGCCGTCTGCGGTACCG CCTTGATCAAGAAAGTACCCAAAGTTGGAATCTGAATCTGCTACGGTGACTACAAGGTCTACGACGTCAAGAAAGATGATCG CCAAGTCTGAACAAAGAAATCGGTAAAGGCCACCGCAAGTACTTCTACTCTAATCATGAACTTCTTCAAGACCGGAA TCACCTTGGCCAAACGGTGAAATCAGAAAGAGACAAATGATCGAACCACCGGTGAACCGGTGAAAATCGTCTGGGACAAG GGTAGAGACTTCGCCACCGTCAGAAAGGTCTGTCTATGCCACAAGTCAACATCGTCAAGAAAGACCGGAATCCAAACCGGT GGTCTCTAAGGAATCTATCTTGCCAAAAGAGAACTCTGACAAGTTGATCGCCAGAAAAGAGGACTGGGACCCCAAGAA GTACGGTGGTTTCGACTCTCCAAACCGTCGCCTACTCTGTCTTGGTCTGCGCCAAAGGTGCAAGAGGTAAAGTCTAAGAAAGTT GAAGTCTGTCAAGGAATTGTGGGTATCACCATCATGGAAGAAATCTTCTCGAAAAGAACCCCAATCGGACTTCTTGGAAAGC </p>

	CAAAGGTTACAAAGGAAGTCAAGAAAGGACTTGATCATCAAGTTGCCAAAGTACTCTTTGTTTCGAAATTGGAAACCGGTAGAA AGAGAAATGTTGGCTCTGCCGTTGAATTCGAAAAGGTAAACGAATGGCCTTGCCATCTAAGTACGTCAACTTCTGTACTT GGCTCTCACTACGAAAGTTGAAGGTTCTCCAGAAAGAACGAAACAAAGCAATTTGTCGCAACAACAAGCACT ACTTGGACGAATCATCGAAACAATCTCTGAATCTCTAAGAGATCATTTGGCCGACGCCAACTTGGACAAGGTCCTTGT CTGCTACAACAAGCACAGAGACAAGCCAAATCAGAGAACAAAGCCGAAACATATCCACTTGTTTACCTTGACCAACTTG GGTCCCCAGCGCTTCAAGTACTTCGACACCACTCAGACAGAAAGAGATACACCTCTACCAAGGAAGTCTTGGACGCC ACCTTGATCCACCAATCTATACCGGTTTGTAGCGAAACCAAGATCGACTTGTCTCAATTGGGTGGTGACGGTGGTGTCTC CAAGAAGAGAGAAAGGTCTAA
511	ATGGACAAAGAAAGTACTCCATCGGCTGGACATCGGCACCAACTCCGTGGGCTGGCCGTGATCACCGACGAGTACAAGGT GCCC'TCCAAAGAAAGTTCAAGGTGCTGGCAACAACCGACCGCACTCCATCAAGAAAGAACCTGATCGGCGCCCTGCTGTTCGA CTCCGGCGAGACCGCCGAGGCCACCCGGCTGAAGCGGACCCCGCGGGGTACACCCGGCGGAAGAACCCGGATCTGCT ACCTGCAGGAGATCTTCCAAACGAGATGGCCAAAGGTGGACGACTCCTTTCTCCACCCGGCTGGAGGATCTTCTCGGTGG AGGAGACAAGAAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGTGGCTACCTGGCCCTGGCCACATG ATCTACCACTTCGGGAAGAGTGGTGACTCCACCGACAAAGCCGACCTGCGCTGATCTACCTGGCCCTGGCCACATG ATCAAATTCCGGGCCACTTCTCTGATCGAGGGCGACCTGAACCCGCAACTCCGACGTGGACAAGCTGTTTCAATCCAGCTG GTGCAGACTTACAACCACTGTTTCGAGGAGAACCCATCAACGCCCTCCGGCTGGACGCCAAGGCCATCTGTCGCCCGG CTGTCAAAGTCCCGGGCTGGAGAACCTGATCGCCACGCTGCCGGCGAGAAAGAACCGGCTGTTTCGGCAACCTGAT CGCCCTGTCCCTGGGCTGACCCCAACTTCAAGTCCAACCTCGACCTGGCGAGGACGCCAAGTGCAGCTGTCCAAGGA CACCTACGACGACGACCTGGACAACCTGCTGGCCCAAGATCGGCGACCACTGACGCCGCTGTTTCTGGCCGCCAAGAACCT GTCCGAGCCATCTGCTGCTCCGACATCTCTGGGGTGAACACCGAGATCACCAAGGCCCCCTGTCGCCCTCCATGATCAA GCGGTACGACGAGCACCAAGGACCTGACCCCTGCTGAAGGCCCTGTTGGGCAAGCTGCCCCGAGAAAGTACAAGGAGA TCTTCTCGACCACTCAAGAAAGGCTACGCCGGCTACATCGACGGCGGCCCTCCAGGAGGATTTCAAGTTTCATCA AGCCCATCCTGGAGAAAGATGGACGGCAACCGAGGAGTCTGTTGAAGCTGAACCCGGGAGGACCTGCTGCGGAAGCAGCG GACCTTCGACAAGGCTTCAATCCCCACCAAGATCCACCTGGGCGAGCTGCACGCCATCTCGCGCGGCAAGGAGGACTTCTA CCCTTCTCTGAAGGACAACCCGGGAGAGATCGAGAAAGATCTGACCTTCGGATCCCC'ACTACGTGGGCCCTGGCCCC GGCAACTCCCGGTTCCGCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCTTGAACCTTCGAGGAGTGGTGACA AGGGCGCTCCCGCCAGTCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCAACGAGAAAGTGTGTCGCCAAG CACTCCCTGCTGTACGAGTATTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGCAATGCGGAAGCCC GCCTTCTGTCCGGGAGCAAGAAAGGCCATCTGTGACCTGCTGTTCAGACCAACCGGAAGGTGACCTGAAGCAGCT GAAAGGAGGACTACTTCAAGAAAGATCGAGTCTGACTCCGTGGAGATCTCCGGCTGGAGACCGGTTCAACGCCCTCCCT GGGCACTACCAAGACCTGCTGAAGATCATCAAGGACAAGGACTTCTTGACAAACGAGGAAACGAGACATCTCGGAGG ACATCGTGTGACCTTGACCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACTGTTTCGACG ACAAGGTGATGAAGCAGCTGAAGCGGGCGGCTACACCGGCTGGGGCGGCTGTCCCGGAAGCTGATCAACGGCATCCCG GACAAAGCTCCCGCAAGACCATCTTGACTTCTGAACTCCGACCGGCTTCGCCAACCGGAACCTTCATGACGTGATCCAC GACGACTCCCTGACCTTCAAGGAGGACATCCAGAAAGGCCCAAGGTGTCGGGCCAGGGCGACTCCCTGCACGAGCACATCGC

	CAACCTGGCCGGCTCCCCGCCATCAAGAAAGGGCATCCTGCGAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGG GCCGGCACAAAGCCGAGAACATCGTATCGAGATGGCCCGGGAGAACAGACCCAGAAAGGGCCAGAGAACTCCCG GGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGAGCTGGGCTCCAGATCCTGAAGAGCACCCCGTGGAGAACACC CAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCGAGCTGGACATCAA CCGGCTGTCCGACTACGACGTGGACACATCGTGGCCAGTCTCTGAAAGGACGACTCCATCGACAAACAAGTGTCTGAC CCGGTCCGACAAAGAACCGGGCAAGTCCGACACGTGCCCTCCGAGGAGTGGTGAAGAGAACTACTGGCGGC AGCTGTGAAGCCAAAGCTGATACCCAGCGGAAGTTTCGACAACTGACCAAGGCGAGGGGGGCTGTCCGAGCTG GACAAAGCCGGCTTCATCAAGCGGACGCTGTGGAGACCCGGCAGATCAACAAAGCAGCTGGCCAGATCCTGGACTCCCG GATGAACACCAAGTACGACGAGAACGACAAAGTGTATCCGGGAGGTGAAGTGTATCACTTGAAGTCCAAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACACCACCCGACGACGCTACCTGAACGCGC TGGTGGGCAACCGCCCTGATCAAGAAAGTACCCCAAGCTGGAGTCCGAGTTCTGTACGGCGACTACAAGGTGTACGACGTGC GGAAAGATGATCGCCAAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTACTTCCAAACATCATGAATCTCT TCAAGACCGAGATCACCTGGCCACCGCGAGATCCGGAAGCGGCCCTGATCGAGACCAACGGCGAGACCGGGGAGATC GTGTGGACAAAGGCCGGGACTTCGCCACCGTGGGGAAGGTGTCTCCATGCCCAGGTGAACATCTGGAAGAAAGACCGA GGTGACAGCCGGCGCTTCTCCAAGGAGTCCATCTGCCCCAAGCGGAACCTCCGACAAAGTGTATCGGCCGGAAGAGGACT GGGACCCCAAGAAAGTACGGCGGCTTCGACTCCCCACCGTGGCCTACTCCGTGCTGGTGGCCAAAGTGGAGAAAGGGC AAGTCCAAAGAAAGTGAAGTCCGTGAAGAGCTGCTGGGCTACCATCATGGAAGCGGTCTCTTCGAGAAAGAACCCCAT CGACTTCTGGAGGCCAAGGGTACAAGGAGTGAAGAGGACCTGATCATCAAGCTGCCAAGTACTCCCTGTTCTGAGCT GGAAACGGCCGGAAAGCGGATGTCTGGCCTCCCGCGCGAGCTGCAAGAGGCAACGAGCTGGCCCTGCCCTCCAAAGTACG TGAATTCCTGTACCTGGCTCCCACTACGAGAAAGCTGAAGGGTCCCCGAGGACACGAGCAAGCAAGCTGTTCTGTGG AGCAGCACAAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGTGATCCTGGCCGACGCCAAC CTGGACAAGGTGTCTCCGCTACAACAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTT CACCTGACCAACCTTGGGCGCCCCCGCCCTTCAAGTACTTCGACACCACTCATCGACCCGGAAGCGGTACACCTCCACCAA GGAGGTGCTGGACGCCACCTGTATCCACCACTCCATCACCGGCTGTACGAGACCCGGATCGACCTGTCTCCAGCTGGGCGG CGACGGCGGGCTCCCCCAAGAAAGAACGCGGAAGGTGTGA
512	ATGGACAAAGAAAGTACAGCATCGGCTGGACATCGGCACCAACAGCTGGGCTGGGCCGTGATCACCGACGAGTACAAGGT GCCAGCAAGAAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAAGAACCTGATCGGCGCTGCTGTTCG ACAAGCGGAGACCGCGAGGGCAACCGGGTGAAGCGGACCGCGCGGGCGGTACACCGCGGGAAGAACCGGATCTG CTACCTGCAGGAGATCTTACGCAACGAGATGGCCAAAGGTGGACGACAGCTTCTTCCACCGGGTGAAGGAGAGCTTCTGTT GGAGGAGGACAAAGAAAGCAGAGCGGCACCCCATCTTCGGCAACATCTGTGGACGAGGTGGCCTACCAAGAGAAAGTACCCCA CCATCTACCACTTCCGGAAGAGCTGGTGACAGCACGACAAAGGCGGACCTGGCGTGTATCTACCTGGCCCTGGCCAC ATGATCAAGTTCCGGGGCACTTCTGTGATCGAGGGCGACTGAACCCGACAAACAGCAGCTGGGACAAAGTGTTCATCCAG CTGGTGCAGACCTACAACAGCTGTTTCGAGGAGAACCCCATCAACGCCAGCGGCTGGACGCCAAGGCCATCCTGAGCGC CCGGCTGAGCAAGAGCGCGGCTGGAGAACCTGTATCGCCAGCTGCCCGGAGAAAGAAAGAACCGCCTGTTCCGCAACC TGATCGCCCTGAGGCTGGGCTGACCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGAGCA

<p>AGGACACCTACGACGACCTGGACAACTGCTGGCCAGATCGGCGACCACTAGCGCCGACCTGTTCTCGCCGCCAAG AACCTGAGCGACGCCATCTGCTGAGCGACATCTCTGGGTGAACACCGAGATACCAAGGCCCTGAGCGCCAGCAT GATCAAGCGGTACGACGACACCAAGACCTGACCTGCTGAAGCCCTGTTGGCGCAGCACTGCCGAGAAAGTACA AGGAGATCTTCTTGACCAAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCCAGCCAGGAGATTCTACAAG TTCATCAAGCCCATCTTGAGAAAGATGACGGCACCGAGGAGCTGCTGTTGAAGCTGAACCGGGAGGACCTGCTGCGGAA GCACGGACCTTGACAAACGGCAGCATCCCCACAGATCCACCTGGCGGAGCTGCACGCTATCTCGGGCGCAGGAGG ACTTACCCCTTCTGAAAGGACAACCGGGAGAAAGATCGAAGATCTGACCTTCCGGATCCCTACTACTCTGGGCCCTC TGGCCCGGGCAACAGCCGTTCCCTGGATGACCCGGAAAGCGAGGAGACCATCACCCCTGGAACCTCGAGGAGGTG GTGGACAAGGGCGCCAGCGCCAGAGCTTCTACGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCT GCCCCAGCACAGCTGCTACGAGTACTTCACTGTTACAAAGCTGACCAAGGTGAAGTACGTGACCGGAGGCTGCTG GGAAAGCCCTTCTGAGCGGCGAGCAAGAAAGGCCATCTGTGACCTGCTGTTCAAGACCAACCGGAAAGGTGACCGTG AAGCAGCTGAAGGAGGACTACTTCAAGAGATCGAGTCTTCGACACGCTGGAGATCAGCGGCTGGAGCCGGTTCAA CGCCAGCTGGCACCTACCAGACCTGCTGAAGATCATCAAGGACAAGGACTTCTTGACAACGAGGAGAACGAGGACA TCCTGGAGGACATCTGCTGACCTGACCTGTTTCGAGGACCGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCAC CTGTTTCGACGACAAGGTGATGAAGCAGCTGAAGCGCGGCTGACACCGGCTGGGGCGGCTGAGCCGAAAGCTGATCAA CGGCATCCGGGACAAGCAGCGGCAAGACCATCTCTGAGTCTTCTGAAGCAGCGCTTCGCCAACCGGAACCTCATGC AGCTGATCCACGACGACAGCTGACCTTCAAGGAGGACATCCAGAAAGGCCAGGTGAGCGGCCAGGCGACAGCTGCAC GAGCACATCGCCAACTGGCGGCGAGCCCGCATCAAGAAAGGCGATCTCGAACCGTGAAGGTGGTGACGAGCTGCT GAAAGGTGATGGCGCGCAAGGCCGAGAACATCTGTATCGAGATGGCCCGGGAGAACCAAGCAACCCAGAAAGGCGCAG AAGAACAGCCGGGACGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGCAGCCAGATCCTGAAGGACACCCCG TGGAAGAACCCAGCTGCAGAACGAGAAGCTGTACTCTGACAGAACGGCGGGACATGTACGTGGACCAAGGAG CTGGACATCAACCGGCTGAGCGACTACGACGTGGACCACTCTGCCCCAGAGCTTCTGAAGGACGACAGCATCGACAA CAAGGTGCTGACCCGAGCGACAAGAACCGGGGCAAGAGCGACAACTGTGCCAGCGAGGAGGTGGTGAAGAAAGATGAAG AACTACTGGCGGAGCTGTGAACGCCAAGCTGATCACCCAGCGGAAGTTTCGACAACCTGACCAAGGCCGAGCGGGCGG CCTGAGCGAGCTGGACAAGCGCGGCTTCAATCAAGCGGAGCTGTTGGAGACCCGGCAGATCACCAGCACGTGGCCCCAG TCCTGGACAGCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCTGAAGAGC AAGCTGCTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACCTACCCACCGCCACGACGC CTACCTGAACGCCGTGGTGGCACCCGCTGATCAAGAAAGTACCCCAAGCTGGAGAGCGGAGTTCTGTACGGCGACTACA AGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCAAGTACTTCTTCTACAGC AACATCATGAACCTTCAAGACCCGAGATCACCTGGCCAACCGCGAGATCCGGAAGCGGCCCTGATCGAGACCAACCG CGAGACCGCGAGATCTGTGGGACAAGGGCCGGGACTTCGCCACCTGCGGAAGGTGCTGAGCATGCCCAAGGTGAACA TCGTGAAGAAGACCGGAGTGCAGACCGGGGCTTACGAAAGGAGATCTCTGCCAACCGGAAACGCGACAGCTGATC GCCCCGAAAGAGGACTGGGACCCCAAGAAAGTACGGCGGCTTCGACAGCCCCACCTGGCTACGCGTGTGTTGGTGGC CAAGGTGAGAAAGGGCAAGAGCAAGAAAGTGAAGCGTGAAGGAGCTGTGGGCATCACCATCATGAGCGGAGCAGC TTCGAGAAGAAACCCCATCGACTTCTCTGGAGGGCAAGGGCTACAAGGAGGTGAAGAAAGGACCTGTATCATCAAGCTGCCCAA</p>
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513	

