

METHODS FOR TREATING PRIMARY HYPEROXALURIA VIA GENETIC EDITING OF HYDROXYACID OXIDASE 1

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 This application claims the benefit of the filing date of U.S. Provisional Application No. 63/617,867, filed January 5, 2024, the entire contents of which is incorporated by reference herein.

SEQUENCE LISTING

- 10 The instant application contains a Sequence Listing which has been filed electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on January 2, 2025, is named 063586-536001WO_Seq-Listing_ST26.xml and is 56,491 bytes in size.

BACKGROUND

- 15 Primary Hyperoxaluria (PH) is a medical condition characterized by recurrent kidney and bladder stones resulting from overproduction of oxalate, which combines with calcium to form calcium oxalate, a main component of kidney and bladder stones. PH is estimated to affect 1 out of 58,000 individuals worldwide. There are three types of PH, PH1, PH2, and PH3,
20 among which PH1 accounts for approximately 80% of the cases.

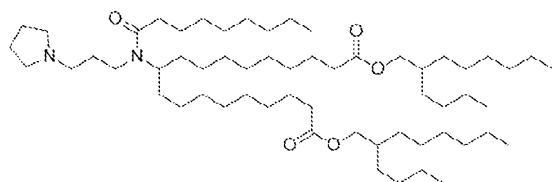
- Primary Hyperoxaluria is associated with genetic mutations in genes such as *AGXT*, *GRHPR*, and *HOGAI* genes, leading to abnormal breakdown of glyoxylate. Glycolate oxidase (GO), encoded by the hydroxyacid oxidase 1 (HAO1) gene, converts glyoxylate to oxalate. Knocking out the HAO1 gene could reduce the level of GO enzyme and, in turn, the level of
25 oxalate, thereby benefiting PH treatment.

SUMMARY OF THE INVENTION

- The present disclosure is based, at least in part, on the development of compositions and methods for genetic editing the hydroxyacid oxidase 1 (HAO1) gene in a subject to benefit
30 treatment of primary hyperoxaluria (PH). Accordingly, provided herein are, *inter alia*, compositions for genetic editing of an HAO1 gene and methods for using the HAO1 gene editing compositions disclosed herein for genetic modification of an HAO1 gene in cells (*e.g.*, in a subject) and/or for treating diseases associated with the HAO1 gene, for example, primary hyperoxaluria (PH).

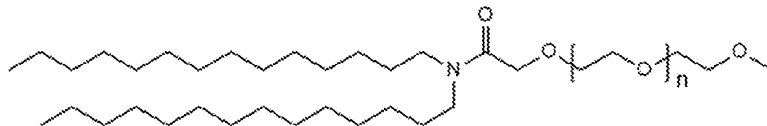
Accordingly, the present disclosure provides, in one aspect, a composition for genetic editing of a hydroxyacid oxidase 1 (HAO1) gene, comprising:

- (a) a messenger RNA (mRNA) comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 8, wherein the mRNA encodes a Cas12i2 polypeptide set forth as SEQ ID NO:7;
 - (b) a guide RNA (gRNA) comprising the nucleotide sequence of SEQ ID NO: 13; and
 - (c) lipid excipients comprising 45-50 mol% of a cationic lipid, 9-11 mol% of a zwitterionic phospholipid, 38-42 mol% of cholesterol, and 2.2-2.8 mol% of a pegylated lipid.
- In some embodiments, the cationic lipid has the structure of:



, or is a pharmaceutically acceptable salt or seteroisomer thereof.

In some embodiments, the pegylated lipid has the structure of:



- , in which the average n is 45-49, inclusive.

In some embodiments, the zwitterionic phospholipid is 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC).

- In some embodiments, the mRNA comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 8. In some embodiments, the mRNA comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 8. In some instances, the mRNA comprises a poly A tail at its 3' end, *e.g.*, a poly A tail containing 60-135 adenosine (A) residues (*e.g.*, ≥ 85 A residues). In specific examples, the mRNA comprises the nucleotide sequence of SEQ ID NO: 23. Alternatively or in addition, the mRNA comprises a 5' cap moiety, and/or one or more modified nucleotides, for example, pseudouridine (*e.g.*, in replacement of uridine residues). In some examples, the mRNA comprises pseudouridine residues at 75% or more of the uridine positions in the mRNA. In one specific example, the mRNA comprises pseudouridine residues

at all uridine positions in the mRNA. In one specific example, the mRNA comprises pseudouridine residues at all uridine positions in the mRNA and a 5' cap moiety.

In some embodiments, the lipid excipients comprising 47.5 mol% of the cationic lipid, 10 mol% of a zwitterionic phospholipid, 40 mol% of cholesterol, and 2.5 mol% of a pegylated lipid.

In any of the HAO1 gene editing compositions disclosed herein, the ratio between the messenger RNA and the gRNA may be $1\pm 20\%$ (*e.g.*, $1\pm 10\%$ or $1\pm 5\%$). In some examples, the ratio can be 1:1 (1). The nucleic acid components (mRNA and gRNA) may be associated with the lipid excipients. In some instances, the lipid excipients may form lipid nanoparticles (LNPs) and the nucleic acid components may be attached to or encapsulated in the LNPs.

In some embodiments, the gRNA in any of the compositions disclosed herein comprises one or more modifications. Examples include phosphorothioate linkage, 2'-O-methylation, or a combination thereof. In some instances, the modifications are located at the 5' and/or 3' nucleotides. In one specific example, the gRNA is a modified nucleic acid comprising the nucleotide sequence of SEQ ID NO: 14.

In some embodiments, the nitrogen:phosphate (N/P) ratio in the composition provided herein can range from 3 to 9. In some examples, the N/P ratio in the composition is 6.0 ± 1.0 . In one example, the N/P ratio of the composition is 6.0 ± 0.5 (*e.g.*, about 6.0).

In another aspects, the present disclosure features a method for genetic editing a hydroxyacid oxidase 1 (HAO1) gene in a subject, the method comprising: administering to a subject in need thereof an effective amount of any of the HAO1 gene editing compositions disclosed herein. In some embodiments, the subject is a human subject having primary hyperoxaluria (PH). In some examples, the human subject has PH1.