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514	

<p> AACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGTGAACACCGAGATCACCAAGGCCCCCTGAGCGCCAGCAT GATCAAGCGGTACGACGACACACAGGACCTGACCTGCTGAAGGCCCTGCTGCGGCGACGAGCTGCCCGAGAAAGTACA AGGAGATCTTCTTGACACAGACAAGAACGGTACGCCGGCTACATCGACGGCGGCCAGCCAGGAGATTCTACAAAG TTTCATCAAGCCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGTTGAAGCTGAACCGGGAGGACCTGCTGCGGAA GCAGCGGACCTTCGACAAACGGCAGCATCCCCACAGATCCACCTGGGCGAGCTGACGCCATCCTGCGGCGCAGGAGG ACTTCTACCCCTTCTGAAAGGACAAACCGGAGAAAGATCGAGAAGATCCTGACCTTCCGGATCCCTTACTACGTGGGCCCC TGGCCCCGGGCAACAGCCGTTCCCTGGATGACCCGGAAAGAGCGAGGAGACCATCAACCCCTGGAATCTCGAGGAGGTG GTGGACAAAGGGGCCAGCGCCAGAGCTTTCATCGAGCGGATGACCAACTTCGACAAGAACTTCGCCAACGAGAGGTGCT GCCCCAGCACAGCCTGCTACGAGTACTTCAACCTGTACAACGAGTGAACAGGTGAAGTACGTGACCGGAGGATGC GGAAAGCCGCTTCTGAGCGCGAGCAGAAAGAGGCCATCTGTGACCTGCTTCAAGACCAACCGGAAAGGTGACCCGTG AAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGATGCTTTCGACAGCTGGAGATCAGCGGCTGGAGACCGGTTCAA CGCCAGCCTGGGCACCTACCAGACCTGCTGAAGATCATCAAGGACAAGGACTTCTTGACACACGAGGAGAAACGAGGACA TCCTGGAGGACATCCTGCTGACCTGACCTGTTTCGAGGACCGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCAC CTGTTGACGACAAAGGTGATGAAGCAGCTGAAGCGCGGCTACACCGCTGGGGCGGCTGAGCCGGAAGCTGATCAA CGGCATCCGGGACAAGAGAGCGGCAAGACCATCTTGACTTCTGAAAGCGGCTTCGCCAACCGGAACCTTCATGC AGCTGATCCACGACGACGCTGACCTTCAAGGAGGACATCCAGAAAGGCCAGGTGAGCGGCCAGGGCGACAGCCTGCAC GAGCACATCGCCAAACCTGGCCGGCAGCCCCGCCATCAAGAAAGGACATCTGCAACCGTGAAGGTGGTGGACGAGCTGGT GAAAGTGTGGCCGGCACAAAGCCGAGAACATCTGTATCGAGATGGCCCGGAGAACAGACACCCAGAGGGCCAG AAGAAACGCCGGGAGCGGATGAAGCGGATCGAGGAGGACATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCG TGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGCCGGGACATGTACGTGGACCCAGGAG CTGGACATCAACCGGCTGAGCGACTACGACCTGGACCACATCTGCCCCAGAGCTTCTGAAAGGACGACAGCATCGACAA CAAGGTGCTGACCCGGAGCGACAAAGACCGGGCAAGAGCGACAACTGTGCCAGCGAGGAGGTGTGAAGAAAGATGAAG AACTACTGGCGCAGCTGCTGAACGCCAAAGCTGATCAACCCAGCGGAAAGTTTCGACAACCTGACCAAGGCCGAGCGGGCGG CCTGAGCGAGCTGGACAAGCCGGCTTCATCAAGCGGCAAGCTGGTGGAGACCCGGCAGATCACCAGACACGTGGCCCA TCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGACAAAGTATCCGGGAGGTGAAGGTATCACCCCTGAAGAGC AAGCTGTTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACCTACCACCGCCACGACCGC CTACCTGAACGCCGTGGTGGCACCCGCTGATCAAGAAGTACCCCAAGCTGGAGAGGAGTTTCGTACGGCGACTACA AGGTGTACGACGTGGGAAGATGATGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAAGTACTTCTTCTACAGC AACATCATGAATCTTCAAGACCGAGATCACCTGGCCACCGCGAGATCCGGAACGCCGCCCTGATCGAGACCAACCG CGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGGGAAGGTGCTGAGCATGCCCAAGGTGAACA TCGTGAAGAAAGACCGGTTGACAGCCGGCTTCAGCAAGGAGAGATCTCTCCCAAGCGGAAACAGACAAAGCTGATC GCCCGGAAGAAGGACTGGGACCCCAAGAAAGTACGGCGGCTTCGACAGCCCCACCTGGCTGCTACAGCTGCTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAAGTGAAGCGCTGAAGGAGTGAAGGAGTGAAGGAGGACCTGATCATCAAGCTGCCAA TTCGAGAAGAACCCCATCGACTTCTGAGGGCCAAAGGCTACAAGGAGGTGAAGAGGACCTGATCATCAAGCTGCCAA GTACAGCCTGTTCTGAGCTGGAGAACCGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAAGGCAACGAGCTGG </p>
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	CCCTGCCAGCAAGTACGTGAACCTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAAACGAG CAGAAAGCAGCTGTTCTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGACAGATCAGCGAGTTCAGCAAGCGGGT GATCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCAACCGGACAAAGCCCATCCGGGAGCAGGCCG AGAACATCATCCACCTGTTACCCCTGACCAACCTGGGGCCCCCGCCCTTCAAGTACTTCGACACCAACCATCGACCCGGA AGCGGTACACAGCACAGGAGGTGTGAGCGCCACCTGATCCACAGAGCATCACCGGCTGTACGAGACCCGGATC GACCTGAGCCAGCTGGCGGCGACGGCAGCGGCAGCCCCAAGAAAGCGGAAGGTGAGCGGACGCCCAAGAAAGAAAGC GGAAGGTGGACAGCGGTGA
515	ATGGACAAAGAGTACAGATCGGCTGGACATCGGCACCAACAGCGTGGCTGGGCCGTGATCACCGACGAGTACAAGGT GCCACGAAAGATTCAAGGTGCTGGCAACACCGACCGGCACAGCATCAAGAAAGAACTGATCGGCGCCCTGTGTTG ACAGCGGAGACCGCGAGGCCACCCGGCTGAAGCGGACCGCCGGCGGTACACCGCGGAAGAACCGGATCTG CTACCTGACGAGATCTTACGAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGCTTCTGCT GGAGGAGCAAGAAAGCAGAGCGGCACCCCATCTTCGGCAACATCTGTGGACGAGGTGGCCTACCAAGAGAAAGTACCCCA CCATCTACCACCTGCGGAAGAGTGTGGACAGCACCGACAAGCCGACCTGCGGTGATCTACCTGGCCCTGGCCAC ATGATCAAGTTCCGGGCCACTTCTGATCGAGGGCGACCTGAACCCCGACACAGCGACGTGGACAAGCTGTTTCATCCAG CTGGTGACAGACTACAACAGCTGTTTCGAGGAGAAACCCATCAACGCCAGCGGCTGGACGCCAAGGCCATCTGAGCGC CCGGTGAGCAAGAGCCGGGCTGGAGAACTGATCGCCCAAGCTGCCCGGAGAAAGAACGGCCTGTTCCGGCAACC TGATCGCCCTGAGCTGGCCCTGACCCCAACTTCAAGAGCAACTTCACTGACCTGGCCGAGGACGCCAAGCTGCACTGAGCA AGGACACCTACGACGACCTGGACAACCTGCTGGCCAGATCGGCGACCACTACGCGGACCTGTTCTTCCGCGCCAAAG AACCTGAGCGACGCCATCTGCTGAGCGACATCTGCGGTGAACACCGAGATCAACAAAGCCCTGAGCGCCAGCAT GATCAAGCGGTACGACGACCAACAGGACCTGACCTGCTGAAGCCCTGGTGGCGCAGCAGTGGCCGAGAAGTACA AGGAGATCTTCTTGACCAAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCCAGCCAGGAGGAGTTCTACAAG TTCATCAAGCCCATCTTGAGAAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAA GCAGCGGACCTTGACAAACGGCAGCATCCCCCAACAGATCCACCTGGCGGAGCTGCACGCCATCCTGCGGCGCAGGAGG ACTTCTACCCCTTCTGAAAGGACAACCGGGAGAAGATCGAGAAGATCTGACCTTCCGGATCCCTACTACGTGGGCCCC TGGCCCGGGCAACAGCCGTTCCCTGGATGACCCGGAAGCGAGGACCATCACCCCTGGAACCTCGAGGAGGTG GTGGACAAGGGCGCCAGCGCCAGAGCTTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCT GCCAAGCACAGCCTGCTACGAGTACTTCACTGTTACAAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGC GGAAGCCCGCTTCTGAGCGGCGAGCAAGAAAGGCCATCTGGACCTGCTGTTCAGACCAACCGGAAGGTGACCGTG AAGCAGCTGAAGGAGGACTACTTCAAGAGATCGAGTCTTCGACAGCGTGGAGATCAGCGGCTGGAGCCCGTTCAA CGCCAGCCTGGGCACTACACGACCTGCTGAAGATCATCAAGGACAAGGACTTCTTGACACACGAGGAGAACGAGGACA TCTTGAGGACATGCTGACCTGACCTGTTGAGGACCGGAGATGATCGAGGAGCGGTGAAGACCTACGCCAC CTGTTGACGACAAGGTGATGAAGCAAGTGAAGCGGCGGCTGACCGGCTGGGGCGGCTGAGCCGGAAGCTGATCAA CGGCATCCGGGACAAGCAGAGCGCAAGACCATCTTCTGAGAGCGACGGCTTCGCCAACCGGAACCTTCATGC AGCTGATCCACGACGACAGCTGACCTTCAAGGAGGACATCCAGAGGCCCAAGGTGAGCGGCCAGGCGACAGCCTGCAC GAGCACATCGCCAACCTGGCCGCGCAGCCCCCGCATCAAGAAAGGGCATCTTCAGACACCTGAAGGTGTTGGTGGACGAGCTGCT

	<p>GAAAGGTGATGGCCGGCACAAGCCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACAGACACCCAGAAGGGCCAG AAGAAACGCCGGGAGCGGATGAAGCGGATCGAGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGAGACACCCCG TGGAGAACACCCAGCTGCAGAACGAGAGCTGTACCTGTACTACCTGCAGAACGCCGGGACATGTACGTGGACACAGGAG CTGGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTCCCCAGAGCTTCTGAAGGACGACAGCATCGACAA CAAGTGCTGACCCGGAGCGACAAGAACCCGGGCAAGAGCGACAACGTGCCAGCGAGGAGGTGTTGAAGAAATGAAG AACTACTGGCGGAGCTGTGAACGCCAAGCTGATCAACAGCGGAAGTTTCGACAACCTGACCAAGCCGAGCGGGCGG CCTGAGCGAGCTGGACAAGGCCGCTTCATCAAGGGCAGCTGGTGAGACCCGGCAGATCACTAAGCAGCTGGGCCAGA TCCTGGACGCCGGATGAACACCAAGTACGACGAGAACGACAAAGCTGATCCGGGAGGTGAAGGTGATCACCTGGAAGAGC AAGCTGCTGAGCGACTTCCGGAAGGACTTCCAGTTTACAAGGTGCGGGAGATCAACAATACCACCCAGCCACGACGC CTACCTGAACGCCGTGGTGGCACCCGCTGTATCAAGAAAGTACCCCAAGCTGGAGAGAGTTTCGTACGGCGACTACA AGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGCAAGGCCACCGCAAGTACTTCTTCTACAGC AACATCATGAATTTCTTCAAGACCGAGATCACCTGGCCAACGGCGAGATCCGGAAAGCGCCCTGATCGAGACCAACGG CGAGACCGCGGAGATCGTGTGGACAAGGGCCGGGACTTCGCCACCGTGGGAAGGTGCTGAGCATGCCCAAGGTGAACA TCGTGAAGAAAGACCGAGGTGCAAGACCGCGGCTTCAGCAAGGAGAGCATCTGCCCAAGCGGAACAGCGACAAGCTGATC GCCGGAAGAAAGGACTGGACCCCAAGAAAGTACGGCGGCTTCGACAGCCCAACCTGGCTACAGCTGTGTTGGTGGC CAAGGTGGAGAAAGGCAAGACAAAGCTGAAGCGTGAAGGAGCTGTGGGCATCACCATCATGAGCGGAGCAGC TTCGAGAAAGAACCCATCGACTTCTGTGAGGCCAAGGGCTACAAGGAGGTGAAGAGGACCTGATCATCAAGCTGCCAA GTACAGCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGTGGCCAGCGCGGAGCTGCAGAAAGGCAACGAGCTGG CCCTGCCAGCAAGTACGTGAATTTCTGTACTGTGCCAGCCACTACGAGAAAGTGAAGGCAAGCCCGAGGACAACGAG CAGAAAGCAGCTGTTCGTGGAGCAGCAAGCACTACCTGGACGAGATCATCGACAGATCAGCGAGTTCAAGCAAGCGGT GATCTGGCCGACGCCAACCTGGACAAGTGTGTAGCGCTACAACAAGCACCAGGACAAGCCCATCCGGGAGCAGGCCG AGAACATCATCCACCTGTACCCCTGACCAACCTGGGCGCCCCGCTTCAAGTACTTCGACACCACTCATCGACCCGGA AGCGGTACACACGACCAAGGAGGTGCTGGACGCCACCTGTATCCACAGAGCATCACCGGCTGTACGAGACCCCGGATC GACCTGAGCCAGCTGGCGGCGACTGA</p>
516	<p>GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACAAGAAAGUACUCCAUCCGGCCUGGACAUCG GCACCAACUCCGUGGGCUGGGCGUGAUCACCGACGAGUAACAAGGUGCCCUCCAAAGAAAGUUAAGGUGCUGGGCAACA CCGACCGGCACUCCAUCAAGAAAGAACCUGAUCGGCGCCUGCUUUCGACUCCGGGAGACCGCGGAGGCCACCCGGCU GAAGCGGACCGCCGGCGGUACACCCGGCGGAAGAACCAGAUUCUACCGGAGGAUUCUUCUCCAACAGAGAU GGCCAAAGGUGGACGACUCCUCCACCGGUGGAGGUCCUUCUGGUGGAGGAGGACAAAGAGCAGCAGCGGCCA CCCCAUUCUGGCAACUUCGUGGACGAGGUGGCCUACACGAGAAGUACCCCAACUACCUACCGCGGAAGAAGCUG GUGGACUCCACCGACAAGGCCGACUUCGCGUGAUCUACUUGGCCUGGCCACAUAUAAGUUCGGGGCCACUUC UGAUCGAGGGGACCUAGAACCAGCAACUCCGACUGGAGCAAGCUGUUAUCCAGCUGGUGGAGCAUACAACAGC UGUUCGAGGAGAACCCAUACAACGCCUCCGGCGUGGAGCCCAAGGCCAUCCUCCGCCCGGCGUCCAAAGUCCCGGCG GCUUGGAGAACCCUAGUCCCAAGUCCCGCGGAGAAAGAAAGAGCGGCGUGUUGGCAACCUAGUCCGCCUGGCGG CUGACCCCAACUUCAGUCCAAUUCGACCCUGGCCGAGGAGCCAGCUGCAGCUGUCCAAAGGACACCUACGACGACG</p>

<p> ACCUGGACAAACCUGUGGGCCCA GAUCGGCGACCAGUACGCGACCUCUGUUCUCCUGGCCGCCAAGAACCUCUGUCCGAGCCCAU CCUGUCUCCGACAUCCUGCGGGUGAACACCGAGAUACACAGGCCCCUCCUGUCCGCCUCCAU GAUCAAGCGGUACGAC GAGCACCAACGAGACCUUGACCCUGGUAAGGCCUUGGUGGCGAGCAGCUGCCCCGAGAAAGUACAAGAGAUUCUUC GACCAUCCAAGAACGGCUACGCCGCUACUACGACGGCGGCCUCCCGAGGAGUUCUACAAGUACAAGCCCA UCCUGGAGAAAGUUGACGGCACCGAGGAGCUGUGGUGAAGCUGAACCGGGAGGACCUGUGCGGAAGCAGCGGACCU UCGACAACGGCUCCAUCCCCACCAAGAUCCUGGGCGAGUGACGCCAUCCUGCGGGCGAGGAGGACUUCUACCC CUUCCUGAAGGACAACCGGAGAGAUUCGAGAGAUAUCCUGACUUCGGAUCCUCCUACUACGUGGGCCCCUGGCCCGG GGCAUCCCGGUGCCUGGUAUACCCCGAAGUCCGAGGAGACCAUACCCCCUGGAACUUCGAGGAGGUGGUGGAC AAGGGCCUCCGCCAGUCCUUAUCGAGCGGAUGACCAUUCGACAAGAACUCCGCCAACGAGAAAGGUGCUGCCCA AGCAUCCUCCUGUGUACGAGUAUUCACCGUGUAACAAGAGCUGACCAAGGUAAGUACGUGACCGAGGGCAUCCGGA AGCCCGCUUCUGUCCGGGAGCAGAAAGAGGCCAUUCGUGGACCUUGUUAAGACCAACCGGAAGGUGACCGUGA AGCAGCUAAGGAGGACUAUUAAGAAUGAGAGUUCUAGCUGGAGUUCGACUCCUGGAGAUUCCGGGUGGAGGCCGUUCA ACGCCUCCUUGGCACCUACACGACCUUGCUGAAGAUACAAGGACAAGGACUUCUGGACAACGAGGAGAACGAGG ACAUCCUGGAGGACAUUGUGUACCCUGACCCUUGUUGAGGACCGGAGAU GAUCGAGGAGCGGCUAGAGACCUACG CCCACUUGUUCGACGACAAGGUGAUGAAGCAUCUGAAGCGGGCGGUACACCGGCUGGGGCCGGUUCGCCGGAAGC UGAUCAACGGCAUCCGGGACAAGCAGUCCGGCAAGACCAUCCUGGACUCCUGAAGUCCGACGGCUUCGCCAACCGGA ACUUAUGCAGCUUCCACGACGACUCCUCCUACCUAAGAGGACAUCCAAGAGGCCAGGUUCCGGCCAGGGCG ACUCCUGCACGAGCACAUCCGCAACCUUGGGCGGUCCCCGCCCAUACAAGAGGGCAUCCUGCAGACCGUGAAGGUGGU GGACGAGCUGGUGAAGGUGAUGGGCCGGCACAGCCCGAGAACAUUCGUGAUCGAGUUGGCCGGGAGAACAGACAC CCAGAAAGGCCAGAAACUCCCGGAGCGGAUGAAGCGGAUCGAGGAGGCAUCAAGGAGCUGGGCUCCCAAGUCCU GAAAGGACACCCCGUGGAGAACACCCAGCUGCAGACGAGAGCUGAACUUGUACUACCUAGCAACCGGCCGGGACAU GUACGUGGACAGGAGCUGGACAUCAACCGGCUGUCCGACUACGACGUGGACCACAUCCUGUCCCAAGUCCUGAA GGACGACUCCAUCCGACAACAAGUGCUGACCCGGUCCGACAGAAACCGGGCAAGUCCCGACAAACGUGCCUCCGAGGA GUGUGUGAAGAAAGAUAGAACAUCUGGGCGCAGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCU GACCAAGGCCGAGCGGGCGGCCUUGUCCGAGCUGGACAAGGCCGGCUUAUCAAGCGGCAGCUGGUGGAGACCCGGCA GAUACCAAGCACGUGGGCCAGAUCCUGGACUCCGGAUGAACACCAAGUACACGAGAACGACAAGCUGAUCCGGGA GGUGAAGGUGAUACCCUGAAGUCCAAGCUGGUGUCCGACUUCGGAAGGACUUCAGUUCUACAAGGUGCGGGAGAU CAACAACUACCAACCGCCACGACGCCUACUUGAACGCCUGGUGGGCACCGCCU GAUCAAGAAUAACCCCAAGCUG GAGUCCGAGUUCUGUACGGCGACUACAAGGUGUACGACGUGCGGAAGAUGAUCGCCAAGUCCGAGCAGGAGUCCGC AAGGCCACCGCCAAAGUACUUCUACUCCACAACAUAGAUCUUAAGACGACGAGAUACCCUGGCCAACGGCGAGA UCCGGAAGCGGCCUGAUCGAGACAACCGCGGAGACCGCGAGAUCCUGGGACAAGGGCCGGACUUCGCCACCG UCGGGAAGGUGUGUCCAUCCGCCAGGUGAACAUCUGUAGAAGACACGAGGUGCAGCGGGCGGUUCUCCAAAGGAGU CCAUCCUGCCCAGCGGAACUCCGACAAGCUGUACCGCCGGAAGAGGACUGGGACCCCAAGAAUAACGCGGGCUUCG ACUCCCCACCGUGGGCUACUCCGUGUGGUGGCAAGGUGGAGAAAGGCAAGUCCAAAGAAAGUAGUCCUGGAG AGGAGCUGCUGGGCAUACCAUCAUGGAGCGGUCCUCCUUCGAGAGAAACCCCAUCGACUUCUUGGAGGCCAAGGGCU </p>

	ACAAAGGAGGUGAAGAAAGGACCUUGAUCAUCAAAGCUGCCCCAAGUACUCCCCUGUUCGAGCUGGAGAACGCGCCGGAAAGCGGA UGCUGGCCUCCCGCGCGAGCUGGCAAGGGCAACGAGCUGGCCCUGCCCUCCAAAGUACGUGAACUUCCUGUACCUUGGC CUCCACUACGAGAGCUGAAGGGCUCCCCGAGGACACGAGCAGAAAGCAGGUGUUCGUGGAGCAGCACAAAGCACUA CCUGGACGAGAUCAUCGAGCAGAUUCUCCGAGUUCUCCAAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCU GUCCGCCUACAAACAGCACCGGGACAAGCCC AUCCGGGAGCAGGCCGAGAACAUCCACCUGUUCACCCUGACCCAAC CUGGGCGCCCCGCCGUUCAAGUACUUCGACACCAACCAUCGACCGGAAGCGGUACACCUCCACCAAGGAGGUGCUGG ACGCCACCUUGAUCCACAGUCCAUACCGGCCUUGUACGAGACCCGGAUCGACCUUCCCCAGCUGGGCGGCGACGGGG CGGCUCUCCCCAAGAAGAAAGCGGAAGGUGUGACUAGCACCGCCUCAAAGAACCCGAAUGGAGUCUUAAGCUACAUA AUACCAACUUACACUUAACAAAUUGUUGUCCCCCAAAAUAGCCAUUCGUUCUGUCUCCUAAUAAAAAGAAAGUUUC UUCACAUUCUCUCGAG
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1.2 Human KLKB1 guide design and human KLKB1 with cynomolgus homology guide design

[00591] Guide RNAs were designed toward human *KLKB1* (ENSG00000164344) targeting the protein coding regions within Exons 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15. Guide RNAs were also designed toward cynomolgus *KLKB1* (ENSMFAT00000002355) targeting the protein coding regions within Exons 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15. Guide RNAs and corresponding target genomic coordinates are provided above (Table 1).

1.2. Cas9 (mRNA/protein) and guide RNA delivery *in vitro*

1.2.1. Cell Preparation, delivery *in vitro*

[00592] Primary human hepatocytes (PHH) (Gibco, Lots Hu8296, Hu8300, and Hu8284, Hu8296, HU8290, and HU8317) and primary cynomolgus hepatocytes (PCH) (Gibco, Lots Cy367, Cy400, and 10281011) were thawed and resuspended in hepatocyte thawing medium with supplements (Gibco, Cat. CM7500) followed by centrifugation. The supernatant was discarded and the pelleted cells resuspended in hepatocyte plating medium (William's E Medium (Invitrogen, Cat. A1217601) with Dexamethosone + cocktail supplement, FBS content, and Plating Supplements (Gibco, Cat. CM3000)). Cells were counted and plated on Bio-coat collagen I coated 96-well plates (ThermoFisher, Cat. 877272) at a density of 30,000-35,000 cells/well for PHH and 40,000-45,000 cells/well for PCH. 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 (William's E Medium with maintenance supplements (Gibco, Cat. CM4000)) or Cellartis Power Primary HEP Medium (Takada, Cat. Y20020).

[00593] Guide RNAs targeting *KLKB1* were delivered to cells using a liposomal system with Cas9 protein, for example, or using an LNP formulation comprising Cas9 mRNA and guide RNA as further described below.

1.2.2. RNP transfection

[00594] RNP transfection was used with a liposomal system (Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) and CRISPR reagents (guide RNA, Cas9 Protein) to shuttle a ribonucleoprotein (RNP) complex across the cell membrane.

[00595] For studies utilizing dual guide (dgRNA), individual crRNA and trRNA was pre-annealed by mixing equivalent amounts of reagent and incubating at 95°C for 2 min and cooling to room temperature. The gRNA consisting of pre-annealed crRNA and trRNA was added to Spy Cas9 protein in the reaction buffer (OptiMem) to form a RNP complex and the formed RNP complex was incubated at room temperature for 10 minutes. The RNP complex was diluted with OptiMem to prepare a 1 μ M RNP complex stock solution. A Transfection Mix including Lipofectamine RNAiMAX and OptiMem was prepared and incubated for at least 5 minutes. The Transfection Mix was added to the RNP complex and incubated at room temperature for 10 minutes, and the Transfection Agent (Transfection Mix and RNP complex) was added to cells. Cells were transfected with the RNP complex containing Spy Cas9 protein (10nM), individual guide/tracer RNA (10 nM), and Lipofectamine RNAiMAX (1.0 μ L/well) and OptiMem.

1.2.3. RNP Electroporation

[00596] RNP Electroporation was used with the cell electroporation system (Lonza 4D Nucleofector™ kit 816B0346) and CRISPR reagents, gRNA and Cas9 protein, to shuttle a ribonucleoprotein (RNP) complex across the cell membrane.

[00597] For studies utilizing dgRNA, individual crRNA and trRNA were pre-annealed by mixing equivalent amounts of reagent and incubating at 95°C for 2 min and cooling to room temperature.

[00598] For studies utilizing sgRNA, a 50uM sgRNA stock solution was prepared by incubating equal amounts of 100 uM sgRNA to water at 95°C for 2 min followed by cooling on ice for 5 minutes. The sgRNA was added to the Spy Cas9 protein in reaction buffer (20 mM Hepes, 100 mM KCl, 1 mM MgCl₂, 10 % glycerol, 1 mM DTT pH 7.5) to form an RNP complex and incubated at room temperature for 10 minutes. Cells were electroporated (Amaxa™ 96-well Shuttle™ Cat. AAM-1001S) with the RNP complex containing Spy Cas9 protein (2uM) and gRNA (4 uM) and Lonza P3 buffer (Catalog #: V4SP-3960). Post electroporation, hepatocyte plating media (Will's E, Cat. A12176-01) was added to the cell plate, the media with cells was transferred to collagen coated plates (Corning 354407). After 4-6 hrs, the media was changed to maintenance media (William's E (Gibco, Cat. A12176-01, Lot 2039733) and maintenance supplement (Gibco, Cat. A12176-01, Lot 2039733) for overnight incubation at 37°C overnight.

1.2.4. Preparation of LNP formulation containing sgRNA and Cas9 mRNA

[00599] 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 in Examples 2-10 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), cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively. 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 in Examples 2-10 comprise a Cas9 mRNA and an sgRNA.

[00600] The LNPs were prepared using a cross-flow technique utilizing impinging jet mixing of the lipid in ethanol with two volumes of RNA solutions and one volume of water. The lipid in ethanol was mixed through a mixing cross with the two volumes of RNA solution. 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 concentrated using tangential flow filtration on a flat sheet cartridge (Sartorius, 100kD MWCO) and then buffer exchanged using PD-10 desalting columns (GE) into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS). The resulting mixture was then filtered using a 0.2 µm 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.

1.2.5. sgRNA and Cas9 mRNA lipofection

[00601] Lipofection of Cas9 mRNA and gRNAs used pre-mixed lipid formulations. The lipofection reagent 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), cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively. This mixture was reconstituted in 100% ethanol then mixed with RNA cargos (e.g., Cas9 mRNA and gRNA) at a lipid amine to RNA phosphate (N:P) molar ratio of about 6.0. Guide RNA was

chemically synthesized by commercial vendors or using standard in vitro synthesis techniques with modified nucleotides. A Cas9 ORF of Table 5 was produced by IVT as described in WO2019/067910, see e.g. paragraph [00354], using a 2 hour IVT reaction time and purifying the mRNA by LiCl precipitation followed by tangential flow filtration. Lipofections were performed with 3% cynomolgus serum and a ratio of gRNA to mRNA of 1:1 by weight.

1.2.6. LNP Transfection

[00602] Modified sgRNAs targeting human KLKB1 were formulated in LNPs as described in Example 1. Primary human hepatocytes were plated as described in Example 1. Cells were incubated at 37°C, 5% CO₂ for 48 hours prior to treatment with LNPs. LNPs were incubated in media containing 3% fetal bovine serum (FBS) at 37°C for 10 minutes. Post-incubation, media was aspirated from cells and the mixture of media with 3% FBS and LNP were added to the hepatocytes. At 72 to 96 hours post-transfection, a portion of the cells were collected and processed for NGS sequencing as described in Example 1.

1.3. Genomic DNA isolation

[00603] Transfected PHH and PCH were harvested at 48 or 72 hours post-transfection. The gDNA was extracted from each well of a 96-well plate using 50 µL/well BuccalAmp DNA Extraction solution (Epicentre, Cat. QE09050) or Zymo's Quick RNA/DNA extraction kit (Cat. R2130) according to manufacturer's protocol. All DNA samples were subjected to PCR and subsequent NGS analysis, as described herein.

1.4. Next-generation sequencing ("NGS") and analysis for on-target cleavage efficiency

[00604] To quantitatively determine the efficiency of editing at the target location in the genome, next generation 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. *KLKB1*), and the genomic area of interest was amplified. Primer sequence design was done as is standard in the field.

[00605] 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 human (e.g., hg38) reference genome 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.

[00606] 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.

[00607] A biochemical assay (See, *e.g.*, Cameron et al., *Nature Methods*. 6, 600-606; 2017) was used to discover potential off-target genomic sites cleaved by Cas9 targeting *KLKB1*. Purified genomic DNA (gDNA) from cells were digested with *in vitro* assembled ribonucleoprotein (RNP) of Cas9 and sgRNA, to induce DNA cleavage at the on-target site and potential off-target sites with homology to the sgRNA spacer sequence. After gDNA digestion, the free gDNA fragment ends were ligated with adapters to facilitate edited fragment enrichment and NGS library construction. The NGS libraries were sequenced and through bioinformatic analysis, the reads were analyzed to determine the genomic coordinates of the free DNA ends. Locations in the human genome with an accumulation of reads were then annotated as potential off-target sites.

[00608] In known off-target detection assays, such as the biochemical assay used above, a large number of potential off-target sites are typically recovered, by design, so as to “cast a wide net” for potential sites that can be validated in other contexts, *e.g.*, in a primary cell of interest. For example, the biochemical assay typically overrepresents the number of potential off-target sites as the assay utilizes purified high molecular weight genomic DNA free of the cell environment and is dependent on the dose of Cas9 RNP used. Accordingly, potential off-target sites identified by these assays were validated using targeted sequencing of the identified potential off-target sites.

[00609] In one approach to targeted sequencing, Cas9 and a sgRNA of interest (*e.g.*, a sgRNA having potential off-target sites for evaluation) were introduced to PHH or PCH cells. The cells were then lysed and primers flanking the potential off-target site(s) were used to generate an amplicon for NGS analysis. Identification of indels at a certain level can be used to validate potential off-target site, whereas the lack of indels found at the potential off-target site can indicate a false positive in the off-target assay that was utilized.

[00610] Guides showing on target indel activity were tested for potential off-target genomic cleavage sites with this assay. Repair structures were manually inspected at loci with statistically relevant indel rates at the off-target cleavage sites to validate the repair structures.

1.5 Transcript analysis by Quantitative PCR

[00611] Quantitative PCR was performed to assess *KLKB1* transcript levels. To isolate mRNA, the Qiagen RNeasy Mini Kit (Qiagen, Cat. 74106) was used. The RNeasy Mini Kit procedure was completed according to the manufacturer's protocol.

[00612] RNA was quantified using a Nanodrop 8000 (ThermoFisher Scientific, Cat. ND-8000-GL). The RNA quantification procedure was completed according to the manufacturer's protocol. RNA samples were stored at -20°C prior to use.

[00613] The Taqman RNA-to-Ct 1-Step Kit (Thermo Fisher Scientific, Cat. 4392938) was used to create the PCR reactions. The reaction set-up was completed according to the manufacturer's protocol. Alternatively, a Cells-to-CT 1-Step TaqMan Kit (Thermo Fisher Scientific, Cat. A25603) was used to produce samples for qPCR. Quantitative PCR probes targeting human or cynomolgus monkey *KLKB1* (Thermo Fisher Scientific, Cat. 4351372, transcript UniGene ID Hs01111828_m1; Thermo Fisher Scientific, Cat. 4331182, transcript UniGene ID Hs00168478_m1), internal control *PPIB* (Thermo Fisher Scientific, Cat. 4351372, transcript UniGene ID Hs00168719_m1; Thermo Fisher Scientific, Cat. 4331182, transcript UniGene ID Mf02802985_m1), internal control *GAPDH* (Thermo Fisher Scientific, Cat. 4351372, transcript UniGene ID Hs02786624_g1), and internal control *18S* (Thermo Fisher Scientific, Cat. 4319413E) were used in the PCR reactions. The StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Cat. 4376600) was used to perform the real-time PCR reaction and transcript quantification according to the manufacturer's protocol.

[00614] Double delta Ct analysis of *KLKB1* mRNA was provided using the Ct values determined from the StepOnePlus Real-Time PCR System. A double delta Ct value was calculated for the Ct values for internal controls within each sample compared to the values for *KLKB1*. The expression fold change was determined based on the double delta Ct value for each sequence.

1.6. Protein analysis of tissue culture media by ELISA

[00615] PHH or PCH were transfected as previously described. Starting at three days post-transfection and plating (96-well plate), media was changed on cells every two days. Seven to ten days post-transfection, media was removed from cells and then replaced with 100 µL of William's E culture media or Cellartis Power Primary HEP Medium (Takada, Cat. Y20020). Twenty-four to forty-eight hours later, media was harvested and stored at -20°C. Total secreted KLKB1 protein levels were determined using a prekallikrein ELISA Kit (Abcam, Cat. ab202405), which detects prekallikrein and kallikrein (also, called total

kallikrein). Kit reagents and standards were prepared using the manufacturer's protocols. Prior to running the ELISA, frozen media was thawed at room temperature and centrifuged at 1000 rpm for 1 minute to pellet debris and then placed on ice. For the ELISA, 10 to 40 μ L of media was diluted with Sample Diluent NS assay diluent to a total volume of 50 μ L. The ELISA procedure was completed according to the manufacturer's protocol. The plate was read on a SpectraMax M5 plate reader. Total kallikrein levels were calculated by SoftMax Pro software ver. 6.4.2 using a four-parameter logistic curve fit of the standard curve. Reduction of total secreted pre-kallikrein protein for cells treated with KLKB1 reagents was determined when compared to wells treated with control reagents or untreated samples.

1.6.1 Protein analysis of serum by ELISA

[00616] Serum pre-kallikrein levels were measured in humanized mice using the following procedure. Six to seven days post-dose, animals were euthanized by exsanguination via cardiac puncture under isoflourane anesthesia. Blood was collected into serum separator tubes, and allowed to clot at room temperature for 2 hours before being spun down at 9000g for 10 min to separate the serum. Samples were stored at -20C until analysis.

[00617] Pre-kallikrein protein levels were determined using a human pre-kallikrein ELISA Kit (Abcam, Cat. ab202405), which detects prekallikrein and kallikrein (also, called total kallikrein). Briefly, sera were serially diluted with kit sample diluent to a final dilution of 1:500 or 1:1000 fold before adding to the ELISA plate. The assay was carried out according to the manufacturer's protocol. The plate was read on a Clariostar plate reader (BMG Labtech). Pre-kallikrein levels were calculated by Mars software using a four-parameter logistic curve fit of the standard curve. Reduction of total secreted pre-kallikrein protein for cells treated with KLKB1 reagents was determined when compared to wells treated with control reagents or untreated samples.

1.6.2. Protein analysis by western blot

[00618] PHH were treated with LNP formulated with select guide RNAs from Table 1 as further described below. LNPs were incubated in Cellartis Power Primary HEP Medium (Takada, Cat. Y20020) containing 3% FBS or cynomolgus serum at 37°C for 10 minutes. Post-incubation the LNPs were added to the human hepatocytes. Starting at three days post-transfection, media was changed on cells every two days. Ten to fourteen days post-transfection, for cells plated in a 96-well plate the media was removed and the cells were lysed with 50 μ L/well RIPA buffer (Boston Bio Products, Cat. BP-115) plus freshly added protease inhibitor mixture consisting of complete protease inhibitor cocktail (Sigma, Cat.

11697498001), 1 mM DTT, and 250 U/ml Benzonase (EMD Millipore, Cat. 71206-3) per 30,000 to 45,000 cells. Cells were kept on ice for 30 minutes at which time NaCl (1 M final concentration) was added. Cell lysates were thoroughly mixed and retained on ice for 30 minutes. The whole cell extracts ("WCE") were transferred to a PCR plate and centrifuged to pellet debris. A Bradford assay (Bio-Rad, Cat. 500-0001) was used to assess protein content of the lysates. The Bradford assay procedure was completed according to the manufacturer's protocol. Extracts were stored at -20°C prior to use.

[00619] Western blots were performed to assess KLKB1 protein levels. Lysates were mixed with Laemmli buffer (Boston BioProducts, Cat. BP-111R) and denatured at 95°C for 10 minutes. Western blots were run using the NuPage system on 4-12% Bis-Tris gels (Thermo Fisher Scientific, Cat. NP0323BOX) according to the manufacturer's protocol followed by wet transfer onto 0.45 µm nitrocellulose membrane (Bio-Rad, Cat. 1620115). After transfer membranes were rinsed thoroughly with water and stained with Ponceau S solution (Boston Bio Products, Cat. ST-180) to confirm complete and even transfer. Blots were blocked using 5% dry milk in TBS for 30 minutes on a lab rocker at room temperature. Blots were rinsed with TBST and probed with rabbit α-kallikrein monoclonal antibody (Abcam, Cat. ab124938) at 1:1000 in TBST. For blots with *in vitro* cell lysate, GAPDH was used as a loading control (Novus, Cat. NB600502) at 1:2500 in TBST and incubated simultaneously with the KLKB1 primary antibody. After incubation, blots were rinsed 3 times for 5 minutes each in TBST. Blots were visualized and analyzed by densitometry using a Licor Odyssey system.

1.6.3. Electrochemiluminescence-based detection of plasma kallikrein levels

[00620] Plasma kallikrein levels in in samples were measured by an immunoassay using an electrochemiluminescence detection platform by MesoScale Discovery (MSD). A 96-well MSD standard plate (Cat. No: L15XA) was coated with 25 or 40 µL of a mouse monoclonal capture antibody for kallikrein (LS-Bio, LS-C38308) at a concentration of 1 µg/mL in PBS over night at 4° C. On the following day, the wells were washed and then blocked with 150 µL of 3% Blocker-A (MSD, Cat. No: R93AA) and incubated for 1 hour at room temperature on a shaker set to 700rpm. After washing, the samples for the determination of kallikrein concentration along with a human kallikrein standard of a known concentration, made in-house, were added to the wells and incubated for 2 hours at room temperature on a shaker set to 700rpm. Both samples and standards were diluted in 1% Blocker-A, optionally with 0.05% Tween20.

[00621] After washing, a 25 μ L of the detection antibody solution was added (LSBio #C185168 at 1 μ g/mL and MSD# R32AG at 500 μ g/mL in 1% Blocker-A with 0.05% Tween20) and incubated for 1 hour at room temperature. The plate was washed and 150 μ L MSD gold Read Buffer (MesoScale Discovery, Cat. No: R92TG) was added to each well. The plate was read using the QuickPlex SQ 120 (MesoScale Discovery). The plate was washed 3 $\times$ with PBS with 0.05% Tween20 between the different steps.

1.7. Fluorometric analysis of plasma kallikrein activity

[00622] Total plasma kallikrein activity levels in samples, such as non-human primate (NHP) samples, were measured using the Fluorometric SensoLyte Rh110 Plasma Kallikrein Activity Assay Kit (Anaspec Cat No: AS-72255). A chloroform pretreatment was performed to inhibit C1-Inhibitor activity by mixing an equal volume of cold chloroform with K2EDTA NHP plasma in a 96-well plate. The plate was then centrifuged for 5 min at 4°C at 16,000X g and 10 μ L of the treated plasma was carefully collected from the top layer. In a 96-well black microplate, the 10 μ L of pretreated plasma was mixed with 30 μ L of assay buffer, 10 μ L of Plasma Prekallikrein Activator and 50 μ L of substrate, all of which are provided in the kit and prepared according to kit protocol. The fluorescence measurements were immediately initiated at Ex/Em = 490 nm/520 nm with a reading every 5 minutes for 1 hour on a SpectraMax M5 plate reader. The slope of the linear portion of the kinetic fluorometric readout for a given post-treatment plasma sample is compared to the slope of the pre-treatment plasma sample from the same animal to calculate % basal.

1.7.1 Electrochemiluminescence-based detection of plasma kallikrein levels in non-human primates

[00623] Plasma kallikrein levels in non-human primates (NHP) were measured by an immunoassay using an electrochemiluminescence detection platform by MesoScale Discovery (MSD). A 96-well MSD standard plate (Cat. No: L15XA) was coated with 40 μ L of a mouse monoclonal capture antibody for kallikrein (LS-Bio, LS-C38308) at a concentration of 1 μ g/mL in PBS over night at 4° C. On the following day, the wells were washed and then blocked with 150 μ L of 3% Blocker-A (MSD, Cat. No: R93AA) and incubated for 1 hour at room temperature on a shaker set to 700rpm. After washing, NHP samples for the determination of kallikrein concentration along with a NHP kallikrein standard of a known concentration, made in-house, were added to the wells and incubated for 2 hours at room temperature on a shaker set to 700rpm. Both samples and standards were diluted in 1% Blocker-A with 0.05% Tween20.

[00624] After washing, 25 μ L of the detection antibody solution was added and incubated for 1 hour at room temperature. The plate was washed and 150 μ L MSD gold Read Buffer (MesoScale Discovery, Cat. No: R92TG) was added to each well. The plate was read using the QuickPlex SQ 120 (MesoScale Discovery). The plate was washed 3 $\times$ with PBS with 0.05% Tween 20 between the different steps.