In some instances, the method may further comprise evaluating the level of HAO1 genetic editing in the subject. Alternatively, the method may further comprise analyzing levels of HAO1 gene product (the HAO1 protein, *a.k.a.*, GO protein or GO enzyme), molecules involved the GO enzyme-mediated catalytic pathway (*e.g.*, glycolate), or a combination thereof.

Also within the scope of the present disclosure is the HAO1 gene editing composition as disclosed herein for use in genetic editing the HAO1 gene in host cells and/or in a subject, who may be a human patient having PH, as well as for use in treating PH in a subject in need of the treatment. Further, the instant application provides uses of any of the HAO1 gene editing composition for manufacturing a medicament for use in treating PH (*e.g.*, PH1) in a subject, such as a human patient having the PH.

The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appended claims.

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BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to the drawing in combination with the detailed description of specific embodiments presented herein.

FIG. 1 is a diagram illustrating the effect of different treatments and doses on HAO1 gene editing in livers of non-human primate (NHP). Target Indel editing set at 55%. N/P: nitrogen:phosphate (N/P) target ratio. guide/RNA: target ratio of gRNA to mRNA.

FIGs. 2A-2C include diagrams illustrating treatment efficacy of an exemplary HAO1 gene editing composition as indicated. **FIG. 2A**: HAO1 gene editing efficiency. **FIG. 2B**: GO enzyme activity. **FIG. 2C**: glycolate levels.

FIG. 3 is a diagram showing correlation between HAO1 knockdown, glycolate and GO protein in mice using a mouse counterpart of the HAO1 gene editing composition provided herein.

FIGs. 4A-4D include diagrams showing HAO1 gene editing in a mouse model for PH1 using a mouse counterpart of the HAO1 gene editing composition provided herein. **FIG. 4A**: increase in HAO1 indels with increase doses. **FIG. 4B**: effects of *Hao1* editing on GO Enzyme activity. **FIG. 4C**: effects of *Hao1* editing on Glycolate levels. **FIG. 4D**: reduction in urinary oxalate that results from *Hao1* gene editing.

FIGs. 5A-5B include diagrams showing long-term durability of HAO1 gene editing and urinary oxalate levels in *Agxt*^{-/-} mice. **FIG. 5A**: a diagram showing endpoint whole liver tissue indels 3 and 6 months after 1.5 mg/kg dose of the composition. **FIG. 5B**: a diagram showing urinary oxalate expressed as % of baseline in *Agxt*^{-/-} mice 3 and 6 months after 1.5 mg/kg dose of the composition.

FIGs. 6A-6B include diagrams showing *Hao1* gene editing and urinary oxalate levels in juvenile *Agxt*^{-/-} mice treated with a HAO1 gene editing composition. **FIG. 6A**: a diagram showing editing of hepatic *Hao1* in *Agxt*^{-/-} mice at different ages after juvenile drug administration at the 2 mg/kg dose. **FIG. 6B**: a diagram showing 3 Month urinary oxalate in *Agxt*^{-/-} mice 3 months after juvenile drug administration at the 2 mg/kg dose.

FIG. 7 is a diagram showing normalized GO protein expression in the liver following 30 to 70% hepatectomy.

DETAILED DESCRIPTION

5 The present disclosure is based, at least in part, on the development of compositions and methods for genetic editing the hydroxyacid oxidase 1 (HAO1) gene in a subject to benefit treatment of primary hyperoxaluria (PH). As reported herein, the HAO1 gene editing composition disclosed herein showed successful genetic editing of the HAO1 gene in non-human primates (NHPs) as evidenced by Indel percentages at the HAO1 locus. Knocking out
10 the HAO1 gene in the animals led to reduced GO enzyme activities and increased glycolate levels in the treated animals, indicating a reduced level of conversion from glycolate to glyoxylate and thus reduced oxalate. Accordingly, the HAO1 gene editing composition provided herein is expected to benefit treatment of diseases such as primary hyperoxaluria (PH) via genetic editing of the HAO1 gene so as to reduce molecules contributing to formation
15 of kidney and bladder stones, one major symptom associated with PH.

 Further, durability of hepatic *Hao1* editing and urinary oxalate reduction was observed in a mouse model of PH1 after treatment of the HAO1 gene editing composition provided herein. Finally, rhampseq of 2 primary hepatocyte donors treated with the editing composition was used to assess 1549 candidate off-target sites nominated by *in silico* search (CALITAS) and
20 Digenome-Seq. Despite the large number of off-target site candidates identified in the *in silico* study, only 3 sites were verified to have editing activity slightly above the limit of detection (0.012%) in 1 or both donors in intronic regions not annotated in the *Catalogue of Somatic Mutations in Cancer* (COSMIC 2024). No *bona fide* off-targets were identified in exonic regions. No structural variations were detected using long range sequencing and optical
25 genome mapping, indicating that the chromosomal structure is maintained after treatment with the *HAO1* editing composition.

 Accordingly, the present disclosure provides, among others, compositions for genetic editing of an HAO1 gene and methods of using such compositions for genetic modification of an HAO1 gene in cells (*e.g.*, in a subject) and/or for treating diseases associated with the
30 HAO1 gene, for example, primary hyperoxaluria (PH).

I. Composition for Genetic Editing of HAO1 Gene

 Hydroxyacid oxidase 1 (“HAO1”), also known as “glycolate oxidase 1,” is a peroxisome protein expressed primarily in the liver and pancreas, and its activities include

oxidation of glycolate and 2-hydroxy fatty acids. The HAO1 gene product is the glycolate oxidase (GO) enzyme, also referred to as the GO protein or HAO1 protein. The structural information of human HAO1 gene can be found under NCBI Reference Sequence NG_046733.1.

5 The present disclosure provides a gene editing composition targeting an HAO1 gene, *e.g.*, the human HAO1 gene. The HAO1 gene editing composition provided herein may comprise: (a) a Cas12i2 polypeptide or a nucleic acid encoding the Cas12i2 polypeptide, (b) a guide RNA targeting a genomic site in an HAO1 gene, and (c) lipid excipients comprising one or more lipids, which may form lipid nanoparticle (LNP) structures. The Cas12i2 polypeptide
10 or the encoding nucleic acid and/or the gRNA can be associated with the lipid excipients. When the lipid excipients form LNPs, the Cas12i2 polypeptide or the encoding nucleic acid and/or the gRNA may be attached to and/or encapsulated by the LNPs.