1.8 Vascular permeability assay

[00625] The Evans Blue vascular permeability assay is an established model of edema and vascular leakage that can be used as a model in the study of HAE (see, e.g., Bhattacharjee et al., 2013). The assay is based on the injection of Evans Blue, an albumin-binding dye, in a test animal, typically a mouse. Under physiologic conditions the endothelium is impermeable to albumin, so the albumin-bound Evans blue remains restricted within blood vessels. In pathologic conditions that promote increased vascular permeability, extravasation of Evans Blue can be readily observed qualitatively e.g., by the presence of blue color in the ears, feet, and nose of mice after intravenous injection, or quantitatively by measurement of dye incorporated into tissue, e.g., intestine.

[00626] Using the huKLKB1 mouse, a model for vascular permeability was developed to evaluate the potential of KLKB1 editing to mitigate the effects of excess bradykinin production (Bhattacharjee et al., 2013). A modified KLKB1 targeting sgRNA and the Cas9 mRNA was administered in a dose response at total RNA doses of 0.03 mg/kg, 0.1 mg/kg and 0.3 mg/kg. Additional groups were treated with 0.3 mg/kg of non-targeting-LNP control and TSS vehicle control. Thirteen days post-dose, vascular permeability was induced using a 2.5 mg/kg intraperitoneal injection of the angiotensin converting enzyme (ACE) inhibitor captopril. After 15 minutes, a mixture of Evans Blue Dye (30mg/kg) and dextran sulfate (0.3 mg/kg) was administered by intravenous tail injection. The animals were euthanized 15 minutes after this injection and evaluated for dye extravasation into the colon by optical density (OD) at the absorbance of 600 nm via the Clariostar plate reader (BMG LabTech). Liver and serum were collected to quantify huKLKB1 gene editing and kallikrein protein, respectively.

Example 2: Screening and *In Vitro* Guide Characterization

2.1. Screening of dual guide RNAs (dgRNAs) that target human *KLKB1*

[00627] Guides targeting human *KLKB1* were prepared as dual guide RNAs and evaluated by transfection into primary human hepatocytes (PHH) and primary cynomolgus

hepatocytes (PCH) as described in Example 1. The cells were lysed 48 hours post treatment for NGS analysis as described in Example 1. The guides shown in Table 6 were tested.

Table 6. Dual guides and single guides in human and cynomolgus

human dgRNA	human guide sequence	SEQ ID NO	human sgRNA	cyno sgRNA
CR005916	ACAGGAAACUGUAGCAAACA	1	G012253	NA
CR005917	AUAGAUAAUUCACUUACCAC	2	G012254	NA
CR005918	UACAUCCCCACCUCUGAAGA	3	G012255	NA
CR005919	AACUGAAUAGCAAACACCUU	89	NA	NA
CR005920	ACAAUUACCAAUUUCUGAAA	90	NA	NA
CR005921	UACAAUUACCAAUUUCUGAA	91	NA	NA
CR005922	UCUUGAGGAGUAGAGGAACU	4	G012256	NA
CR005923	GGUGUUUUCUUGAGGAGUAG	92	NA	NA
CR005924	ACCAGGUAAAGUUCUUUUGC	5	G012257	NA
CR005925	GGGUAAAUUUUAGAAUGGCA	6	G012258	NA
CR005926	CGGGUAAAUUUUAGAAUGGC	93	NA	G013884
CR005927	CUCCCGGGUAAAUUUUAGAA	94	NA	G013925
CR005928	AUUUACCCGGGAGUUGACUU	7	G012259	NA
CR005929	UACCCGGGAGUUGACUUUGG	8	G012260	NA
CR005930	UAUGGGACACAAGGGAGCUC	95	NA	NA
CR005931	UCUUUGAGAUUGUGUAAACAC	9	G012261	NA
CR005932	CUUUGAGAUUGUGUAAACACU	10	G012262	NA
CR005933	UUUGAGAUUGUGUAAACACUG	11	G012263	NA
CR005934	UUGGAGGAACAAACUCUUCU	96	NA	G013912
CR005935	UGGAGGAACAAACUCUUCUU	97	NA	NA
CR005936	CAAACUCUUCUUGGGGAGAG	98	NA	NA
CR005937	CUAUGAGUGACCCUCCACAC	99	NA	G013886
CR005938	CUGUGUGGAGGGUCACUCAU	100	NA	G013938
CR005939	GGUCACUCAUAGGACACCAG	101	NA	G013946
CR005940	GUCACUCAUAGGACACCAGU	102	NA	G013896
CR005941	ACUGCUGCCCACUGCUUUGA	103	NA	NA
CR005942	ACACUUACCCAUCAAAGCAG	104	NA	G013902
CR005943	UACAUACCAGUGUAAUUCAA	12	G012264	NA
CR005944	AGGAACACCUACCGCUAUAA	105	NA	G013871
CR005945	CUCCGGGACUGUACUUUAAU	106	NA	G013889
CR005946	GUCCCAUACGCAAUCCUAGU	107	NA	G013890
CR005947	CUCAGCACCUUUAUAGCGGU	108	NA	G013892
CR005948	UAUAGCGGUAGGUGUCCUC	109	NA	G013874
CR005949	CUCCAACUAGGAUUGCGUAU	13	G012265	G013933
CR005950	CUAUUAAAGUACAGUCCCGG	110	NA	G013875
CR005951	AGGAUUGCGUAUGGGACACA	14	G012266	G013904
CR005952	GAUUGCGUAUGGGACACAA	15	G012267	G013901
CR005953	GUGCUGAGUAAACGUGGAAUC	111	NA	G013883
CR005954	UAUAAAGGUGCUGAGUAAACG	112	NA	G013878
CR005955	UCUCCAACUAGGAUUGCGUA	113	NA	G013908

human dgRNA	human guide sequence	SEQ ID NO	human sgRNA	cyno sgRNA
CR005956	GUUACUCAGCACC UUU AUAG	16	G012268	G013945
CR005957	AUAGCGGUAGGUGUCCUCC	114	NA	G013873
CR005958	CUGCCAAAAGUACAUCGAAC	115	NA	G013877
CR005959	UGCCUAUUAAAAGUACAGUCC	17	G012269	NA
CR005960	CUAUGGAUGGUUCUCCAACU	18	G012270	G013922
CR005961	ACCAAUUUCUGAAAAGGGCAC	116	NA	NA
CR005962	GUGUUUCUUAAGAUUAUCUA	117	NA	NA
CR005963	GAUGUUUGGCGCAUCUAUAG	19	G012271	G013921
CR005964	CCAAUUUCUGAAAAGGGCACA	118	NA	NA
CR005965	UUCUUAAGAUUAUCUAUGGA	119	NA	G013940
CR005966	CUGUUCGAUGUACUUUUGGC	120	NA	NA
CR005967	UGUUCGAUGUACUUUUGGCA	121	NA	G013880
CR005968	GGUGGAAUGUGCACCUCUAC	122	NA	G013939
CR005969	GUCCGACACACAAAAGCAUC	123	NA	G013894
CR005970	AUGCGCCAAACAUCUGCAG	20	G012272	G013885
CR005971	AAACUGGCAGCGAAUGUUAC	124	NA	G013930
CR005972	UGCCACGCAAAACAUUCACA	125	NA	NA
CR005973	GCACCGUUCGAUGUACUUU	126	NA	G013870
CR005974	AGAUGCGCCAAACAUCUGC	127	NA	NA
CR005975	GCACCUCUUCUGGCAGUAUU	128	NA	NA
CR005976	CAUCUGAGAACGCAAGAUGC	129	NA	G013934
CR005977	AUGCCCAAUACUGCCAGAUG	130	NA	NA
CR005978	UGCACCUCUUCUGGCAGUAU	131	NA	G013944
CR005979	CUCCUUUAUAAAUGUCUCGA	21	G012273	G013905
CR005980	AUGUCAUUGAUUGAACUUGC	132	NA	G013936
CR005981	ACAAGCACACGCAUUGUUGG	133	NA	G013893
CR005982	UGUUACUGGUGCACCUUUUU	22	G012274	NA
CR005983	GAUGCGCCAAACAUCUGCA	23	G012275	G013876
CR005984	UAUCGCCUUGAUAAAACUCC	134	NA	G013926
CR005985	CCUCAAGAAAACACCAUAUC	135	NA	G013906
CR005986	AAACGCCUUCUUCAGAGGUG	136	NA	NA
CR005987	AAAACAAGCACACGCAUUGU	137	NA	G013891
CR005988	CAUCGAACAGGUGCAGUUUC	138	NA	G013879
CR005989	GGCUUCCCCUGCAGGAUGUU	139	NA	G013881
CR005990	UUGAUGACCACAUUGCUUCA	140	NA	G013937
CR005991	AGGAGCCUGGAGUUUUUAUCA	141	NA	NA
CR005992	AUCUGGCAGUAUUGGGCAUU	24	G012276	G013915
CR005993	UGCCAUCGAGACAUUUUAAA	142	NA	G013899
CR005994	GCGUGGCAUAUGAAAAAAC	25	G012277	NA
CR005995	UAUAAAAGGAGUUGAUUAGAG	26	G012278	G013913
CR005996	AGCAAGUUCAAUCAUGACA	143	NA	G013897
CR005997	GGACAUUCCUUGAAGCAAUG	144	NA	NA
CR005998	ACACCUUGAAUUGUACUCAC	27	G012279	NA
CR005999	GUUGGGGUGAUAGGUGCAGA	145	NA	NA
CR006000	GAAAACGCCUUCUUCAGAGG	146	NA	NA

human dgRNA	human guide sequence	SEQ ID NO	human sgRNA	cyno sgRNA
CR006001	UAUGAAAACGCCUUCUUCAG	147	NA	NA
CR006002	CUCAGAUGUGGAUGUUGCCA	148	NA	NA
CR006003	CUCUCCUAGGCUUCCCCUGC	149	NA	NA

[00628] Editing was determined for dgRNAs in two separate sets of PHH and PCH populations. The screening data for the guide sequences are listed in Table 6 above. Table 7A and Figs. 1A-1B show the percent editing for the *KLKB1* targeting guides co-transfected with Spy Cas9 protein in primary human hepatocytes (PHH) (N=2) and Table 7B and Figs. 1C-1D for primary cynomolgus hepatocytes (PCH) (N=2).

[00629] The top performing guide RNAs and corresponding editing data from Set 2 are marked with an asterisk (*) in Table 7A and 7B. When compared, the sets were determined to be highly correlated (Spearman R =0.985).

Table 7A: *KLKB1* editing data for dual guides delivered to primary human hepatocytes: Primer sets 1 & 2

GUIDE ID	SET 1		GUIDE ID	SET 2	
	Avg % Edit	Std Dev % Edit		Avg % Edit	Std Dev % Edit
CR005916*	44.8	4.67	CR005916*	46.8	1.7
CR005917*	45.75	1.77	CR005917*	49.9	4.38
CR005918	ND	ND	CR005918	ND	ND
CR005919	24.6	0.28	CR005919	28.9	4.81
CR005920	9.35	0.35	CR005920	10.7	0.99
CR005921	6.15	1.06	CR005921	5.55	0.64
CR005922	ND	ND	CR005922	19.25	1.91
CR005923	19.25	4.74	CR005923	20.35	2.76
CR005924	6.1	0.71	CR005924	5.55	0.78
CR005925*	35.55	1.06	CR005925*	36.3	1.41
CR005926	11.95	4.45	CR005926	11.2	2.83
CR005927	29.2	4.24	CR005927	30.4	2.97
CR005928	ND	ND	CR005928	33.45	4.03
CR005929	ND	ND	CR005929	51.35	0.07
CR005930*	32.45	0.21	CR005930*	32.6	8.06
CR005931*	37.85	5.02	CR005931*	33.15	1.63
CR005932*	62.25	3.04	CR005932*	63.25	4.6
CR005933*	70.05	1.91	CR005933*	62.45	2.47
CR005934	17	2.12	CR005934	16.5	0.71
CR005935	26.25	0.35	CR005935	26.45	3.75

	SET 1			SET 2	
CR005936	4.55	0.64	CR005936	5	0.71
CR005937*	32.6	0.99	CR005937*	32.45	1.63
CR005938*	39.25	8.7	CR005938*	36.85	5.87
CR005939	27	0.57	CR005939	24.85	3.46
CR005940	16.7	2.12	CR005940	17.15	1.91
CR005941	8.9	0.85	CR005941	8.95	1.48
CR005942	19.8	0.71	CR005942	20.05	0.49
CR005943*	38.3	6.08	CR005943*	38.05	3.32
CR005944	23.55	1.91	CR005944	22.05	4.17
CR005945	21.95	1.63	CR005945	23.05	1.63
CR005946	29.35	2.47	CR005946	27.4	2.55
CR005947	12.4	2.97	CR005947	12.55	1.63
CR005948	15.2	1.56	CR005948	14.5	0.85
CR005949	19.15	2.33	CR005949	19.5	0.99
CR005950	21.6	2.12	CR005950	19.2	1.7
CR005951*	44.9	1.41	CR005951*	43.45	3.75
CR005952*	63.4	3.11	CR005952*	64.4	2.26
CR005953	5.7	0	CR005953	6.35	1.06
CR005954	12.5	1.7	CR005954	12.8	0.57
CR005955	24.65	3.04	CR005955	24.95	0.64
CR005956*	31.35	0.92	CR005956*	30.55	4.88
CR005957	22.95	2.05	CR005957	24.8	1.41
CR005958	16.45	1.2	CR005958	12.05	2.33
CR005959*	42.4	3.11	CR005959*	42.95	4.17
CR005960*	38.7	0.57	CR005960*	41.1	4.38
CR005961	15.2	0.71	CR005961	13.8	3.25
CR005962*	31.4	0	CR005962*	29.65	0.92
CR005963	ND	ND	CR005963	45.25	9.97
CR005964	17.45	1.91	CR005964	15.65	3.89
CR005965	25.25	2.33	CR005965	22.25	0.21
CR005966	8.35	2.9	CR005966	6.15	0.49
CR005967	19.5	2.55	CR005967	16.7	3.11
CR005968	11.55	2.33	CR005968	11.65	1.91
CR005969	13.5	2.26	CR005969	12.35	3.75
CR005970	ND	ND	CR005970	22.25	4.17
CR005971	1.1	0.42	CR005971	1.15	0.64
CR005972	13.65	1.63	CR005972	12.1	2.97
CR005973	6.45	0.21	CR005973	5.25	1.06
CR005974	ND	ND	CR005974	16.7	1.27
CR005975	7.95	1.34	CR005975	6.75	0.92
CR005976	ND	ND	CR005976	14.6	0.99
CR005977	12.2	2.69	CR005977	13.65	3.32
CR005978	9.95	1.06	CR005978	9.25	0.92

	SET 1			SET 2	
CR005979	22.35	1.63	CR005979	22.15	0.21
CR005980	18.2	2.26	CR005980	21.25	0.78
CR005981	18.2	1.27	CR005981	17.8	0.42
CR005982	6.25	1.77	CR005982	3.95	2.76
CR005983*	53	NA	CR005983*	43.3	9.9
CR005984	17.7	7.07	CR005984	18.45	5.73
CR005985	15.1	8.91	CR005985	16.3	0.71
CR005986	ND	ND	CR005986	ND	ND
CR005987	10.6	2.12	CR005987	9.8	0.85
CR005988	8.55	2.05	CR005988	6.4	1.13
CR005989	ND	ND	CR005989	4.9	0.42
CR005990	ND	ND	CR005990	20.2	6.36
CR005991	28.15	2.9	CR005991	26.9	4.81
CR005992	1.65	0.21	CR005992	4.85	0.92
CR005993*	38	1.27	CR005993*	31.35	1.2
CR005994*	33.4	1.98	CR005994*	36.4	1.98
CR005995	22.55	2.47	CR005995	30.65	7.14
CR005996	16.55	0.64	CR005996	13.75	3.46
CR005997	22.05	1.2	CR005997	16.45	3.61
CR005998*	56.65	2.33	CR005998*	59.05	14.07
CR005999	25	3.68	CR005999	22.45	2.19
CR006000	ND	ND	CR006000	ND	ND
CR006001	ND	ND	CR006001	ND	ND
CR006002	23.25	3.75	CR006002	20.85	2.05
CR006003	ND	ND	CR006003	7	1.13

* “selected dgRNA”, a subset of the tested guide RNAs

Table 7B: *KLKB1* editing data for crRNAs delivered to primary cynomolgus hepatocytes: Sets 1 & 2

	SET 1			SET 2	
GUIDE ID	Avg % Edit	Std Dev % Edit	GUIDE ID	Avg % Edit	Std Dev % Edit
CR005916*	26.6	2.4	CR005916*	26.1	1.8
CR005917*	31.5	3.3	CR005917*	33.2	2.8
CR005918*	0.6	0.2	CR005918	ND	ND
CR005919*	2.5	ND	CR005919*	3.2	0.3
CR005920*	0.4	0.1	CR005920	0.1	0.1
CR005921	0.1	0.0	CR005921	0.1	0.1
CR005010	72.8	0.2	CR005010	65.7	0.9
CR005922*	0.5	0.1	CR005922*	0.6	0.3
CR005923	0.4	0.1	CR005923	0.3	0.1
CR005924	0.2	0.0	CR005924	0.2	0.2
CR005925*	0.7	0.4	CR005925*	1.5	0.3
CR005926	0.0	0.0	CR005926	0.0	0.0

	SET 1			SET 2	
CR005927*	3.1	0.8	CR005927*	3.2	0.2
CR005928*	3.3	1.6	CR005928*	2.2	0.4
CR005929*	13.0	2.7	CR005929*	12.5	0.0
CR005930*	5.0	0.9	CR005930*	3.6	1.3
CR005931*	1.3	0.0	CR005931*	2.3	0.0
CR005932*	15.5	3.5	CR005932*	12.2	5.7
CR005933*	21.5	2.6	CR005933*	16.6	ND
CR005934	ND	ND	CR005934	0.3	0.2
CR005935	ND	ND	CR005935*	2.0	0.1
CR005936	0.1	0.0	CR005936	ND	ND
CR005937*	2.8	0.1	CR005937*	2.9	0.1
CR005938*	5.8	0.4	CR005938*	6.2	0.0
CR005939*	2.3	0.1	CR005939*	1.9	0.4
CR005940*	1.2	0.3	CR005940*	1.2	0.3
CR005025	31.3	10.8	CR005025	36.5	ND
CR005941	0.4	0.1	CR005941*	0.3	0.0
CR005942*	1.2	0.6	CR005942*	1.0	0.8
CR005943*	4.0	0.4	CR005943*	3.1	0.0
CR005020	29.5	1.5	CR005020	30.7	0.6
CR003187	14.5	0.6	CR003187	15.2	2.2
CR005964	0.3	0.4	CR005964	0.1	0.1
CR005032	5.3	1.6	CR005032	4.5	0.9

* "selected dgRNA", a subset of the tested guide RNAs

2.1.1 Cross screening of sgRNAs in PHH and PCH, editing

[00630] Selected guide sequences targeting *KLKB1* were prepared as sgRNAs and further evaluated in PHH and PCH. PHH and PCH (Gibco, Lot Hu8298) were prepared and transfected with RNP as described in Example 1. The cells were lysed at 48 and 72 hours, respectively, post-treatment for NGS analysis as described in Example 1. Table 8A and FIGS. 2A-2B show percent editing in PHH and Table 8B and FIGS. 2C-2D in PCH.

[00631] **Table 8A: *KLKB1* editing data for sgRNAs delivered to primary human hepatocytes: Sets 1 & 2**

	SET 1			SET 2	
GUIDE ID	Avg % Edit	Std Dev % Edit	GUIDE ID	Avg % Edit	Std Dev % Edit
G012321*	64.7	5.8	G012321*	62	5.5
G012102	64	5.7	G012102	63.6	4.7

	SET 1			SET 2	
G012293*	63.5	4.4	G012293*	61.6	3.9
G009246	63.3	3.1	G009246	62.3	4.6
G012308*	59.0	1.6	G012308*	59	1.5
G012253*	58.6	3.5	G012253*	59.1	1.7
G012319*	55.3	0.9	G012319*	55.1	3.6
G012298*	53.1	4.2	G012298*	54	4.9
G012320*	52.5	5.5	G012320*	51.9	7.7
G012290*	52.0	1.4	G012290*	51.7	0.9
G012304*	48.7	2.7	G012304	NA	NA
G012323*	47.4	1.4	G012323*	49.1	1.1
G012280*	46.2	4.0	G012280*	45.4	5.4
G012305*	46.1	5.2	G012305	NA	NA
G012303*	45.4	7.4	G012303	NA	NA
G012285*	45.1	7.6	G012285*	45.2	8.6
G012335*	45.1	2.4	G012335*	44.7	3.2
G012286*	44.7	5.3	G012286*	43.9	3.5
G000644	42.4	9.9	G000644	42.7	9.5
G012294*	42.0	1.3	G012294*	43	2.4
G009267	41.6	0.6	G009267	45.8	1.3
G009285	37.7	1.7	G009285	35.6	1.3
G012334*	36.4	2.5	G012334*	36	5.3
G012325*	36.2	1.8	G012325*	36.4	3.6
G012296*	35.9	6.2	G012296*	35.4	7.8
G012331	35.5	3.0	G012331*	35.7	1.2
G012306	34.9	1.8	G012306	NA	NA
G012313	34.9	1.3	G012313*	36.3	3.3

	SET 1			SET 2	
G012297	34.4	4.7	G012297	32.7	4.7
G012322	32.9	0.4	G012322	33.2	0.4
G012299	32.4	0.7	G012299	NA	NA
G012333	31.3	0.4	G012333	32.3	3.3
G012328	31.2	2.3	G012328*	34.9	0.1
G012309	30.4	0.1	G012309	29.9	2.3
G009321	29.9	2	G009321	31.8	4.5
G012338	29.0	2.2	G012338	29.7	2.3
G012283	26.2	4.2	G012283	24.9	4.1
G012291	25.3	2.3	G012291	24.7	3.7
G012302	23.2	NA	G012302	24.6	3.1
G012337	23.0	2.3	G012337	23.6	0.5
G012311	22.8	0.8	G012311	23.9	0.2
G012144	22.6	1.5	G012144	22.9	0.9
G012327	22.4	3.5	G012327	21.7	3.4
G012316	22.3	0.1	G012316	21.1	0.5
G000645	22	0.7	G000645	22.5	1.6
G012281	20.9	0.1	G012281	21.5	0.1
G012300	19.2	2.6	G012300	NA	NA
G012310	19.2	2.5	G012310	18.2	0.1
G012329	19.2	1.6	G012329	21.7	0.8
G012318	18.8	0.7	G012318	17.5	0.6
G012314	18.6	0.7	G012314	19.6	2.7
G012326	18.4	3.2	G012326	18.5	3.2
G012295	17.7	3.6	G012295	17.6	5.7
G012287	17.2	0.4	G012287	17	2.4
G012288	16.7	0.1	G012288	15.4	0.3
G012339	16.5	2.6	G012339	17.6	1.3
G012284	15.3	0.3	G012284	15.4	1.5
G012324	15.3	2.2	G012324	19.1	3.5
G012289	14.3	1.8	G012289	13.6	0.8
G012312	14.0	0.8	G012312	14.4	NA
G012292	13.4	3.6	G012292	12.9	3
G012330	13.3	1.9	G012330	12.9	0.6
G012315	13.0	0.1	G012315	12.8	1.9
G012332	12.6	1.0	G012332	11.5	0.6
G012340	10.5	2.3	G012340	13.9	1.8
G012301	9.2	0.1	G012301	NA	NA
G012336	7.5	1.6	G012336	6.3	1.8
G012282	7.3	1.2	G012282	8.6	2.3
G012307	5.3	0.2	G012307	NA	NA

	SET 1			SET 2	
G012317	3.5	0.6	G012317	3.5	0.7
G012260	1.9	0.6	G012260	1.8	0.1
G012254	1.6	0.2	G012254	0.7	0.3
G012266	1.0	0.1	G012266	1.9	0.6
G012256	0.8	0.4	G012256	0.2	0
G012274	0.8	0.1	G012274	0.7	0.1
G012259	0.6	0.1	G012259	0.6	0.1
G012262	0.5	0.1	G012262	0.6	0.2
G012276	0.5	0.0	G012276	0.8	NA
G012257	0.4	0.1	G012257	0.1	0
G012277	0.4	0.1	G012277	0.4	0.1
G012261	0.2	0.1	G012261	0.2	0.1
G012270	0.2	0.1	G012270	0.1	0
G012255	0.1	0.1	G012255	0.1	0.1
G012258	0.1	0.0	G012258	0.1	0.1
G012263	0.1	0.0	G012263	0.1	0
G012264	0.1	0.0	G012264	0.2	0.1
G012265	0.1	0.0	G012265	0.1	0
G012267	0.1	0.0	G012267	0.2	0.1
G012268	0.1	0.0	G012268	0.1	0.1
G012269	0.1	0.0	G012269	0.1	0.1
G012272	0.1	0.1	G012272	0.1	0
G012273	0.1	0.1	G012273	0.1	0.1
G012275	0.1	0.0	G012275	0.2	0.1
G012278	0.1	0.0	G012278	0.1	0.1
G012279	0.0	0.0	G012279	0.1	0.1
G012271	NA	NA	G012271	0.1	0

* “selected dgRNA”, a subset of the tested guide RNAs

Table 8B: *KLKB1* editing data for sgRNAs delivered to primary cynomolgus hepatocytes: Sets 1 & 2

	SET 1			SET 2	
GUIDE ID	Avg % Edit	Std Dev % Edit	GUIDE ID	Avg % Edit	Std Dev % Edit
G000644	35.9	3.5	G000644	37.4	2.3
G000645	64.1	0.2	G000645	65	0.1
G009246	92.7	1.2	G009246	93	0.9
G009267	84	1.6	G009267	83.3	1.9
G009285	82.5	0.3	G009285	80.7	0.7
G009321	46.8	17	G009321	46.3	12.8

	SET 1			SET 2	
G012102	91.4	0.5	G012102	92.4	0.1
G012144	45.3	0.6	G012144	45.4	4.2
G012253*	92	0.3	G012253*	90.4	1.3
G012254	2.3	0.1	G012254*	2.8	0.1
G012255	0	0	G012255	0.1	0
G012256	0.1	0	G012256	0.1	0
G012257	0.1	0	G012257	0.2	0
G012258	NA	NA	G012258	0.1	0.1
G012259	0.3	0.1	G012259	0.3	0.1
G012260	0.6	0	G012260*	0.8	0.1
G012261	0.2	0.1	G012261	0.2	0.1
G012262	0.3	0.2	G012262	0.4	0.1
G012263	0.2	0.1	G012263	0.2	0.1
G012264	0.1	0	G012264	0.1	0
G012265	NA	NA	G012265	0.1	0
G012266	0.3	0.1	G012266	0.3	0.1
G012267	0.2	0.1	G012267	0.1	0
G012268	0.1	0.1	G012268	0.1	0
G012269	0.1	0	G012269	0.1	0
G012270	NA	NA	G012270*	4.8	0.7
G012271	0.2	0.1	G012271	0	0
G012274	NA	NA	G012274	0.6	0.2
G012277	0.6	0.1	G012277	0.6	0
G012278	0	0	G012278	0.2	0.1
G012279	0.2	0.1	G012279	0.1	0
G012280*	42.2	1.6	G012280*	39.1	5.6
G012282*	8.8	0.3	G012282*	10.3	NA
G012283*	50.5	NA	G012283*	53.7	3.2
G012284	0.3	0.2	G012284	0.2	0
G012285*	30.2	4.9	G012285*	30.5	0.8
G012287	0.1	0	G012287	0.1	0
G012291	5.5	0.8	G012291*	4.8	1.3
G012292	6.4	0.3	G012292*	6.3	0.7
G012293*	86.6	0.1	G012293*	83.3	6.8
G012294*	53.6	1.5	G012294*	52.1	0.3
G012295*	6.7	0.1	G012295*	7	0.8
G012296*	51.4	2.1	G012296*	49	3.4
G012297	NA	NA	G012297*	41.9	0.4
G012299*	38.2	0.9	G012299	NA	NA
G012300*	28.5	4.6	G012300	NA	NA
G012301*	7.6	2	G012301	NA	NA
G012302*	39.9	2.8	G012302	NA	NA
G012303*	13.8	1.1	G012303	NA	NA

	SET 1			SET 2	
G012304*	69.7	2.8	G012304	NA	NA
G012305*	16	0.1	G012305	NA	NA
G012307	0.3	0.2	G012307	NA	NA
G012308*	57.7	3.9	G012308*	58.1	0.7
G012309*	55.9	7.3	G012309*	55.1	3.7
G012310*	27	2.3	G012310*	27	1.9
G012311	4	0.1	G012311*	3.6	1.8
G012312*	14.4	0.5	G012312*	14.7	0.3

* “selected sgRNA”, a subset of the tested guide RNAs

2.2. Screening of sgRNA in Primary Human Hepatocytes (PHH), editing and protein knockdown

[00632] Three PHH lots (Hu8296, Hu8300, and Hu8284) were individually plated as described in Example 1 and incubated at 37°C, 5% CO₂ for 24 hours prior to lipofection. A mixture of 6.88 µL of 10 µM sgRNA guide and 4.5 µL of 500 ng/µl Cas9 mRNA was prepared in a total volume of 11.4 µl water. A lipofection reagent as described in Example 1 was thawed to room temperature. The guide/Cas9 mRNA mix was sequentially added with 4.8 µL of 50mM sodium citrate/200 mM NaCl (pH 5), 4.8 µL of lipofection reagent, and 54 µL of molecular grade water, to prepare a total volume 75 µL per sample. The lipofection sample was pre-incubated with media, William’s E or Cellartis Power Primary HEP Medium (Takada, Cat. Y20020), containing 3% FBS or cynomolgus serum for 10 min at 37°C prior to addition to cells. Cells were transfected with 10 µL of prepared lipofection sample containing 300 ng of Cas9 mRNA and 302 ng guide sgRNA.

[00633] The cells were lysed 72 hours post-transfection for NGS analysis was conducted as described in Example 1.

[00634] For cells to be utilized for secreted protein analysis by ELISA or intracellular protein analysis by western blotting, at 72 hours post-transfection the media was aspirated and replaced with Cellartis Power Primary HEP Medium (Takada, Cat. Y20020). Media was aspirated and replaced every two days. For samples used to determine reduction of secreted protein, media was aspirated from wells and replaced with fresh media which was incubated for 24-48 hrs prior to harvest. Media was collected and transferred to 96-well PCR plates and stored at -20°C prior to use in assays.

[00635] Table 8C and FIGS. 3A-3B show percent editing and secreted KLKB1 protein levels based on transfection of three PHH lots. Twenty guides were compared in pairs of

PHH lots, and determined to be highly correlated (Spearman $R > 0.8$) as shown in FIGS. 3C-3E.

Table 8C. KLKB1 indel frequency and secreted KLKB1 protein levels in PHH

GUIDE ID	PHH Lot 1 - HU8296				PHH Lot 2 - HU8284				PHH Lot 3 - HU8300			
	Indel Freq	SD	Secreted KLKB1	SD	Indel Freq	SD	Secreted KLKB1	SD	Indel Freq	SD	Secreted KLKB1	SD
G012253	0.32	0.01	1.54	0.03	0.63	0.06	0.24	0.00	0.49	0.01	0.43	0.11
G012254	0.12	0.01	7.22	0.33	0.40	0.09	0.57	0.05	0.15	0.00	1.61	0.06
G012255	0.02	0.00	15.72	0.70	0.22	0.02	5.05	0.26	0.03	0.01	5.33	0.08
G012256	0.10	0.03	9.26	0.13	0.43	0.05	1.57	0.31	0.11	0.00	2.69	0.07
G012257	0.00	0.00	22.99	0.45	0.08	0.04	8.75	0.28	0.00	0.00	3.71	0.21
G012258	0.13	0.01	4.24	0.02	0.26	0.02	3.89	0.03	0.09	0.02	2.76	0.00
G012259	0.27	0.01	2.18	0.01	0.55	0.07	1.02	0.01	0.35	0.01	0.91	0.13
G012260	0.69	0.03	0.07	0.02	0.73	0.03	0.05	0.01	0.82	0.03	-0.50	0.01
G012261	0.06	0.00	14.26	0.77	0.38	0.03	3.39	0.11	0.07	0.02	3.84	0.28
G012262	0.48	0.03	1.65	0.05	0.74	0.04	0.68	0.09	0.53	0.03	1.40	0.10
G012263	0.42	0.02	2.48	0.06	0.67	0.03	0.78	0.02	0.53	0.03	0.55	0.16
G012264	0.32	0.02	1.97	0.02	0.65	0.07	0.54	0.03	0.26	0.01	2.02	0.09
G012265	0.11	0.00	6.24	0.02	0.29	0.09	1.36	0.10	0.11	0.01	1.10	0.03
G012266	0.42	0.01	1.30	0.02	0.61	0.05	0.87	0.03	0.45	0.02	0.15	0.02
G012267	0.72	0.00	0.23	0.02	0.79	0.02	0.14	0.00	0.82	0.00	-0.52	0.01
G012268	0.20	0.01	4.78	0.14	0.44	0.01	1.65	0.06	0.30	0.01	1.05	0.03
G012269	0.18	0.03	4.10	0.06	0.68	0.01	0.52	0.07	0.25	0.00	2.79	0.04
G012270	0.48	0.01	0.46	0.02	0.77	0.02	0.21	0.00	0.67	0.06	-0.13	0.04
G012271	0.10	0.02	3.16	0.02	0.58	0.06	0.79	0.04	ND	0.00	0.87	0.05
G012272	0.12	0.02	6.42	0.09	0.53	0.06	1.16	0.03	0.21	0.01	0.09	0.02
G012273	0.07	0.01	9.39	0.03	0.16	ND	0.82	0.04	0.07	0.00	2.14	0.06
G012274	0.04	0.00	15.14	0.43	0.38	0.11	2.73	0.03	ND	0.00	4.13	0.04
G012275	0.54	0.00	2.35	0.02	0.75	0.03	0.89	0.00	0.62	0.02	0.33	0.00
G012276	0.01	0.00	20.54	0.24	0.09	0.00	4.56	0.18	0.03	0.00	3.92	0.03
G012277	0.28	0.05	3.39	0.08	0.57	0.00	1.61	0.01	0.22	0.03	2.22	0.01
G012278	0.22	0.01	1.61	0.03	0.64	0.04	0.50	0.00	0.42	0.02	-0.08	0.01
G012279	0.40	0.05	2.56	0.08	0.59	0.03	1.51	0.02	0.53	0.03	-0.12	0.01
G012280	0.39	0.02	1.34	0.06	0.55	0.04	0.52	0.01	0.44	0.02	0.08	0.00
G012285	0.24	0.00	2.53	0.01	0.56	ND	1.23	0.06	0.26	0.00	1.60	0.06
G012286	0.26	0.01	2.56	0.05	0.48	0.12	1.06	0.01	0.33	0.01	0.55	0.02
G012290	0.13	0.00	14.44	0.06	0.60	0.01	5.45	0.06	0.29	0.00	2.41	0.04
G012293	0.00	0.00	21.78	0.26	0.00	0.00	6.39	0.02	0.54	0.04	0.21	0.02
G012294	0.23	0.01	2.12	0.16	0.62	0.02	0.39	0.06	0.34	0.02	0.29	0.08
G012296	0.22	0.00	2.89	0.11	0.58	0.05	0.90	0.04	0.35	0.01	0.55	0.04
G012298	0.37	0.01	2.19	0.01	0.68	0.05	0.72	0.03	0.63	0.01	-0.04	0.00
G012303	0.40	0.03	0.52	0.02	0.70	0.05	0.38	0.00	0.38	0.03	0.04	0.00
G012304	0.30	0.02	0.89	0.09	0.26	ND	0.20	0.01	0.27	0.03	1.31	0.06
G012305	0.38	0.03	0.79	0.07	0.73	0.03	0.32	0.01	0.51	0.02	0.04	0.00
G012308	0.18	0.02	1.79	0.13	0.72	0.03	0.53	0.04	0.33	0.01	-0.03	0.01
G012319	0.26	0.00	1.45	0.03	0.25	0.02	3.62	0.76	0.32	0.03	0.80	0.00

	PHH Lot 1 - HU8296				PHH Lot 2 - HU8284				PHH Lot 3 - HU8300			
G012320	0.21	0.00	2.24	0.11	0.63	0.03	0.88	0.01	0.32	0.01	1.17	0.02
G012321	0.45	0.00	0.49	0.07	0.72	0.01	0.49	0.09	0.60	0.07	-0.09	0.02
G012323	0.28	0.00	1.32	0.01	0.48	0.12	0.75	0.17	0.33	0.00	0.01	0.02
G012325	0.40	0.02	2.51	0.01	0.62	0.00	1.37	0.68	0.50	0.05	0.27	0.02
G012334	0.10	0.00	7.52	0.18	0.19	ND	2.86	0.14	0.06	0.02	3.07	0.19
G012335	0.27	0.02	3.78	0.26	0.47	0.10	1.42	0.09	0.42	0.01	0.11	0.02
G009321 (HOX9)	0.10	0.03	19.59	1.30	0.30	0.32	6.57	0.00	0.37	0.03	3.80	0.11
Untreated			24.03	0.03			7.58	0.10			3.47	0.03

2.3. Screening of sgRNAs in PHH

[00636] Primary human hepatocytes (PHHs) were transfected with Cas9 mRNA and sgRNA as described in Example 1. The cells were lysed 72 hours post transfection and NGS analysis was conducted as described in Example 1.

[00637] Percent editing was determined for sgRNAs comprising guide sequences of Table 1 for two primer sets. The average percent editing for each guide in the two data sets is shown in Table 9A and FIGS. 4A-4B.