 In some embodiments, the HAO1 gene editing composition may comprise a messenger RNA (mRNA) molecule encoding the Cas12i2 polypeptide, which may comprise an amino
15 acid sequence at least 95% identical to a parent Cas12i2 CRISPR nuclease set forth as SEQ ID NO: 1 and comprise at least amino acid substitutions at positions D581, I926, and V1030 (*e.g.*, D581R, I926R, and V1030G) relative to SEQ ID NO: 1. Alternatively or in addition, the gRNA in the HAO1 gene editing composition may comprise a spacer sequence, which is specific to a target sequence of 5'-CAAAGTCTATATATGACTAT-3' (SEQ ID NO: 10) in the HAO1
20 gene. The mRNA and the gRNA in the composition may have a ratio of 1±20%, for example, 1±10%, 1±5% or 1±2%. In some examples, the ratio is about 1:1 (*e.g.*, 1). Further, both the mRNA and the gRNA can be associated with (*e.g.*, attached to or encapsulated by) the lipid excipients, which may form LNPs.

25 A. *Cas12i2 Polypeptides and Encoding Nucleic Acids*

 The HAO1 gene editing composition provided herein involves a Cas12i2 polypeptide or a nucleic acid encoding the Cas12i2 polypeptide. In specific examples, the HAO1 gene editing composition comprises a messenger RNA (mRNA) molecule encoding the Cas12i2 polypeptide.

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(a) Cas12i2 Polypeptide

 The Cas12i2 polypeptide disclosed herein comprises a Cas12i2 nuclease domain and optionally one or more additional functional motifs, for example, nuclear localization signal(s), peptide linkers, or a combination thereof. Cas12i2 nuclease is a Class 2 type V CRISPR-Cas

endonuclease that is capable of cleaving target double-strand DNAs guided by guide RNAs. Cas12i2 nucleases can be found, *e.g.*, in WO2021/202800, and WO2022/256642, the relevant disclosures of each of which are incorporated by reference for the subject matter and purpose referenced herein.

- 5 In some embodiments, the Cas12i2 polypeptide involved in the HAO1 gene editing compositions disclosed herein may comprise a Cas12i2 nuclease, which is a variant of a parent Cas12i2 enzyme set forth in SEQ ID NO: 1. See the **Sequence Table** below. Such a variant Cas12i2 nuclease may comprise an amino acid sequence at least 90% (*e.g.*, at least 95%, at least 97%, at least 98%, at least 99%, or above) identical to SEQ ID NO: 1. Homology or
10 identity can be determined by amino acid sequence alignment, *e.g.*, using a program such as BLAST, ALIGN, or CLUSTAL, as described herein.

- The “percent identity” of two nucleic acids or of two amino acid sequences is determined using the algorithm of Karlin and Altschul Proc. Natl. Acad. Sci. USA 87:2264-68, 1990, modified as in Karlin and Altschul Proc. Natl. Acad. Sci. USA 90:5873-77, 1993. Such
15 an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. J. Mol. Biol. 215:403-10, 1990. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid
20 sequences homologous to the protein molecules of the invention. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul et al., Nucleic Acids Res. 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

- The variant Cas12i2 nuclease may comprise one or more amino acid substitutions
25 relative to SEQ ID NO:1, for example, at positions D581, I926, V1030, and optionally further at positions G624, F626, P868, E1035, and S1046 in SEQ ID NO: 1. In some instances, the original amino acid residue in SEQ ID NO: 1 at the just-noted positions (*e.g.*, D581, G624, F626, I926, and/or E1035 positions) may be replaced by R or a conservative substitution thereof (*e.g.*, K). Alternatively or in addition, the original amino acid residue in SEQ ID NO: 1
30 at the noted positions (*e.g.*, V1030 and/or S1046) may be replaced with G or a conservative substitution thereof such as A. Further, the original amino acid residue at position P868 may be replaced with T or a conservative substitution thereof such as S.

 As used herein, a “conservative amino acid substitution” refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which

the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, *e.g.*, Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

10 In one example, the variant Cas12i2 nuclease comprises D581R, I926R, and V1030G relative to SEQ ID NO:1. Such a Cas12i2 nuclease may comprise the amino acid sequence of SEQ ID NO: 2. See the **Sequence Table** below.

In another example, the variant Cas12i2 nuclease comprises D581R, G624R, F626R, P868T, I926R, V1030G, E1035R, and S1046G relative to SEQ ID NO: 1. Such a Cas12i2 nuclease may comprise the amino acid sequence of SEQ ID NO: 3.

15 Any of the Cas12i2 polypeptide may further comprise one or more functional motifs in addition to the nuclease domain. For example, the Cas12i polypeptide comprises at least one (*e.g.*, two, three, four, five, six, or more, identical or different) nuclear localization signal (NLS). In some embodiments, the Cas12i polypeptide comprises at least one (*e.g.*, two, three, four, five, six, or more, identical or different) nuclear export signal (NES). In some
20 embodiments, the Cas12i polypeptide comprises at least one (*e.g.*, two, three, four, five, six, or more) NLS and at least one (*e.g.*, two, three, four, five, six, or more) NES. In some examples, the Cas12i2 polypeptide may comprise one NLS at the C-terminus. In other examples, the Cas12i2 polypeptide may comprise one NLS at the N-terminus and one NLS at the C-terminus. In some instances, the N-terminus and C-terminus NLS motifs can be identical. Suitable NLS
25 motifs known in the art can be used in the Cas12i2 polypeptides disclosed herein. An example is provided in the **Sequence Table** below.

In some embodiments, the Cas12i2 polypeptide may comprise one or more flexible peptide linkers between the nuclease domain and the one or more additional functional motifs such as the NLS motifs. Any suitable flexible peptide linkers (*e.g.*, a G/S rich peptide linker as
30 those known in the art) can be used. One example is provided in the **Sequence Table** below.

In one specific example, the Cas12i2 polypeptide may comprise, from N-terminus to C-terminus, a Cas12i2 nuclease domain and an NLS. In another specific example, the Cas12i2 polypeptide may comprise, from N-terminus to C-terminus, a first NLS, a first flexible peptide linker, a Cas12i2 nuclease domain, a second flexible peptide linker, and a second NLS. Such a

Cas12i2 polypeptide may comprise the amino acid sequence of SEQ ID NO: 7. In another example, the Cas12i2 polypeptide may comprise the amino acid sequence of SEQ ID NO: 17. See **Sequence Table** below. The NLS motif is italicized and in boldface and the peptide linker is underlined.