Table 9A. KLKB1 editing data in primary human hepatocytes

Guide ID	Set 1			Guide ID	Set 2		
	% Editing	SD	N		% Editing	SD	N
G013946*	49.9	2.2	2	G013946	50.8	9.1	2
G000644	49.3	5.7	2	G000644	44.7	3.7	2
G013945*	48.6	1.9	2	G013945*	50.3	1.6	2
G012102	48.1	4.4	2	G012102	45.8	0.3	2
G012320*	45	14	2	G012320*	41.9	15	2
G013938*	44.5	9.5	2	G013938	ND	NA	2
G009285	42.1	0.7	2	G009285	41.4	4.9	2
G009246	40.5	0.6	2	G009246	39.1	3.1	2
G000502	36.5	15	2	G000502	34.9	18	2
G000502	36.5	15	2	G000502	34.9	18.4	2
G012304*	36.2	3.3	2	G012304	ND	NA	2
G013886*	30.5	4	2	G013886*	37.2	0.8	2
G012325*	28.7	3.3	2	G012325*	33.9	0.9	2

	Set 1			Set 2			
G013896*	28.4	3.7	2	G013896*	28.7	1.2	2
G009321	27.7	10	2	G009321	26.9	11	2
G012323*	27.4	7.2	2	G012323*	30.6	6.5	2
G013925*	25.9	11	2	G013925*	28.7	12	2
G013922*	21	7	2	G013922*	19.3	3.3	2
G012327	20.5	2.3	2	G012327*	19.5	4.6	2
G012322	18.6	3.8	2	G012322	13.9	4.5	2
G009267	18.3	1.9	2	G009267	20.4	4.8	2
G013924*	17.4	9.3	2	G013924	ND	NA	2
G013943*	14.2	9.5	2	G013943*	12.1	6.1	2
G000645	13.5	3.2	2	G000645	9.1	4	2
G013895*	13.4	3.1	2	G013895	14	6.6	2
G013941*	13	4.8	2	G013941	14.3	2.6	2
G013882*	10.4	8.1	2	G013882	ND	NA	2
G012329*	9.8	0.7	2	G012329*	8.9	0.9	2
G012300*	9.7	1.8	2	G012300	ND	NA	2
G013899	9.3	0.2	2	G013899*	7.1	4.4	2
G013874	9	2.3	2	G013874*	9.5	3.6	2
G013931	8.9	1.6	2	G013931	ND	NA	2
G012315	8.8	5.4	2	G012315	ND	NA	2
G013902	6.2	3.9	2	G013902*	4.9	5.2	2
G013917	6.1	4.6	2	G013917*	7.1	6.5	2
G013916	5.6	0.6	2	G013916	4	0.8	2
G012324	5.2	2.9	2	G012324	4.8	3.9	2
G013912	5.1	2.9	2	G013912	4.6	1.1	2
G013913	4.8	0.4	2	G013913*	5	0.2	2
G013932	4.8	4.9	2	G013932	ND	NA	2
G013900	4.6	2.1	2	G013900	4.6	0.7	2
G012340	3.3	1.1	2	G012340	2.2	1.7	2
G013873	2.8	0.8	2	G013873	3.6	1.1	2
G013884	2.3	1.1	2	G013884	3	0.3	2
G013903	1.4	0.4	2	G013903	1.3	0.5	2

	Set 1			Set 2			
G013901	1.2	1.3	2	G013901	1.4	1.3	2
G013889	0.9	0.2	2	G013889	1.1	1.2	2
G013930	0.9	1.1	2	G013930	1.3	0.1	2
G013893	0.8	0.3	2	G013893	1.8	0.4	2
G013919	0.8	0.2	2	G013919	0.6	0.4	2
G013891	0.7	0.1	2	G013891	0.3	0	2
G013934	0.6	0.2	2	G013934	0.9	1.1	2
G013894	0.3	0.4	2	G013894	0.6	0.8	2
G013906	0.3	0	2	G013906	0.7	0.3	2
G013914	0.3	0.1	2	G013914	ND	NA	2
G013928	0.3	0.2	2	G013928	0.5	NA	2
G013875	0.2	0.1	2	G013875	0.3	0.1	2
G013878	0.2	0.1	2	G013878	0.3	0.1	2
G013880	0.2	0.1	2	G013880	0.1	0	2
G013939	0.2	0.2	2	G013939	0.2	0.1	2
G013942	0.2	0.1	2	G013942	0.2	0.1	2
G013870	0.1	0	2	G013870	0.1	0	2
G013871	0.1	0	2	G013871	0.1	0	2
G013883	0.1	0	2	G013883	0.6	0.4	2
G013897	0.1	0	2	G013897	0.1	0	2
G013927	0.1	0	2	G013927	0	0	2
G013929	0.1	0	2	G013929	ND	NA	2
G013935	0.1	0.1	2	G013935	0.1	0	2
G013936	0.1	0	2	G013936	0.1	0	2
G013940	0.1	0	2	G013940	0.1	0.1	2
G013921	ND	ND	2	G013921	0.3	0.2	2
G013926	ND	ND	2	G013926	0.2	0.1	2

* “selected sgRNA”, a subset of the tested guide RNAs

2.3.1 Screening of sgRNAs in PCH

[00638] Primary cynomolgus hepatocytes (PCHs) were transfected with Cas9 mRNA and sgRNA as described in Example 1 using increasing amounts of prepared lipofection sample to assay a dose responsive effect. The cells were lysed 72 post transfection and NGS analysis was conducted as described in Example 1. Percent editing was determined for sgRNAs comprising guide sequences in Table 1 using two primer sets for amplification and detection of indels. The average percent editing for each guide in the two data sets is shown in Table 9B and FIGS. 4C-4D.

[00639] The selected guide RNAs and corresponding editing data from Sets 1 and 2 are marked with an asterisk (*) in Table 9B. When compared the datasets were determined to be highly correlated (Spearman R = 0.987).

Table 9B. KLKB1 editing data in primary cynomolgus hepatocytes

Guide ID	Set 1			Set 2		
	% Editing	SD	N	% Editing	SD	N
G013923	4.3	0	2	5	0.6	2
G013877	1.5	0	2	1.3	0.2	2
G013884	0.4	0	2	0.2	0.1	2
G013929	0.3	0	2	0.5	0.1	2
G013940	0.2	0	2	0.3	0.1	2
G013915	0.1	0	2	0.2	ND	2
G013939	9.3	0.1	2	10.1	0.3	2
G013870	4.8	0.1	2	4	1	2
G013879	3.4	0.1	2	3.1	0.6	2
G012327	1.4	0.1	2	1.5	0.1	2
G013942	0.7	0.1	2	0.2	ND	2
G013883	0.3	0.1	2	0.2	0.1	2
G013926	0.1	0.1	2	0	0	2
G013887	0.9	0.2	2	0.7	0.7	2
G013930	3.1	0.3	2	3.2	1.3	2
G013902	0.9	0.3	2	0.7	0.4	2
G013903	0.7	0.3	2	0.9	0.1	2
G013875	6.8	0.4	2	5.6	0.2	2
G013881	5.8	0.4	2	5.7	0	2
G013914	1.4	0.4	2	ND	ND	2
G013936	8.2	0.6	2	8.1	1.3	2
G013916	7.1	0.6	2	6.4	1.9	2
G013874	6.9	0.6	2	6	0.4	2
G012340	3.6	0.6	2	3.7	0.9	2
G013880	1.9	0.6	2	1.9	0.1	2
G013919	0.9	0.6	2	0.7	0.4	2
G013878*	14.3	0.7	2	11.8	3	2
G013931	4	0.8	2	ND	ND	2
G013872	11.1	0.9	2	11.7	1	2
G013909	4.8	0.9	2	5.3	0.5	2
G013899	3	0.9	2	3	1.6	2
G013886*	15.4	1.1	2	15.2	0.4	2
G013944	2.1	1.1	2	2.3	0.5	2
G013876*	23.4	1.3	2	21.4	3.3	2
G013921	4.1	1.3	2	3.1	3.3	2
G013913*	12.1	1.5	2	11.8	ND	2
G013871*	25.9	1.6	2	23.9	4	2
G013904	9	1.6	2	10.4	2.3	2

	Set 1			Set 2		
G013906	3.2	1.6	2	3.3	1.9	2
G013928	4.6	1.7	2	4.1	1.6	2
G012324	3.9	1.8	2	4.4	2.4	2
G013910	3.9	1.8	2	4.8	1.8	2
G013892	2.7	1.8	2	2.8	1.8	2
G013943	3.1	2	2	3.7	2.3	2
G013920	2	2	2	2.9	1.4	2
G013889*	29.4	2.1	2	31.4	0.5	2
G013938*	24.9	2.1	2	24.2	0.6	2
G012329*	12.4	2.1	2	11.4	0.5	2
G012315	4.1	2.1	2	ND	ND	2
G013927	4	2.1	2	5.2	0.3	2
G013918	2.3	2.1	2	2	1.6	2
G013935	4.1	2.2	2	4.3	1.1	2
G013941	7	2.3	2	8.7	1.3	2
G012322	10.8	2.4	2	10.4	3.4	2
G013890	4.9	2.5	2	5.5	2.1	2
G013925	5.8	2.6	2	6.9	2.3	2
G012325*	11.8	2.8	2	11.6	3.4	2
G013888	6.7	2.8	2	7.1	1.6	2
G013897	11	3	2	11.9	1.6	2
G013900	6.6	3	2	7.7	4.2	2
G013917*	16.4	3.2	2	18.3	3.8	2
G013937*	30.4	3.8	2	26.5	6	2
G013932	5.6	4.4	2	ND	ND	2
G012304*	11.7	4.7	2	ND	ND	2
G013895	6.8	4.7	2	6	3.2	2
G013896*	11.5	5	2	10.3	3.8	2
G013907*	36.1	5.5	2	36.2	5.7	2
G012320	8.9	5.7	2	9.2	4.6	2
G013945*	19	6.6	2	20	6.1	2
G013924*	13.9	6.7	2	ND	ND	2
G013882*	24.7	9	2	ND	ND	2
G013946*	27.2	ND	2	22.4	4.2	2
G013873*	23.6	ND	2	20.1	2.9	2
G012323*	12.3	ND	2	11.8	1.5	2
G013934	8.3	ND	2	7.1	1.6	2
G013905	6	ND	2	4	3.3	2
G013898	5.7	ND	2	6	2.1	2
G013908	1.8	ND	2	3.6	1.3	2
G013911	1.8	ND	2	3.4	1.6	2
G013894	0.3	ND	2	0.2	0.1	2
G013885	ND	ND	2	5.9	0.2	2
G013891	ND	ND	2	3.3	0.7	2
G013893	ND	ND	2	1.2	0.9	2

	Set 1			Set 2		
G013901	ND	ND	2	42.2	4.2	2
G013912	ND	ND	2	0.4	ND	2
G013922	ND	ND	2	11.2	1.1	2
G013933	ND	ND	2	12.2	4	2

* “selected sgRNA”, a subset of the tested guide RNAs

Example 3: Dose Response Assays

3.1 Cross screening of sgRNAs in PCH and PHH in 4-point dose response assays

[00640] Modified sgRNAs targeting human KLKB1 and the cynomolgus matched sgRNA sequences were tested in PHH and PCH in a dose response assay, using 16 guides from the PHH guide screen described in Example 2.2. Lipofection samples including Cas9 mRNA and sgRNAs were prepared as described in Example 2.2. Primary human and cynomolgus hepatocytes were plated as described in Example 1. Both cell lines were incubated at 37°C, 5% CO₂ for 48 hours prior to treatment with lipofection samples. Lipofection samples were incubated in Cellartis Power Primary HEP Medium (Takada, Cat. Y20020) containing 3% FBS at 37°C for 10 minutes.

[00641] Post-incubation the lipofection samples were added to the human or cynomolgus hepatocytes in a 4-point dose response assay. The PHH were lysed 120 hours post-transfection and the PCH were lysed 168 hrs post-transfection and gDNAs were subjected to quantified PCR for NGS analysis as described in Example 1.

[00642] The indel frequency of sgRNAs at concentrations 0.4 nM, 3.3 nM, 30 nM, and 90 nM in PHH cells is shown in Table 10 and FIGS. 5A-5B. Secreted KLKB1 protein levels of the sgRNAs determined by ELISA is shown in Table 10 and FIGS. 5C-5D.

Table 10: KLKB1 editing data and secreted KLKB1 protein levels in PHH

GUIDE ID	Guide concentration (nM)	Avg Indel Freq	SD	Avg secreted KLKB1	SD
G012253	90	0.54	0.04	5.84	0.29
	30	0.64	0.05	4.06	0.09
	3.3	0.31	0.01	17.29	0.35
	0.4	0.02	0.00	26.56	0.00
G012259	90	0.30	0.02	8.70	0.10
	30	0.52	0.06	4.90	0.00
	3.3	0.20	0.03	18.90	0.20
	0.4	0.02	0.00	26.60	0.00
G012260	90	0.73	0.04	2.20	0.00

GUIDE ID	Guide concentration (nM)	Avg Indel Freq	SD	Avg secreted KLKB1	SD
	30	0.84	0.00	0.30	0.30
	3.3	0.71	0.02	4.00	0.20
	0.4	0.20	0.01	25.10	0.30
G012267	90	0.80	0.03	2.10	0.10
	30	0.89	0.01	0.30	0.00
	3.3	0.76	0.05	4.00	0.20
	0.4	0.29	0.03	20.70	0.20
G012278	90	0.47	0.10	4.80	0.10
	30	0.57	0.02	1.80	0.10
	3.3	0.24	0.02	10.80	0.10
	0.4	0.02	0.01	26.50	0.10
G012279	90	0.00	0.00	18.70	0.40
	30	0.00	0.00	21.10	0.30
	3.3	0.00	0.00	26.60	0.00
	0.4	0.00	0.00	26.60	0.00
G012280	90	0.42	0.08	6.90	0.10
	30	0.69	0.01	4.70	0.10
	3.3	0.21	0.00	23.30	0.10
	0.4	0.01	0.00	26.60	0.00
G012293	90	0.48	0.05	5.10	0.10
	30	0.70	0.00	4.20	0.00
	3.3	0.32	0.02	15.20	0.60
	0.4	0.03	0.00	26.60	0.00
G012294	90	0.39	0.08	7.37	0.08
	30	0.48	0.04	6.44	0.06
	3.3	0.13	0.01	22.57	0.25
	0.4	0.01	0.00	23.49	0.73
G012298	90	0.48	0.14	9.30	0.60
	30	0.52	0.00	6.20	0.10
	3.3	0.20	0.07	21.50	0.50
	0.4	0.01	0.00	25.50	0.50
G012303	90	0.68	0.05	6.00	0.00
	30	0.75	0.04	3.80	0.30
	3.3	0.43	0.03	15.80	0.20
	0.4	0.02	0.00	23.20	2.70
G012304	90	0.54	0.02	6.00	0.10
	30	0.52	0.02	7.20	0.20
	3.3	0.21	0.04	21.30	0.30
	0.4	0.01	0.00	25.60	0.00
G012305	90	0.61	0.03	7.90	0.00
	30	0.66	0.01	4.40	0.10
	3.3	0.33	0.01	18.00	0.00
	0.4	0.01	0.00	26.10	0.20
G012308	90	0.41	0.01	10.70	0.20
	30	0.42	0.10	9.10	0.10

GUIDE ID	Guide concentration (nM)	Avg Indel Freq	SD	Avg secreted KLKB1	SD
	3.3	0.08	0.01	24.30	0.10
	0.4	0.01	0.00	26.60	0.00
G012321	90	0.43	0.00	9.70	0.10
	30	0.61	0.13	5.20	0.00
	3.3	0.17	0.01	21.70	0.20
	0.4	0.02	0.02	26.50	0.10
G012323	90	0.28	0.03	9.90	0.30
	30	0.28	0.10	7.60	0.10
	3.3	0.14	0.03	22.40	0.30
	0.4	0.01	0.00	26.60	0.00
untreated	NA			22.61	0.21

[00643] The indel frequency of sgRNAs at concentrations 0.4 nM, 10 nM, 30 nM, and 90 nM in PCH cells is shown in Table 11 and FIGS. 5E-5F. Secreted KLKB1 protein levels of the sgRNAs determined by ELISA is shown in Table 11 and FIGS. 5G-5H.

Table 11: KLKB1 editing data and secreted KLKB1 protein levels in PCH

guide	Guide concentration (nM)	Mean Indel Freq	SD	Mean secreted KLKB1	SD
G012253	90	0.25	0.01	0.82	0.01
	30	0.38	0.05	0.77	0.00
	10	2.76	0.09	0.44	0.07
	0.4	13.26	0.32	0.01	0.00
G012259	90	1.13	0.02	0.70	0.01
	30	0.81	0.12	0.69	0.01
	10	3.95	0.41	0.37	0.04
	0.4	12.88	0.96	0.00	0.00
G012260	90	0.36	0.02	0.82	0.02
	30	0.23	0.00	0.81	0.01
	10	1.56	0.13	0.56	0.06
	0.4	11.51	1.03	0.01	0.00
G012267	90	1.28	0.10	0.67	0.02
	30	0.98	0.07	0.67	0.00
	10	3.18	0.37	0.42	0.04
	0.4	11.88	0.28	0.01	0.00
G012278	90	1.01	0.06	0.61	0.03
	30	0.82	0.01	0.63	0.02
	10	3.16	0.19	0.30	0.05
	0.4	12.08	1.14	0.00	0.00
G012279	90	9.88	1.25	0.00	0.00
	30	9.44	1.07	0.00	0.00

guide	Guide concentration (nM)	Mean Indel Freq	SD	Mean secreted KLKB1	SD
	10	12.49	0.14	0.00	0.00
	0.4	12.12	0.25	0.00	0.00
G012280	90	4.32	0.20	0.44	0.02
	30	3.08	0.18	0.46	0.00
	10	7.70	0.21	0.13	0.02
	0.4	11.69	1.13	0.00	0.00
G012293	90	0.80	0.22	0.80	0.03
	30	0.54	0.09	0.80	0.02
	10	2.56	0.16	0.59	0.00
	0.4	11.79	0.23	0.01	0.00
G012294	90	6.96	0.48	0.39	0.09
	30	6.21	0.29	0.41	0.03
	10	15.19	1.13	0.12	0.00
	0.4	24.96	3.85	0.00	0.00
G012298	90	18.40	0.36	ND	ND
	30	25.03	2.87	ND	ND
	10	24.81	2.89	ND	ND
	0.4	23.59	3.91	ND	ND
G012303	90	18.34	4.33	0.16	0.06
	30	16.05	4.43	0.17	0.00
	10	26.04	3.42	0.03	0.00
	0.4	27.82	2.74	0.00	0.00
G012304	90	5.75	1.35	0.52	0.04
	30	6.14	0.68	0.54	0.01
	10	17.23	1.75	0.21	0.04
	0.4	24.90	0.95	0.00	0.00
G012305	90	19.27	4.43	0.07	0.02
	30	18.57	3.56	0.11	0.01
	10	22.11	2.31	0.02	0.00
	0.4	27.30	1.17	0.00	0.00
G012308	90	6.97	2.00	0.44	0.04
	30	7.52	0.00	0.49	0.03
	10	14.43	0.07	0.18	0.05
	0.4	22.80	2.56	0.00	0.00
G012321	90	4.95	1.25	ND	ND
	30	5.96	0.07	ND	ND
	10	10.71	0.16	ND	ND
	0.4	23.83	1.78	ND	ND
G012323	90	9.93	2.24	ND	ND
	30	8.97	3.13	ND	ND
	10	15.75	0.20	ND	ND
	0.4	22.16	0.27	ND	ND

[00644] Indel frequency and secreted KLKB1 protein levels were shown to be inversely correlated in both PHH and PCH as shown in FIGS. 5I-5J.

3.2 Cross screening of sgRNAs in PHH and PCH in 7-point dose response assays

[00645] Lipid nanoparticle (LNP) formulations of sgRNAs targeting human KLKB1 sgRNA sequences were tested in PHH and PCH in a dose response assay.

[00646] The LNPs were formulated as described in Example 1. The final LNPs were characterized to determine the encapsulation efficiency, polydispersity index, and average particle size according to the analytical methods provided above.

[00647] Primary human and cynomolgus hepatocytes were plated as described in Example 1. Both cell lines were incubated at 37°C, with 5% CO₂ for 24 hours prior to treatment with LNPs. LNPs were incubated in media containing 3% FBS at 37°C for 10 minutes. Post-incubation the LNPs were added to the human or cynomolgus hepatocytes in a 7 point 3-fold dose response curve. The cells were lysed 72 hours post-transfection and gDNAs were subjected to quantified PCR for NGS analysis as described in Example 1.

[00648] The indel frequency of sgRNAs at concentrations, 0.04 nM, 0.13 nM, 0.40 nM, 1.19 nM, 3.58 nM, 10.75 nM, and 32.25 nM are shown in Table 12 and corresponding dose response curves in FIGS 6A-B for PHH and FIGS 6C-D for PCH.