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(b) Cas12i2-Encoding mRNA

In some embodiments, the HOA1 gene editing composition disclosed herein may comprise a nucleic acid such as an mRNA molecule that encodes the Cas12i2 polypeptide disclosed herein.

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In some examples, the nucleotide sequence encoding the Cas12i polypeptide described herein can be codon-optimized for use in a particular host cell or organism, *e.g.*, in human liver cells. Codon usage tables are readily available, for example, at the “Codon Usage Database” available at www.kazusa.or.jp/codon/ and these tables can be adapted in a number of ways. See Nakamura *et al. Nucl. Acids Res.* 28:292 (2000), which is incorporated herein by reference for the subject matter and purpose referenced herein. Computer algorithms for codon optimizing a particular sequence for expression in a particular host cell are also available, such as Gene Forge (Aptagen; Jacobus, PA). In some examples, the nucleic acid encoding the Cas12i polypeptides such as Cas12i2 polypeptides as disclosed herein can be an mRNA molecule, which can be codon optimized. Exemplary coding sequences, SEQ ID NO: 4 (encoding the Cas12i2 polypeptide of SEQ ID NO:7) and SEQ ID NO: 18 (encoding the Cas12i2 polypeptide of SEQ ID NO: 17), are provided in the **Sequence Table** below.

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In addition to the coding sequence for the Cas12i2 polypeptide, the nucleic acid such as the mRNA molecule may further comprise an untranslated region (UTR) at the 5' end and/or the 3' end. For example, the mRNA molecule may comprise 5' cap modification. mRNAs typically contain a 5'-cap for efficient translation, stabilization, and transportation in eukaryotic cells. In some examples, the mRNA molecule disclosed herein may contain the native 5' m7G cap. Alternatively, the mRNA molecule may contain a non-naturally occurring, synthetic 5' cap such as those known in the art for use in mRNAs, *e.g.*, the synthetic caps provided by TriLink BioTechnologies, See also Ishikawa et al., Nucleic Acid Symposium Series No. 53, pages 129-130, the relevant disclosures of which are incorporated by reference for the purpose and subject matter referenced herein. Examples include, but are not limited to, CAP (G(5')ppp(5')G), mCAP (m7G(5')ppp(5')G), ARCA (3'-O-Me-m7G(5')ppp(5')G), m7(3'OMeG)(5')ppp(5')m6(2'OMeA)pG, m7G(5')ppp(5')(2'OMeA)pU, and m7G(5')ppp(5')(2'OMeA)pG.

Alternatively or in addition, the mRNA molecules disclosed herein may comprise one or more modifications, *e.g.*, modified nucleotides and/or modified backbone linkages. In one example, the mRNA molecule may comprise pseudouridine residues. In some instances, the content of pseudouridine residues in the mRNA may be at least 50% (*e.g.*, at least 60%, at least 70%, at least 80%, at least 90%, or higher) at the uridine positions in the mRNA. In some examples, the mRNA may comprise pseudouridine residues at all uridine positions in the mRNA.

In one specific example, the mRNA in the HAO1 gene editing composition disclosed herein may comprise a nucleotide sequence at least 70% identical to SEQ ID NO: 8 and encode a Cas12i2 polypeptide (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 7). For example, the mRNA may comprise a nucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or above identical to SEQ ID NO: 8 and encodes the Cas12i2 polypeptide of SEQ ID NO: 7. In one specific example, the mRNA comprises (*e.g.*, consists of) the nucleotide sequence of SEQ ID NO: 8.

In another specific example, the mRNA in the HAO1 gene editing composition disclosed herein may comprise a nucleotide sequence at least 70% identical to SEQ ID NO: 18 and encode a Cas12i2 polypeptide (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 17). For example, the mRNA may comprise a nucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or above identical to SEQ ID NO: 18 and encodes the Cas12i2 polypeptide of SEQ ID NO: 17. In one specific example, the mRNA comprises (*e.g.*, consists of) the nucleotide sequence of SEQ ID NO: 18.

Any of such mRNA molecules may contain a 5' cap, *e.g.*, the m7G(5')ppp(5')(2'OMeA)pG cap, and at least 75% (*e.g.*, at least 80%, at least 85%, at least 90%, at least 95% pseudouridine residues at uridine positions in the mRNA. In one example, the mRNA contains pseudouridine residues at all uridine positions.

Additional modifications to RNA molecules as known in the art (*e.g.*, provided herein) may be introduced into the Cas12i2-encoding mRNA molecules disclosed herein.

Alternatively or in addition, the Cas12i2 polypeptide-encoding mRNA may contain a 3' poly A tail. In some instances, the poly A tail in the mRNA may contain about 50-150 adenosine (A) residues. In some examples, the poly A tail may have a length of about 60-140 A residues. In other examples, the poly A tail may have a length of about 70-140 A residues or about 80-140 A residues. In some examples, the poly A tail may have a length of about 85-135 A residues. In some examples, the poly A tail may have a length equal to or greater than 85 A residues. In one specific example, the Cas12i2 polypeptide-encoding mRNA may comprise the

nucleotide sequence of SEQ ID NO: 23.

B. RNA Guides

The HAO1 gene editing composition disclosed herein also involves a guide RNA (gRNA) that targets the HAO1 gene or a nucleic acid encoding such. As used herein, the term “RNA guide” or “RNA guide sequence” refers to any RNA molecule (e.g., a modified RNA molecule) that facilitates the targeting of a CRISPR nuclease such as any of the Cas12i2 polypeptides described herein to a target sequence (e.g., a sequence of an HAO1 gene). For example, an RNA guide can be a molecule targeting a nucleic acid sequence (target sequence) in a target gene (e.g., an HAO1 gene in the present disclosure). More specifically, the RNA guide comprises a domain (spacer sequence) that is specific to the target sequence (i.e., being the RNA counterpart of the target sequence) in the target gene. In addition to the spacer sequence, an RNA guide may further comprise a direct repeat (DR) sequence, which is recognizable by the CRISPR nuclease. In some instances, the RNA guide can be a modified RNA molecule comprising one or more deoxyribonucleotides, for example, in the spacer sequence, which binds a sequence complementary to the target sequence. In some examples, the spacer sequence may contain a DNA sequence or a DNA/RNA hybrid sequence.

As used herein, the term “complementary” refers to a first polynucleotide (e.g., a spacer sequence of an RNA guide) that has a certain level of complementarity to a second polynucleotide (e.g., the complementary sequence of a target sequence) such that the first and second polynucleotides can form a double-stranded complex *via* base-pairing to permit an effector polypeptide that is complexed with the first polynucleotide to act on (e.g., cleave) the second polynucleotide. In some embodiments, the first polynucleotide may be substantially complementary to the second polynucleotide, i.e., having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementarity to the second polynucleotide. In some embodiments, the first polynucleotide is completely complementary to the second polynucleotide, i.e., having 100% complementarity to the second polynucleotide.

In some embodiments, the RNA guide may comprise a spacer sequence followed by a direct repeat sequence, referring to the sequences in the 5' to 3' direction. In some embodiments, the RNA guide may comprise a first direct repeat sequence followed by a spacer sequence and a second direct repeat sequence, referring to the sequences in the 5' to 3' direction. In some embodiments, the first and second direct repeats of such an RNA guide can be identical. In some embodiments, the first and second direct repeats of such an RNA guide

may be different.

In some embodiments, the spacer sequence and the direct repeat sequence(s) of the RNA guide are present within the same RNA molecule. In some embodiments, the spacer and direct repeat sequences are linked directly to one another. In some embodiments, a short linker is present between the spacer and direct repeat sequences, *e.g.*, an RNA linker of 1, 2, or 3 nucleotides in length. In some embodiments, the spacer sequence and the direct repeat sequence(s) of the RNA guide are present in separate molecules, which are joined to one another by base pairing interactions.

10 (a) Spacer Sequence

In some embodiments, the RNA guide in the HAO1 gene editing composition provided herein comprises a spacer sequence specific to a target sequence in exon 1 of the human HAO1 gene, the nucleotide sequence of which is provided in the **Sequence Table** below. The target sequence is adjacent to a 5'-NTTN-3' or 5'-TTN-3' "protospacer adjacent motif" or "PAM" sequence, in which N represents any nucleotide sequence. In some instances, the target sequence is downstream to the PAM sequence, for example, at the 3' end of the PAM sequence.

A PAM sequence is a DNA sequence adjacent to a target sequence, to which a complex comprising an RNA guide (*e.g.*, an HAO1-targeting RNA guide) and a Cas12i2 polypeptide provided herein binds. In a double-stranded DNA molecule, the strand containing the PAM motif is called the "PAM-strand" and the complementary strand is called the "non-PAM strand." The spacer sequence in an RNA guide can bind to a site in the non-PAM strand that is complementary to a target sequence disclosed herein.

In some embodiments, the PAM strand is a coding (*e.g.*, sense) strand. In other embodiments, the PAM strand is a non-coding (*e.g.*, antisense strand). Since an RNA guide binds the non-PAM strand via base-pairing, the non-PAM strand is also known as the target strand, while the PAM strand is also known as the non-target strand.

The spacer sequence of the RNA guide may have a length of between 15-30 nucleotides (*e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) and is complementary to a non-PAM strand sequence. In some embodiments, the spacer sequence is designed to be complementary to a specific DNA strand, *e.g.*, of a genomic locus, which may be within exon 1 of the human HAO1 gene. In specific examples, the target sequence is set forth as 5'-CAAAGTCTATATATGACTAT-3' (SEQ ID NO: 10) in the HAO1 gene.

In some embodiments, the spacer sequence in the RNA guide is substantially identical to a complementary strand of a target sequence (*e.g.*, SEQ ID NO: 10). In some embodiments,

the spacer sequence may have at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5%

- 5 sequence identity to a complementary strand of a target sequence such as SEQ ID NO: 10. The percent identity between two such nucleic acids can be determined manually by inspection of the two optimally aligned nucleic acid sequences or by using software programs or algorithms (e.g., BLAST, ALIGN, CLUSTAL) using standard parameters.

- 10 In some examples, the spacer sequence in the RNA guide disclosed herein may comprise the nucleotide sequence of 5'-CAAAGUCUAUAUAUGACUAU-3' (SEQ ID NO: 11). Alternatively, the spacer sequence may comprise a nucleotide sequence at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or above identical to SEQ ID NO: 11.

- 15 In some embodiments, a spacer sequence described herein comprises an uracil (U). In some embodiments, a spacer sequence described herein comprises a thymine (T). Any nucleotide sequences provided here, unless explicitly noted, refers to a nucleic acid having the defined nucleotide sequence, regardless of modifications, if any, contained therein.

(b) Direct Repeat

- 20 Any of the RNA guides disclosed herein may further comprise a direct repeat sequence. In some embodiments, the direct repeat sequence of the RNA guide has a length of 15-30 nucleotides. In some examples, the direct repeat sequence may have a length of 20-30 nucleotides, for example, 23-26 nucleotides.

- 25 In some embodiments, the direct repeat sequence may comprise the nucleotide sequence of 5'-AGAAAUCCGUCUUUCAUUGACGG-3' (SEQ ID NO: 12). Alternatively, the spacer sequence may comprise a nucleotide sequence at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or above identical to SEQ ID NO: 12.

- 30 (c) Modifications

The RNA guide (and the mRNAs where applicable) provided herein may include one or more covalent modifications with respect to a reference sequence, in particular the parent polyribonucleotide, which are included within the scope of the present disclosure.

Exemplary modifications can include any modification to the sugar, the nucleobase, the

internucleoside linkage (*e.g.*, to a linking phosphate/to a phosphodiester linkage/to the phosphodiester backbone), and any combination thereof. Some of the exemplary modifications provided herein are described in detail below.

The RNA guide may include any useful modification, such as to the sugar, the nucleobase, or the internucleoside linkage (*e.g.*, to a linking phosphate/to a phosphodiester linkage/to the phosphodiester backbone). One or more atoms of a pyrimidine nucleobase may be replaced or substituted with optionally substituted amino, optionally substituted thiol, optionally substituted alkyl (*e.g.*, methyl or ethyl), or halo (*e.g.*, chloro or fluoro). In certain embodiments, modifications (*e.g.*, one or more modifications) are present in each of the sugar and the internucleoside linkage. Modifications may be modifications of ribonucleic acids (RNAs) to deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs) or hybrids thereof. Additional modifications are described herein.