Table 12: Indel frequency and secreted KLKB1 protein for LNPs targeting *KLKB1* in vitro

GUIDE ID	Guide conc. (nM)	PHH				PCH			
		Mean Indel Freq	SD	Mean Secreted KLKB1	SD	Mean Indel Freq	SD	Mean Secreted KLKB1	SD
G012253	32.25	0.91	0.00	-1.38	0.22	0.90	0.01	-1.58	0.10
	10.75	0.89	0.00	-1.30	0.08	0.89	0.01	-1.57	0.07
	3.58	0.89	0.02	-1.23	0.19	0.84	0.00	-1.39	0.07
	1.19	0.83	0.01	-0.48	0.11	0.55	0.03	5.01	0.24
	0.40	0.64	0.04	2.49	0.07	0.18	0.02	22.35	0.29
	0.13	0.34	0.03	9.34	0.01	0.05	0.01	27.18	0.07
	0.04	0.12	0.01	15.04	0.17	0.01	0.00	27.18	0.07
G012259	32.25	0.84	0.01	-1.12	0.21	0.95	0.01	-1.57	0.07
	10.75	0.86	0.01	-1.17	0.11	0.93	0.01	-1.57	0.02
	3.58	0.85	0.02	-1.00	0.06	0.80	0.00	-0.71	0.70
	1.19	0.79	0.01	-0.28	0.04	0.44	0.04	8.93	0.23
	0.40	0.63	0.02	2.37	0.19	0.12	0.03	25.94	0.05
	0.13	0.36	0.03	8.32	0.31	0.01	0.00	27.18	0.07
	0.04	0.13	0.01	12.97	0.41	0.00	0.00	27.18	0.07
G012260	32.25	0.93	0.00	-1.36	0.21	0.96	0.00	-1.32	0.18

GUIDE ID	Guide conc. (nM)	PHH				PCH			
		Mean Indel Freq	SD	Mean Secreted KLKB1	SD	Mean Indel Freq	SD	Mean Secreted KLKB1	SD
	10.75	0.92	0.01	-1.35	0.15	0.95	0.01	-1.41	0.37
	3.58	0.92	0.01	-1.43	0.19	0.87	0.02	-0.94	0.03
	1.19	0.92	0.01	-1.37	0.27	0.50	0.00	7.33	0.02
	0.40	0.90	0.00	-1.03	0.17	0.13	0.01	24.00	1.03
	0.13	0.84	0.02	0.30	0.03	0.03	0.00	27.18	0.07
	0.04	0.63	0.03	4.51	0.20	0.00	0.00	26.95	0.39
G012267	32.25	0.94	0.03	-1.58	0.13	0.95	0.02	-1.88	0.02
	10.75	0.94	0.01	-1.50	0.08	0.94	0.02	-1.94	0.02
	3.58	0.93	0.01	-1.57	0.13	0.84	0.00	-1.48	0.10
	1.19	0.91	0.05	-1.62	0.25	0.52	0.01	7.68	0.29
	0.40	0.91	0.01	-1.48	0.22	0.15	0.01	24.74	1.33
	0.13	0.87	0.01	-0.77	0.07	0.04	0.01	27.00	0.05
G012278	0.04	0.78	0.02	1.28	0.07	0.01	0.00	27.00	0.05
	32.25	0.95	0.00	-1.63	0.18	0.95	0.01	-1.87	0.04
	10.75	0.94	0.01	-1.64	0.10	0.94	0.01	-1.91	0.14
	3.58	0.94	0.00	-1.59	0.05	0.82	0.02	-1.41	0.16
	1.19	0.88	0.01	-1.38	0.20	0.44	0.01	8.23	0.17
	0.40	0.65	0.03	0.29	0.14	0.08	0.03	26.13	1.19
G012279	0.13	0.34	0.02	5.68	0.20	0.02	0.00	27.00	0.05
	0.04	0.12	0.01	12.54	0.43	0.01	0.00	27.00	0.05
	32.25	0.00	0.00	-0.99	0.39	0.00	0.00	0.02	0.11
	10.75	0.00	0.00	-0.56	0.05	0.00	0.00	8.49	0.34
	3.58	0.00	0.00	3.54	0.08	0.00	0.00	26.50	0.03
	1.19	0.00	0.00	12.69	0.56	0.00	0.00	27.00	0.05
G012280	0.40	0.00	0.00	15.91	0.26	0.00	0.00	26.85	0.17
	0.13	0.00	0.00	17.60	0.18	0.00	0.00	27.00	0.05
	0.04	0.00	0.00	17.32	0.00	0.00	0.00	27.00	0.05
	32.25	0.93	0.01	-1.68	0.27	0.87	0.01	-1.91	0.01
	10.75	0.90	0.03	-1.56	0.20	0.83	0.01	-1.62	0.30
	3.58	0.91	0.03	-1.55	0.11	0.56	0.02	2.88	0.53
G012293	1.19	0.85	0.01	-0.61	0.27	0.15	0.00	21.86	0.24
	0.40	0.61	0.02	4.05	0.45	0.02	0.00	26.36	0.86
	0.13	0.26	0.01	11.55	0.19	0.01	0.00	27.00	0.05
	0.04	0.08	0.00	15.47	0.38	0.00	0.00	27.00	0.05
	32.25	0.91	0.00	-1.79	0.18	0.98	0.00	-1.82	0.06
	10.75	0.91	0.01	-1.63	0.19	0.96	0.02	-1.84	0.02
G012294	3.58	0.92	0.02	-1.65	0.27	0.90	0.00	-1.21	0.55
	1.19	0.89	0.00	-1.27	0.55	0.54	0.02	3.40	0.13
	0.40	0.82	0.02	-0.36	0.32	0.19	0.02	22.12	0.61
	0.13	0.60	0.00	3.31	0.19	0.04	0.02	26.91	0.09
	0.04	0.32	0.01	10.09	0.50	0.01	0.00	27.00	0.04
	32.25	0.93	0.01	-1.58	0.28	0.90	0.02	-1.84	0.12
	10.75	0.91	0.01	-1.41	0.14	0.89	0.00	-1.86	0.04

GUIDE ID	Guide conc. (nM)	PHH				PCH			
		Mean Indel Freq	SD	Mean Secreted KLKB1	SD	Mean Indel Freq	SD	Mean Secreted KLKB1	SD
	3.58	0.91	0.01	-1.46	0.23	0.60	0.00	-0.15	0.19
	1.19	0.82	0.02	-0.96	0.32	0.23	0.03	13.68	0.43
	0.40	0.60	0.03	1.61	0.43	0.07	0.03	25.94	1.04
	0.13	0.30	0.01	7.91	0.02	0.01	0.00	26.73	0.16
	0.04	0.12	0.00	12.18	1.01	0.01	0.00	27.00	0.05
G012298	32.25	0.95	0.00	-0.50	0.11	NA	NA	15.77	0.15
	10.75	0.95	0.00	-0.47	0.23	NA	NA	27.00	0.05
	3.58	0.91	0.00	-0.15	0.21	NA	NA	27.00	0.05
	1.19	0.83	0.01	0.88	0.40	NA	NA	27.00	0.05
	0.40	0.59	0.01	6.54	0.52	NA	NA	26.39	0.82
	0.13	0.24	0.02	12.35	0.21	NA	NA	27.00	0.05
G012303	0.04	0.08	0.00	16.29	1.12	NA	NA	27.00	0.05
	32.25	0.96	0.01	-1.27	0.14	0.88	0.01	-1.55	0.10
	10.75	0.94	0.00	-1.39	0.18	0.83	0.02	-1.36	0.12
	3.58	0.93	0.01	-1.28	0.15	0.40	0.02	8.20	0.71
	1.19	0.90	0.01	-0.60	0.18	0.08	0.01	26.23	0.95
	0.40	0.74	0.02	2.18	0.05	0.02	0.00	27.18	0.07
G012304	0.13	0.36	0.01	9.45	0.14	0.00	0.00	27.18	0.07
	0.04	0.13	0.01	15.14	0.26	0.00	0.00	27.18	0.07
	32.25	0.94	0.01	-1.37	0.32	0.96	0.00	-1.70	0.19
	10.75	0.95	0.01	-1.39	0.10	0.94	0.01	-1.72	0.08
	3.58	0.91	0.00	-1.41	0.21	0.87	0.00	-1.52	0.01
	1.19	0.84	0.02	-0.38	0.15	0.52	0.02	6.62	0.71
G012305	0.40	0.61	0.02	3.74	0.19	0.13	0.02	25.35	1.70
	0.13	0.22	0.01	11.39	0.18	0.02	0.00	27.18	0.07
	0.04	0.06	0.00	15.41	0.72	0.00	0.00	27.18	0.07
	32.25	0.95	0.01	-1.26	0.16	0.86	0.01	-1.53	0.11
	10.75	0.94	0.00	-1.37	0.13	0.75	0.02	-0.43	0.12
	3.58	0.92	0.02	-1.30	0.15	0.33	0.01	15.42	0.72
G012308	1.19	0.86	0.01	-0.26	0.07	0.06	0.00	27.18	0.07
	0.40	0.65	0.03	3.85	0.04	0.01	0.00	27.18	0.07
	0.13	0.27	0.04	10.94	0.24	0.00	0.00	27.18	0.07
	0.04	0.08	0.01	15.12	0.54	0.00	0.00	27.18	0.07
	32.25	0.95	0.01	-1.07	0.19	0.93	0.02	-1.66	0.12
	10.75	0.95	0.01	-0.92	0.07	0.92	0.01	-1.44	0.02
G012321	3.58	0.88	0.02	-0.77	0.27	0.60	0.07	-0.18	0.32
	1.19	0.75	0.01	1.00	0.10	0.34	0.03	14.44	0.04
	0.40	0.31	0.05	7.89	0.27	0.08	0.02	26.77	0.64
	0.13	0.07	0.00	12.76	0.59	0.03	0.02	27.18	0.07
	0.04	0.02	0.01	14.75	0.43	0.00	0.00	27.17	0.05
	32.25	0.93	0.00	-1.17	0.24	0.96	0.01	-1.68	0.19
	10.75	0.93	0.01	-1.26	0.14	0.92	0.02	-1.60	0.12
	3.58	0.88	0.02	-1.07	0.21	0.80	0.02	-0.84	0.10

GUIDE ID	Guide conc. (nM)	PHH				PCH			
		Mean Indel Freq	SD	Mean Secreted KLKB1	SD	Mean Indel Freq	SD	Mean Secreted KLKB1	SD
	1.19	0.85	0.00	-0.17	0.17	0.40	0.02	14.33	0.57
	0.40	0.63	0.02	3.56	0.06	0.09	0.01	23.98	1.24
	0.13	0.25	0.03	10.27	0.82	0.03	0.02	27.17	0.05
	0.04	0.09	0.01	13.27	0.79	0.01	0.00	27.18	0.07
G012323	32.25	0.89	0.01	-1.17	0.28	0.93	0.00	-1.74	0.12
	10.75	0.90	0.00	-1.35	0.14	0.93	0.00	-1.72	0.03
	3.58	0.84	0.01	-1.14	0.17	0.76	0.54	-0.36	0.32
	1.19	0.73	0.01	0.20	0.14	0.35	0.01	15.43	0.17
	0.40	0.49	0.01	4.28	0.04	0.07	0.01	25.63	1.00
	0.13	0.23	0.02	10.12	1.03	0.01	0.00	27.18	0.07
G013901	0.04	0.08	0.01	14.08	0.80	0.00	0.00	27.18	0.07
	32.25	0.29	0.01	7.11	0.34	0.97	0.00	-1.58	0.48
	10.75	0.29	0.00	8.89	0.40	0.98	0.00	-1.76	0.26
	3.58	0.22	0.02	10.94	0.34	0.95	0.01	-1.81	0.19
	1.19	0.15	0.02	14.44	0.70	0.79	0.01	-0.82	0.32
	0.40	0.08	0.01	13.76	0.22	0.46	0.00	8.67	0.27
	0.13	0.05	0.00	14.80	0.40	0.19	0.01	21.35	0.16
	0.04	0.02	0.00	14.97	0.59	0.05	0.00	26.84	0.19
untreated	NA	0.00	0.00	13.62	0.95	0.00	0.00	25.77	2.09

3.3 Cross screening of lead sgRNAs in PCH and PHH in 7-point dose response assays

[00649] Lipid nanoparticle (LNP) formulations of modified sgRNAs were tested in PHH and PCH in a dose response assay.

[00650] The LNPs described in Example 3.2 were used in this study.

[00651] Post-incubation, the LNPs were added to the human or cynomolgus hepatocytes in a 7 point, 3-fold dose response curve. The cells were lysed 72 hours post-transfection and gDNAs were subjected to quantified PCR for NGS analysis as described in Example 1. For KLKB1 protein analysis the cells were lysed at day 8 post-transfection and whole cell extracts were subject to western blotting analysis as described in Example 1.

[00652] The indel frequency of sgRNAs at concentrations, 0.04 nM, 0.13 nM, 0.40 nM, 1.19 nM, 3.58 nM, 10.75 nM, and 32.25 nM for PHH and PCH is shown in Table 13 and dose response curve data is illustrated in FIGS. 7A and 7B. Secreted KLKB1 protein levels of the sgRNAs determined by ELISA is shown in Table 13 and FIG. 7C for PHH and FIG 7D for PCH.

Table 13. Indel frequency and secreted KLKB1 protein for LNPs targeting KLKB1 *in vitro*

GUIDE ID	PHH					PCH				
	Guide conc. (nM)	Mean Indel Freq	SD	Mean secreted KLKB1	SD	Guide conc. (nM)	Mean Indel Freq	SD	Mean secreted KLKB1	SD
G012260	32.25	0.88	0.03	-0.07	0.04	32.25	0.97	0.01	-1.42	0.04
	10.75	0.86	0.03	0.05	0.09	10.75	0.98	0.01	-1.32	0.03
	3.58	0.82	0.08	0.03	0.04	3.58	0.95	0.00	-1.41	0.04
	1.19	0.81	0.07	0.06	0.00	1.19	0.68	0.07	-0.08	0.03
	0.40	0.84	0.00	0.14	0.00	0.40	0.26	0.04	14.46	1.24
	0.13	0.79	0.04	0.55	0.01	0.13	0.06	0.01	24.37	0.74
	0.04	0.73	0.02	1.80	0.01	0.04	0.01	0.00	25.32	0.79
G012267	32.25	0.88	0.05	0.11	0.02	32.25	0.98	0.00	-1.46	0.01
	10.75	0.89	0.04	0.17	0.01	10.75	0.97	0.02	-1.28	0.14
	3.58	0.87	0.05	0.16	0.05	3.58	0.94	0.00	-1.36	0.08
	1.19	0.89	0.04	0.12	0.02	1.19	0.61	0.01	1.06	0.00
	0.40	0.86	0.02	0.23	0.02	0.40	0.20	0.03	15.86	0.74
	0.13	0.87	0.02	0.54	0.00	0.13	0.05	0.00	25.73	1.85
	0.04	0.80	0.03	1.54	0.06	0.04	0.02	0.00	26.39	0.16
G012293	32.25	0.85	0.00	-0.09	0.07	32.25	0.98	0.01	-1.43	0.02
	10.75	0.76	0.15	-0.04	0.01	10.75	0.98	0.01	-1.32	0.06
	3.58	0.78	0.02	0.06	0.08	3.58	0.98	0.00	-1.37	0.07
	1.19	0.85	0.06	0.39	0.30	1.19	0.81	0.01	-0.89	0.04
	0.40	0.74	0.08	0.71	0.01	0.40	0.35	0.03	9.93	0.49
	0.13	0.71	0.02	2.52	0.12	0.13	0.09	0.00	23.26	0.06
	0.04	0.45	0.03	7.55	0.09	0.04	0.02	0.00	25.17	0.05
G013901	32.25	0.31	0.07	9.02	0.05	32.25	0.98	0.00	-1.47	0.02
	10.75	0.31	0.02	8.64	0.30	10.75	0.98	0.00	-1.35	0.04
	3.58	0.26	0.03	11.28	0.62	3.58	0.98	0.00	-1.45	0.03
	1.19	0.19	0.01	12.75	0.42	1.19	0.89	0.02	-1.32	0.09
	0.40	0.13	0.02	15.06	0.73	0.40	0.58	0.05	2.65	0.28
	0.13	0.06	0.01	15.61	1.11	0.13	0.23	0.05	15.10	0.22
	0.04	0.04	0.00	16.27	0.46	0.04	0.08	0.00	23.21	0.82
untreated	NA	0.00	0.00	18.29	1.14	NA	0.00	0.00	25.96	1.35

[00653] For KLKB1 protein analysis, PHH were transfected with human KLKB1 guide, G012267, and lysed at day 8 post-transfection and whole cell extracts were subject to western blotting analysis as described in Example 1. Human KLKB1 protein levels across the 7-point dose response curve were compared to untreated control and normalized to GAPDH is shown in FIG. 7E.

Example 4 – Off-target analysis of KLKB1 guides

[00654] The biochemical method described in Example 1 was used to determine potential off-target genomic sites cleaved by Cas9 targeting KLKB1. Guides selected based on results from experiments described above were tested for potential off-target genomic cleavage sites. Sixteen KLKB1 targeting guides were evaluated for off-target genomic cleavage against genomic DNA from HEK293 cells at a 16 nM concentration (ATCC, Cat. #CRL-1573). KLKB1 guide G012267 and control guides with known off-target profiles were included in experiments (G000644 targeted to EMX1 for which 281 off-target sites have been detected, G00045 targeted to VEGFA for which 6602 off-target sites have been detected); Frock et al., 2015, Tsai et al., 2015) were evaluated for off-target genomic cleavage against genomic DNA from pooled human PBMC at a 64 nM concentration. The number of potential off-target sites detected in the biochemical assay using genomic DNA from HEK 293 cells are shown in Table 14A. The number of potential off-target sites detected in the biochemical assay using genomic DNA from PBMCs are shown in Table 14B. The percent of off-target sites detected by the assay performed herein as compared to the number of off-target sites noted in the literature for the EMX1 guide and the VEGFA guide are noted.

Table 14A. Biochemical Off-Target Analysis with HEK293 cell genomic DNA

Guide ID	Target	Guide Concentration (nM)	Off-target sites
G012253	KLKB1	16	122
G012259	KLKB1	16	77
G012260	KLKB1	16	153
G012267	KLKB1	16	136
G012278	KLKB1	16	153
G012279	KLKB1	16	0
G012280	KLKB1	16	292
G012293	KLKB1	16	126
G012294	KLKB1	16	132
G012298	KLKB1	16	155
G012303	KLKB1	16	45
G012304	KLKB1	16	42

Guide ID	Target	Guide Concentration (nM)	Off-target sites
G012305	KLKB1	16	48
G012308	KLKB1	16	116
G012321	KLKB1	16	107
G012323	KLKB1	16	14

Table 14B. Biochemical Off-Target Analysis with human PBMC genomic DNA

Guide	Target	Off-Target sites (percent*)
G012267	KLKB1	61
G000644	EMX1	242/281 (86%)
G000645	VEGFA	4431/6602 (67%)

*Percent is relative to the known number of off-target sites for each of the guide target sites.

Example 5. Targeted sequencing for validating potential off-target sites

[00655] KLKB1 guides were selected based on experiments above for further evaluation. The targeted off-target approach described in Example 1 was used to evaluate the target indel activity for the potential off-targets associated with these guides. The off-target sites tested in the experiment were identified via the biochemical assay experiments described in Example 4 or *in silico* prediction as described in Example 1.

[00656] In this experiment, 3 sgRNAs targeting human KLKB1 were evaluated. PHH were cultured and transfected with LNPs comprising Cas9 mRNA and sgRNA of interest (e.g., a sgRNA having potential off-target sites for evaluation) as described in Example 1. Genomic DNA was isolated from the PHH and subjected to NGS and targeted off-target analysis as described in Example 1.

[00657] The number of potential off-target sites evaluated in the assay and of those sites, off-targets that were successfully characterized by the assay followed by sites that were validated via manual inspection are shown in Table 15A.

Table 15. Targeted Off-Target Analysis

Guide ID	Off-targets evaluated	Off-targets characterized	Validated off-targets
G012260	223	206	5
G012267	181	171	1
G012293	360	347	4

Example 6. In vivo editing of the humanized KLKB1 locus in Hu KLKB1 mouse model

[00658] Humanized mice that express human KLKB1 protein (Hu KLKB1 mouse model) were used in this study. The Hu KLKB1 mouse model comprises a humanized *KLKB1* locus in which the region from start codon to stop codon of mouse KLKB1 was replaced with the corresponding human genomic sequence. Animals were weighed and dosed at volumes specific to individual body weight. There were 5 groups total (N=4 with 2 male and 2 female mice).

[00659] LNPs containing modified sgRNAs (G12260, G12267, G12293, G12303, and G12321) and the Cas9 mRNA were dosed via the lateral tail vein at 0.3 mg/kg based on total RNA cargo in a volume of 10ml per kilogram body weight. The final LNPs were characterized to determine the encapsulation efficiency, polydispersity index, and average particle size according to the analytical methods described in Example 1. At day 11 post-LNP administration, mice were euthanized, and liver tissue was collected for DNA extraction. The tissues were lysed using a Zymo Research Bashing Bead Lysis Rack, and DNA was extracted using the Zymo Research DNA Extraction Kit according to the manufacturer's protocol. The extracted DNA was subject to PCR to be submitted for sequencing.

[00660] Blood was collected into serum separator tubes and allowed to clot for 2 hours at room temperature followed by centrifugation. ELISA was performed on the serum aliquoted and diluted in a 96-well plate.

[00661] Editing observed in treated mice is shown in FIG. 8A and Table 16A.