In some embodiments, the modification may include a chemical or cellular induced modification. For example, some nonlimiting examples of intracellular RNA modifications are described by Lewis and Pan in “RNA modifications and structures cooperate to RNA guide-protein interactions” from *Nat Reviews Mol Cell Biol*, 2017, 18:202-210.

Different sugar modifications, nucleotide modifications, and/or internucleoside linkages (*e.g.*, backbone structures) may exist at various positions in the sequence. One of ordinary skill in the art will appreciate that the nucleotide analogs or other modification(s) may be located at any position(s) of the sequence, such that the function of the sequence is not substantially decreased. The sequence may include from about 1% to about 100% modified nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, *i.e.*, any one or more of A, G, U or C) or any intervening percentage (*e.g.*, from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%).

In some embodiments, sugar modifications (*e.g.*, at the 2' position or 4' position) or

replacement of the sugar at one or more ribonucleotides of the sequence may, as well as backbone modifications, include modification or replacement of the phosphodiester linkages. Specific examples of a sequence include, but are not limited to, sequences including modified backbones or no natural internucleoside linkages such as internucleoside modifications, including modification or replacement of the phosphodiester linkages. Sequences having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this application, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In particular embodiments, a sequence will include ribonucleotides with a phosphorus atom in its internucleoside backbone.

Modified sequence backbones may include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates such as 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates such as 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. In some embodiments, the sequence may be negatively or positively charged.

The modified nucleotides, which may be incorporated into the sequence, can be modified on the internucleoside linkage (*e.g.*, phosphate backbone). Herein, in the context of the polynucleotide backbone, the phrases "phosphate" and "phosphodiester" are used interchangeably. Backbone phosphate groups can be modified by replacing one or more of the oxygen atoms with a different substituent. Further, the modified nucleosides and nucleotides can include the wholesale replacement of an unmodified phosphate moiety with another internucleoside linkage as described herein. Examples of modified phosphate groups include, but are not limited to, phosphorothioate, phosphoroselenates, boranophosphates, boranophosphate esters, hydrogen phosphonates, phosphoramidates, phosphorodiamidates, alkyl or aryl phosphonates, and phosphotriesters. Phosphorodithioates have both non-linking oxygens replaced by sulfur. The phosphate linker can also be modified by the replacement of a linking oxygen with nitrogen (bridged phosphoramidates), sulfur (bridged phosphorothioates), and carbon (bridged methylene-phosphonates).

The α -thio substituted phosphate moiety is provided to confer stability to RNA and

DNA polymers through the unnatural phosphorothioate backbone linkages. Phosphorothioate DNA and RNA have increased nuclease resistance and subsequently a longer half-life in a cellular environment.

In specific embodiments, a modified nucleoside includes an alpha-thio-nucleoside (*e.g.*, 5'-*O*-(1-thiophosphate)-adenosine, 5'-*O*-(1-thiophosphate)-cytidine (a-thio-cytidine), 5'-*O*-(1-thiophosphate)-guanosine, 5'-*O*-(1-thiophosphate)-uridine, or 5'-*O*-(1-thiophosphate)-pseudouridine).

Other internucleoside linkages that may be employed according to the present disclosure, including internucleoside linkages which do not contain a phosphorous atom, are described herein.

In some embodiments, the sequence may include one or more cytotoxic nucleosides. For example, cytotoxic nucleosides may be incorporated into sequence, such as bifunctional modification. Cytotoxic nucleoside may include, but are not limited to, adenosine arabinoside, 5-azacytidine, 4'-thio-aracytidine, cyclopentenylcytosine, cladribine, clofarabine, cytarabine, cytosine arabinoside, 1-(2-C-cyano-2-deoxy-beta-D-arabino-pentofuranosyl)-cytosine, decitabine, 5-fluorouracil, fludarabine, floxuridine, gemcitabine, a combination of tegafur and uracil, tegafur ((RS)-5-fluoro-1-(tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione), troxacitabine, tezacitabine, 2'-deoxy-2'-methylidenecytidine (DMDC), and 6-mercaptopurine. Additional examples include fludarabine phosphate, N4-behenoyl-1-beta-D-arabinofuranosylcytosine, N4-octadecyl-1-beta-D-arabinofuranosylcytosine, N4-palmitoyl-1-(2-C-cyano-2-deoxy-beta-D-arabino-pentofuranosyl) cytosine, and P-4055 (cytarabine 5'-elaidic acid ester).

In some embodiments, the sequence includes one or more post-transcriptional modifications (*e.g.*, capping, cleavage, polyadenylation, splicing, poly-A sequence, methylation, acylation, phosphorylation, methylation of lysine and arginine residues, acetylation, and nitrosylation of thiol groups and tyrosine residues, etc). The one or more post-transcriptional modifications can be any post-transcriptional modification, such as any of the more than one hundred different nucleoside modifications that have been identified in RNA (Rozenski, J, Crain, P, and McCloskey, J. (1999). The RNA Modification Database: 1999 update. Nucl Acids Res 27: 196-197) In some embodiments, the first isolated nucleic acid comprises messenger RNA (mRNA). In some embodiments, the mRNA comprises at least one nucleoside selected from the group consisting of pyridin-4-one ribonucleoside, 5-aza-uridine, 2-thio-5-aza-uridine, 2-thiouridine, 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxyuridine, 3-methyluridine, 5-carboxymethyl-uridine, 1-carboxymethyl-pseudouridine,