Table 16A. In vivo Editing Data in Hu KLKB1 Mouse Model

Guide	%Editing	Editing SD	N
G12260	32.9	10.96	4
G12267	72.83	1.17	4
G12293	43.05	5.59	4
G12303	14.38	5.60	4
G12321	35.53	11.11	4

[00662] Serum human KLKB1 protein levels, pre- and post-dose, were measured using the ELISA assay as described in Example 1. The results are shown in Table 16B and FIG. 8B.

Table 16B. Secreted KLKB1 protein levels in Hu KLKB1 Mouse Model

Guide	Dose (mpk)	Pre-dose	SD	Post-dose	SD	N
G12260	0.3	19.04	6.20	13.10	7.96	4
G12267	0.3	22.48	9.32	1.42	0.41	4
G12293	0.3	18.54	5.41	7.59	2.10	4
G12303	0.3	21.21	9.98	23.11	8.58	4
G12321	0.3	18.07	5.21	11.22	4.51	4

[00663] Serum human KLKB1 protein levels from the samples were measured using the electrochemiluminescence-based array (MSD) as described in Example 1 and compared to baseline levels. The results are shown in Table 17 and FIG. 8C.

Table 17. KLKB1 protein level *in vivo*

Guide	Dose (mpk)	Pre-dose	SD	Post-dose	SD	% serum KD	N
G12260	0.3	10.44	0.43	6.84	0.09	38	4
G12267	0.3	10.59	0.37	BLOD*	-	97**	2
G12293	0.3	10.74	0.24	3.92	0.04	64	4
G12303	0.3	13.86	0.34	7.78	0.35	35**	4
G12321	0.3	8.38	0.1	3.92	0.16	55	4

* Below limit of detection; ** approximate

[00664] KLKB1 mRNA levels for each sequence were measured by quantitative PCR as described in Example 1 and shown in Table 18 and FIG. 8D. Protein reduction was confirmed by western blot analysis as described in Example 1.

Table 18. qPCR results

Guide	Fold change	SD	N
G12260	1.20	0.35	4
G12267	0.51	0.41	4
G12293	0.73	0.22	4
G12303	1.10	0.23	4
G12321	1.18	0.41	4
TSS	1.01	0.17	2

Example 7. *In vivo* editing activity of human KLKB1 guides in Hu KLKB1 mouse model

[00665] Humanized KLKB1 mice described in Example 6 were used in this study and prepared using the same protocol. There were 5 groups total (N=5 with 2 male and 3 female mice or vice versa). LNPs containing modified sgRNAs and mRNA encoding the Cas9 protein were dosed via the lateral tail vein at 0.3 mg/kg and characterized as described in Example 6.

[00666] At day 13 post-LNP administration, mice were euthanized. Liver tissue and blood was processed as described in Example 6 for sequencing and ELISA analysis.

[00667] Table 19 and FIGS. 9A-9D show levels of KLKB1 editing, serum prekallikrein protein (detected using an ELISA that detects both prekallikrein and kallikrein) (ug/ml), prekallikrein protein as percent of KLKB1 protein level in control TSS in treated mice, and the correlation of percent liver editing to percent prekallikrein protein, respectively. G012323 and G012253 guides were tested; however, editing was not detected due to a failure of the NGS method.

Table 19. Percent Editing and Serum Prekallikrein of Certain Guides in Hu-KLKB1 mouse model

Dose (mpk)	Guide	Sample	% Edit	Serum Prekallikrein (ug/ml)	Serum Prekallikrein (% TSS Mean)
0	TSS	Mean	0.1	19.49	100
		Animal 1	0.1	14.77	76
		Animal 2	0.1	18.29	94
		Animal 3	0.1	14.82	76
		Animal 4	0.1	26.82	138
		Animal 5	0.1	22.76	117
0.3	G012304	Mean	21.9	12.4	64
		Animal 1	24.7	9.53	49
		Animal 2	21.0	9.88	51
		Animal 3	29.3	7.94	41
		Animal 4	18.8	20.32	104
		Animal 5	15.9	14.34	74
	G012305	Mean	26.0	10.58	54
		Animal 1	20.6	9.09	47
		Animal 2	32.5	8.85	45
		Animal 3	27.9	8.13	42
		Animal 4	22.7	14.08	72
		Animal 5	26.5	12.75	65
	G012259	Mean	24.2	10.88	56

Dose (mpk)	Guide	Sample	% Edit	Serum Prekallikrein (ug/ml)	Serum Prekallikrein (% TSS Mean)
		Animal 1	41.0	5.83	30
		Animal 2	20.3	9.96	51
		Animal 3	27.6	7.42	38
		Animal 4	9.4	17.83	91
		Animal 5	22.8	13.36	69
	G012278	Mean	24.0	10.91	56
		Animal 1	29.8	7.15	37
		Animal 2	35.2	6.99	36
		Animal 3	28.6	6.16	32
		Animal 4	18.2	17.21	88
		Animal 5	8.0	17.07	88
	G012280	Mean	13.8	14.71	75
		Animal 1	21.4	10.70	55
		Animal 2	24.0	8.92	46
		Animal 3	4.8	20.46	105
		Animal 4	8.9	17.34	89
		Animal 5	9.7	16.11	83
	G012294	Mean	15.9	15.4	79
		Animal 1	21.1	10.38	53
		Animal 2	15.9	9.69	50
		Animal 3	15.2	15.37	79
		Animal 4	14.9	19.13	98
		Animal 5	12.4	22.44	115
	G012298	Mean	36.9	9.05	46
		Animal 1	40.8	4.99	26
		Animal 2	46.6	5.26	27
		Animal 3	44.9	5.38	28
		Animal 4	25.3	15.01	77
		Animal 5	26.9	14.61	75

Example 8. *In vivo* dose response of KLKB1 gene editing in Hu KLKB1 mouse model

[00668] Humanized mice described in Example 6 were used in this study and prepared using the same protocol. There were 5 groups total (N=5 with 2 male and 3 female mice or vice versa). LNPs containing G12267 and mRNA encoding the Cas9 protein were dosed at 0.3, 0.1, 0.03 and 0.01 mg per kg bodyweight and characterized as described in Example 6.

[00669] At day 13 post-LNP administration, mice were euthanized. Liver tissue was processed as described in Example 6 for DNA sequencing. Blood was processed as described

in Example 6 and secreted human prekallikrein was measured via an ELISA, which detects prekallikrein and kallikrein (also, called total kallikrein), as described in Example 1.

[00670] For RNA analysis, liver tissue was lysed using a Zymo Research Bashing Bead Lysis Rack, and RNA was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Cat. 74106) according to the manufacturer's protocol. RNA was quantified using a Nanodrop 8000 (ThermoFisher Scientific, Cat. ND-8000-GL). RNA samples were stored at -20°C prior to use.

[00671] The SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen, Cat. 11732-088) was used to create the PCR reactions. Quantitative PCR probes targeting Hu *KLKB1* and internal control Ms *PPIB* were used in the reactions. The quantitative PCR assay was performed according to the manufacturer's specifications, scaled to the appropriate reaction volume, as well as using the Hu *KLKB1* and Ms *PPIB* probes specified above. The StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Cat. 4376600) was used to perform the real-time PCR reaction and transcript quantification according to the manufacturer's protocol.

[00672] Hu *KLKB1* mRNA was quantified using a standard curve starting at 20 ng/uL of pooled mRNA from the vehicle control group, with five further 3-fold dilutions ending at 0.06ng/uL. Ct values were determined from the StepOnePlus Real-Time PCR System. Reduction of total secreted human prekallikrein protein for cells treated with *KLKB1* reagents was determined by ELISA as described in Example 1.

[00673] Table 20 and FIG. 10 show percent editing, serum prekallikrein levels as a percent of TSS vehicle control treated mice, and mRNA transcript levels as a percent of TSS vehicle control treated animals.

Table 20 Percent Editing, *KLKB1* mRNA (% of basal level) and Serum Prekallikrein Protein Levels (% of basal level) in Hu *KLKB1* Mouse Model

Guide	Dose (mpk)		%Editing	%TSS protein	%TSS mRNA	SD
TSS	0	Mean	0.1	100	100.5	9.7
		Animal 1	0.1	75.8		
		Animal 2	0.1	93.8		
		Animal 3	0.1	76.0		
		Animal 4	0.1	137.6		
		Animal 5	0.1	116.8		

Guide	Dose (mpk)		%Editing	%TSS protein	%TSS mRNA	SD
G12267	0.01	Mean	3.9	91.9	100.1	9.5
		Animal 1	4.4	55.3		
		Animal 2	3.8	57.3		
		Animal 3	4.5	126.2		
		Animal 4	4.2	122.2		
		Animal 5	2.6	98.6		
	0.03	Mean	19.0	64.2	69.3	13.8
		Animal 1	22.1	38.6		
		Animal 2	0.3	51.0		
		Animal 3	26.9	78.9		
		Animal 4	21.3	80.5		
		Animal 5	24.3	72.2		
	0.1	Mean	55.4	23.3	48	11.4
		Animal 1	52.1	17.6		
		Animal 2	52.7	19.6		
		Animal 3	56.5	25.3		
		Animal 4	57.5	25.6		
		Animal 5	58.0	28.3		
	0.3	Mean	72.9	3.1	23	13
		Animal 1	73.9	2.7		
		Animal 2	70.4	2.9		
		Animal 3	72.7	3.1		
		Animal 4	73.1	3.4		
		Animal 5	74.3	3.4		

Example 9. Vascular Leakage study

[00674] A study was performed to evaluate *KLKB1* gene editing, total kallikrein protein expression, and vascular leakage in humanized mice. Humanized mice described in Example 1 were used in this study. There were 6 groups (N=5 with 2 male, 3 female mice per group). Animals were weighed and dosed at volumes specific to individual body weight.

[00675] LNPs containing a modified *KLKB1* targeting sgRNA (G12267) and the Cas9 mRNA were dosed via the lateral tail vein at 0.03 mg/kg, 0.1 mg/kg, or 0.3 mg/kg based on total RNA cargo in a volume of 10ml per kilogram body weight or vehicle control (TSS).

[00676] At one day prior to the vascular leakage study, blood was collected and processed as described in Example 6, and secreted human prekallikrein was measured via an ELISA, which detects prekallikrein and kallikrein (also, called total kallikrein), as described in Example 1.

[00677] The vascular leakage assay was performed as described in Example 1. At necropsy, liver tissue was collected and DNA extracted as described in Example 6 to measure KLKB1 editing. For dye quantification in the vascular leakage model, colon tissue was collected and processed as described in Example 1.

[00678] The results for percent editing, serum hu KLKB1 protein levels, and vascular leakage are shown in Table 21 and FIGS. 11A-11B.

Table 21. Percent editing, KLKB1 protein levels, and vascular leakage in huKLKB1 mice

	Dose (mg/kg)	% Editing	Serum Prekallikrein (ug/ml)	Colon (OD)
TSS-1	0	0.02	100.00	0.07
TSS-2	0	0.06	94.92	0.22
Control	0.3	0.1	92.99	0.30
G012267	0.03	16.48	82.97	0.26
G012267	0.1	40.46	48.97	0.20
G012267	0.3	68.66	13.88	0.09

[00679] A separate study was conducted using similar methods to assess the percent editing, serum prekallikrein levels, and vascular leakage for durability over a 9-month period. Mice were dosed with modified KLKB1 targeting sgRNA (G12267) and the Cas9 mRNA or a non-targeting sgRNA were dosed via the lateral tail vein at 0.1 mg/kg or 0.3 mg/kg based on total RNA cargo in a volume of 10ml per kilogram body weight. The durability of the dose response was observed where increased editing, decreased protein levels, and decreased vascular leakage levels were maintained for the length of the study.

Example 10. *In vivo* testing of KLKB1 gene editing in non-human primates (NHPs)

[00680] In this example, a study was performed to evaluate *KLKB1* gene editing and total kallikrein protein expression, and total kallikrein activity levels in cynomolgus monkeys following administration of CRISPR/Cas9 lipid nanoparticles (LNP) with mRNA for Cas9 protein and various guides to the *KLKB1* gene. Cynomolgus monkeys were treated in cohorts of n=3. This study was conducted with LNP formulations according to Example 1. Each LNP formulation contained a polyadenylated Cas9 mRNA (comprising SEQ ID NO: 516) and gRNA (G013901, a cynomolgus specific KLKB1 guide RNA) with an mRNA:gRNA ratio of 2:1 by weight. Animals were dosed at 1.5, 3, or 6 mg per kg doses based on total RNA

cargo. Indel formation (percent editing) was measured by NGS. Total kallikrein activity and serum kallikrein protein level were measured as described in Example 1.

[00681] The study showed that knockout of KLKB1, which is part of a biological pathway that results in release of bradykinin, with G013901 produced up to a 90% reduction in kallikrein activity in NHP groups, or more, a robust response that exceeds the target activity shown to achieve a therapeutically meaningful impact on HAE attack rates (60% kallikrein activity reduction; Banerji, 2017). This study showed a dose-dependent correlation between increased editing rates, reduced plasma kallikrein levels, and reduced kallikrein activity. The response has been durable through one year in NHPs. Circulating kallikrein protein and activity levels are provided in Tables 22 and 23; and FIGS. 12A-12B.

Table 22. Kallikrein Activity (% of basal activity)

Day	TSS (n=3)		1.5 mpk (n=3)		3 mpk (n=3)		6 mpk (n=3)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	100	0	100	0	100	0	100	0
7	108.4	9	67.8	7	42.8	12.4	18.2	10.6
14	101.8	18.3	31.1	3.2	15.9	11	5.3	1.6
28	124.4	7.9	26.8	6.5	10.6	5.4	3.9	1.4
42	117.8	5.8	19.8	11.9	8.6	4.4	3.6	0.4
56	130.3	32.5	8.8	5	3.4	0.6	4.2	2.2
70	109.2	15	6	1.7	3	0.4	3.9	1.6
84	121.3	25.7	10.6	3.8	4.2	2.1	4.7	2.5
105	130.8	15.5	23.3	4.9	6	3.6	4.7	2.5
119	88.2	9.1	12.2	10.2	3.4	0.5	3	0.5
147	101.1	3.3	11.9	6.6	4.5	1.4	3	0.5
161	113.2	13.4	21	1.8	6	2.8	5.6	1.8
180	122.3	8.7	19.2	9.5	4.7	1	5	1.9
238	125.5	27.3	16.6	6.5	4.7	1.4	3.2	0.7
252	122.7	27	19.1	7.3	8.2	4.4	3.1	0.7
266	115	23.3	13.9	3.3	8.9	5.2	2.7	0.6
280	112.2	22.7	17.5	2.3	6.8	3.6	3	1
294	126.3	34.1	17	5.8	7.9	4.1	2.9	0.6
308	122.9	28.9	18.2	2.3	7.4	3.3	3	0.8
326	111.6	23	13.3	5.2	5.5	2.9	3.5	0.4
333	127.3	22.4	15.9	1.8	6.8	2.8	3.3	0.4
347	108.4	11.7	16.9	1	4.1	1.8	2.9	0.3
365	118	2.2	24.1	10.2	10	5.7	3.4	0.7

Table 23. Plasma Kallikrein Protein Levels (% of basal level)

	TSS (n=3)		1.5 mpk (n=3)		3 mpk (n=3)		6 mpk (n=3)	
Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	100	0	100	0	100	0	100	0
14	101.3	14.9	28.8	7.4	27.1	8.2	13.7	3.3
28	117.7	7	32.7	8.5	16.9	6.8	6.6	2.3
42	112.6	23.5	31.5	9.1	16	7	6.3	2.8
56	111	16	30.1	8.8	15.4	6	5.8	2.5
70	112.5	14.1	29.8	8	15.4	5.8	5.8	2.8
84	133.6	9	38.7	11.1	17.1	6.7	6.7	2.5
105	118.2	20.1	47	12.7	22.3	10.3	7.6	3.8
119	96	9.8	31.6	8.7	18.1	7.2	6.4	2.2
147	110.7	12	32.1	8.3	18.3	8.1	6.7	2.2
161	119.3	6.1	35.8	10.5	7.5	6.8	7.4	3.5
180	131	10.1	33.8	11.4	18	10.1	2.9	1
238	106.2	3.7	27.4	10.8	16.4	9.8	3.7	0.9
252	114.3	21.4	27.9	9.1	15.6	7.1	3.9	0.8
266	117.6	8.7	28.7	9.7	21.8	10.4	3.7	0.5
280	93.4	22.1	33.7	7.1	24.6	9.8	6.3	2.9
294	122.1	23.7	30.4	13.8	24.6	14	7.4	4
308	93.2	12.8	20.6	13.8	27.6	14.2	6.2	3.1
326	N/A		29.7	7.3	23.8	12.5	8	2.8
333	130.2	25.5	30.9	6.4	25.3	12.4	7.8	2.3
347	106.9	15	26	8.2	22.1	11.2	7.3	3.2
365	108.5	41.3	22.7	8	11.4	5.3	4.3	1.6

[00682] Tests of select NHP serum samples found no observed impact on coagulation pathway biomarkers with *KLKB1* knockout in NHPs at weeks 10 or 15 (based on measuring prothrombin, APTT, and fibrinogen (all at week 10), and Factor XII (at week 15)) when comparing TSS buffer control groups to treated groups.

[00683] The NHP study was repeated to evaluate *KLKB1* total kallikrein protein expression, and total kallikrein activity levels in cynomolgus monkeys using guide G012267 which includes a guide sequence fully complementary to human *KLKB1*. The guide sequence of G012267 has one nucleotide difference when compared to the G013901 which has a guide sequence fully complementary to cynomolgus *KLKB1*. The experimental protocol and LNP formulations in this study were essentially the same as described in the above experiment, except animals (n=3) were only dosed at 3 mg per kg based on total RNA cargo. Total

kallikrein activity and serum kallikrein protein levels were measured using the methods described in Example 1.

[00684] The study showed that knockdown of *KLKB1* with G012267 produced up to a 65% reduction in kallikrein activity in NHP groups. The response was durable through 9 months in NHPs. Circulating kallikrein protein and activity levels are provided in Tables 24 and 25, and FIGS. 13A-13B.

Table 24. Kallikrein Activity (% of basal activity)

Day	TSS (n=3)		3 mpk (n=3)	
	Mean	Std. Dev	Mean	Std. Dev
0	100	N/A	100	N/A
7	90.9	13.7	68.7	11.3
15	87.7	6.6	51.3	18.7
28	90.5	2.5	39.3	24
42	96.5	4.8	31.9	19.5
56	94.5	1.6	34.1	16.7
70	96.2	7.2	42.3	25.7
91	100.2	11.7	45.3	27.6
106	100.2	6.3	45.7	14.3
120	99.8	7.4	46.5	11.1
134	115.2	8.5	47.5	32.4
148	91.5	2	52.9	34.7
162	86.1	21.1	50.1	43.2
180	97.9	1.8	47.3	22.1
192	87.9	1.9	43	24.6
208	94.4	5.8	48	23
222	97	2.9	34.6	21.1
236	97.2	2.2	43.5	19.1
250	91.8	12	46.5	18.3
264	99.9	10.6	51.3	25.2
278	105.3	13.4	60.8	32.3

Table 25. Plasma Kallikrein Protein Levels (% of basal level)

Day	TSS (n=3)		3 mpk (n=3)	
	Mean	Std. Dev	Mean	Std. Dev
0	100	N/A	100	N/A
7	99.6	5.1	72.3	13.3

	TSS (n=3)		3 mpk (n=3)	
15	89.7	13.1	50.6	9
28	93.9	5.2	52.8	22.3
42	90.3	8.6	52.7	25.5
56	104.2	17.3	49.9	19.1
70	93.9	5.2	52.8	22.3
91	90.3	8.6	52.7	25.5
106	99.3	21.9	43.1	17.3
120	121.7	12.6	41.1	6.2
134	104.2	12	49.7	5.5
148	93	15.8	47.5	11.8
162	100.3	15.7	56.2	26.7
180	106.8	10.9	44.1	33.2
192	96.5	20.5	52	26.6
208	105.6	25	52.9	28.5
250	103.7	21.5	63.3	17.4
264	101.4	7	67.3	15.4
278	94.3	14.4	62.6	16.7