5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyluridine, 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine, 1-taurinomethyl-4-thio-uridine, 5-methyl-uridine, 1-methyl-pseudouridine, 4-thio-1-methyl-pseudouridine, 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, 5 dihydrouridine, dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, and 4-methoxy-2-thio-pseudouridine. In some embodiments, the mRNA comprises at least one nucleoside selected from the group consisting of 5-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine, N4-acetylcytidine, 5-formylcytidine, N4-methylcytidine, 5-hydroxymethylcytidine, 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine, 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-1-methyl-1-deaza-pseudoisocytidine, 1-methyl-1-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxycytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, and 4-methoxy-1-methyl-pseudoisocytidine. In some embodiments, the mRNA comprises at least one nucleoside selected from the group consisting of 2-aminopurine, 2, 6-diaminopurine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-aminopurine, 7-deaza-8-aza-2-aminopurine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyladenosine, N6-methyladenosine, N6-isopentenyladenosine, N6-(cis-hydroxyisopentenyl)adenosine, 2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine, N6-glycylcarbamoyladenosine, N6-threonylcarbamoyladenosine, 2-methylthio-N6-threonyl carbamoyladenosine, N6,N6-dimethyladenosine, 7-methyladenine, 2-methylthio-adenine, and 2-methoxy-adenine. In some embodiments, mRNA comprises at least one nucleoside selected from the group consisting of inosine, 1-methyl-inosine, wyosine, wybutosine, 7-deaza-guanosine, 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine, 6-thio-7-methyl-guanosine, 7-methylinosine, 6-methoxy-guanosine, 1-methylguanosine, N2-methylguanosine, N2,N2-dimethylguanosine, 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 1-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, and N2,N2-dimethyl-6-thio-guanosine.

30 The sequence may or may not be uniformly modified along the entire length of the molecule. For example, one or more or all types of nucleotides (*e.g.*, naturally-occurring nucleotides, purine or pyrimidine, or any one or more or all of A, G, U, C, I, pU) may or may not be uniformly modified in the sequence, or in a given predetermined sequence region thereof. In some embodiments, the sequence includes a pseudouridine. In some embodiments,

the sequence includes an inosine, which may aid in the immune system characterizing the sequence as endogenous versus viral RNAs. The incorporation of inosine may also mediate improved RNA stability/reduced degradation. See for example, Yu, Z. et al. (2015) RNA editing by ADAR1 marks dsRNA as “self”. Cell Res. 25, 1283-1284, which is incorporated by reference in its entirety.

In some embodiments, one or more of the nucleotides of an RNA guide comprises a 2'-*O*-methyl phosphorothioate modification. In some embodiments, each of the first three nucleotides of the RNA guide comprises a 2'-*O*-methyl phosphorothioate modification. In some embodiments, each of the last four nucleotides of the RNA guide comprises a 2'-*O*-methyl phosphorothioate modification. In some embodiments, each of the first to last, second to last, and third to last nucleotides of the RNA guide comprises a 2'-*O*-methyl phosphorothioate modification, and wherein the last nucleotide of the RNA guide is unmodified. In some embodiments, each of the first three nucleotides of the RNA guide comprises a 2'-*O*-methyl phosphorothioate modification, and each of the first to last, second to last, and third to last nucleotides of the RNA guide comprises a 2'-*O*-methyl phosphorothioate modification.

When a gene editing system disclosed herein comprises nucleic acids encoding the Cas12i polypeptide disclosed herein, *e.g.*, mRNA molecules, such nucleic acid molecules may contain any of the modifications disclosed herein, where applicable.

20

(d) Exemplary RNA Guides

In some examples, the RNA guide provided herein may comprise the direct repeat of SEQ ID NO: 12 and a spacer sequence having a length of 20 nucleotides, which is specific to a target sequence (*e.g.*, SEQ ID NO: 10) within exon 1 of the HAO1 gene. In some specific examples, the spacer sequence may consist of the nucleotide sequence of SEQ ID NO: 11. In some instances, the RNA guide may comprise, from 5' to 3', the direct repeat sequence and the spacer sequence.

In some specific examples, the RNA guide may comprise (*e.g.*, consists of) the nucleotide sequence of SEQ ID NO: 13 provided in the **Sequence Table** below. Alternatively, the RNA guide may be a variant of SEQ ID NO: 13 comprising (*e.g.*, consisting of) a nucleotide sequence at least 80% identical to SEQ ID NO: 13, for example, at least 85%, at least 90%, at least 95%, at least 97% or above identical to SEQ ID NO: 13. In some examples, the variant of SEQ ID NO: 13 may contain up to 5 nucleotide variations relative to SEQ ID NO: 13, for example, up to 4, 3, 2, or 1 nucleotide variations relative to SEQ ID NO: 13.

The RNA guide provided herein may comprise one or more modifications such as those disclosed herein. In some embodiments, the modifications comprise 2'-O-methylation and/or phosphorothioate linkage. In some instances, such modifications can be located at the 5' end and/or 3' end of the RNA guide. One specific example is SEQ ID NO: 14 provided in the
5 **Sequence Table** below.

C. Lipid Excipients and Lipid Nanoparticles (LNPs)

In some embodiments, the HAO1 gene editing compositions provided herein may comprise lipid excipients, which may comprise one or more types of lipids, *e.g.*, a cationic
10 lipid, a phospholipid such as a zwitterionic phospholipid, a pegylated lipid, cholesterol, or a combination thereof. The nucleic acid components (mRNA and RNA guide) can be associated with the lipid excipients.

The lipid excipients may form lipid nanoparticles ("LNPs"), which are particles comprising one or more lipids. In some embodiments, the lipid nanoparticles may comprise a
15 monolayer lipid membrane. Examples of such LNPs include micelles and reverse micelles. In other embodiments, the LNPs may comprise a bilayer lipid membrane (*e.g.*, liposomes). In yet other embodiments, the LNPs are multilamellar vesicles, which contain multiple lamellar phase lipid bilayers. Still in other embodiments, the LNP may be solid lipid nanoparticle, which comprises a solid lipid core matrix that can solubilize lipophilic molecules. In some instances, a
20 solid lipid nanoparticle can also be used to solubilize molecules such as nucleic acid, which may be encapsulated based on charges. In a solid lipid nanoparticle, the lipid core can be stabilized by surfactants (emulsifiers) and cargos can be distributed into the lipid core.

The LNPs disclosed herein refer to particles, which may have at least one dimension on the order of nanometers (*e.g.*, 1-1,000 nm). The LNPs may contain a number of lipids, for
25 example, cationic lipids, ionizable lipids, anionic lipids, neutral lipids, amphipathic lipids, conjugated lipids (*e.g.*, PEGylated lipids), amphipathic lipids such as phospholipids, and/or cholesterol. In some embodiments, the LNPs provided herein are included in the gene editing composition and used to deliver an active agent or therapeutic agent, such as a nucleic acid (*e.g.*, mRNA) to a target site of interest (*e.g.*, cell, tissue, organ, tumor, and the like). In some
30 embodiments, the active agent or therapeutic agent, such as a nucleic acid (*e.g.*, mRNA), may be encapsulated in the lipid portion of the lipid nanoparticle or an aqueous space enveloped by some or all of the lipid portion of the lipid nanoparticle, thereby protecting it from enzymatic degradation or other undesirable effects induced by the mechanisms of the host organism or cells, *e.g.*, an adverse immune response.

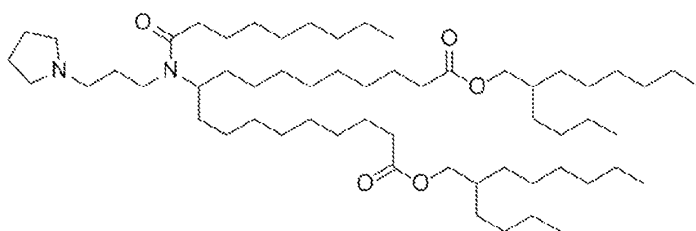
In some embodiments, the LNPs provided herein may comprise a cationic lipid, a neutral lipid (zwitterionic lipid), cholesterol, and a pegylated lipid.

(a) Cationic Lipids

5 In some embodiments, the lipid excipients comprise one or more cationic lipids, which are positively charged lipids.

A “cationic lipid” refers to a lipid capable of being positively charged. Exemplary cationic lipids include one or more amine group(s) which bear the positive charge. Exemplary cationic lipids are ionizable such that they can exist in a positively charged or neutral form
10 depending on pH. The ionization of the cationic lipid affects the surface charge of the lipid nanoparticle under different pH conditions. This charge state can influence plasma protein absorption, blood clearance and tissue distribution (Semple, S.C., et al., Adv. Drug Deliv Rev 32:3-17 (1998)) as well as the ability to form endosomolytic non-bilayer structures (Hafez, I.M., et al., Gene Ther 8:1188-1196 (2001)) critical to the intracellular delivery of nucleic
15 acids.

In one specific example, the cationic lipid is bis(2-butyloctyl) 10-(N-(3-(pyrrolidin-1-yl)propyl)nonanamido)nonadecanedioate, the structure of which is provided below:



, a pharmaceutically

acceptable salt or stereoisomer thereof.

20 In some examples, the cationic lipid is an amino lipid. In some instances, the cationic lipid is the only amino lipid in the lipid excipients contained in the HAO1 gene editing composition.

(b) Amphipathic Lipids

25 In some embodiments, the lipid excipients disclosed herein comprises one or more amphipathic lipid, *i.e.*, a lipid having a polar part and a non-polar part. Exemplary amphipathic lipids suitable for use in nanoparticles of the disclosure include, but are not limited to, sphingolipids, phospholipids, fatty acids, and amino lipids. In some examples, the lipid excipients comprise one or more phospholipids, for example, one or more saturated or

(poly)unsaturated phospholipids or a combination thereof. In general, phospholipids comprise a phospholipid moiety and one or more fatty acid moieties.

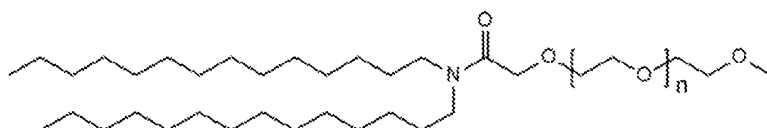
In some examples, the phospholipids in the lipid excipients may be zwitterionic phospholipids. Examples include, but are not limited to, diacylphosphatidylcholine (or 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC)).

In one example, the lipid excipients in the HAO1 gene editing composition provided herein comprise a zwitterionic phospholipid, which can be DSPC.

(c) PEGylated Lipids

In some embodiments, the lipid excipients disclosed herein comprise one or more PEGylated lipid. A PEGylated lipid (also known as a PEG lipid or a PEG-modified lipid) is a lipid modified with polyethylene glycol.

In some examples, the lipid excipients in the HAO1 gene editing composition provided herein comprise a PEG lipid, which is 2-[2-(ω -methoxy (polyethyleneglycol2000) ethoxy]-N,N-ditetradecylacetamide, the structure of which is provided below:



(Average $n=45-49$, inclusive)

(d) Exemplary LNPs

In some examples, the LNPs may comprise (*e.g.*, consists of) a cationic lipid (*e.g.*, bis(2-butyloctyl) 10-(N-(3-(pyrrolidin-1-yl)propyl)nonanamido)nonadecanedioate), a zwitterionic phospholipid (*e.g.*, DSPC), a pegylated lipid (*e.g.*, 2-[2-(ω -methoxy (polyethyleneglycol2000) ethoxy]-N,N-ditetradecylacetamide), and cholesterol. In certain examples, the molar ratio of the cationic lipid to the phospholipid lipid ranges from about 2:1 to about 8:1, the molar ratio of the cationic lipid to cholesterol ranges from about 2:1 to 1:1 and/or the molar ratio of the cationic lipid to the pegylated lipid ranges from about 100:1 to about 25:1.

In some embodiments, the lipid excipients in the HAO1 gene editing composition may comprise about 45-50 mol% of the cationic lipid, for example, about 45-48 mol%, about 46-49 mol%, about 47-48 mol% or about 48-50% of the cationic lipid, based on total mol of the lipid excipients in the LNP. In some examples, the lipid excipients in the gene editing composition

may contain about $47.5 \pm 10\%$ mol% of the cationic lipid. In other examples, the lipid excipients in the gene editing composition may contain about $47.5 \pm 5\%$ mol% of the cationic lipid. In one specific examples, the lipid excipients in the gene editing composition may contain about 47.5 mol% of the cationic lipid.

- 5 Alternatively, or in addition, the lipid excipients in the HAO1 gene editing composition may comprise about 9-11 mol% of the zwitterionic phospholipid, for example, about 9-10 mol%, about 9.5-10.5 mol%, or about 10-11 mol% of the zwitterionic phospholipid, based on total mol of the lipid excipients in the LNP. In other examples, the lipid excipients in the gene editing composition may comprise about $10 \pm 10\%$ mol% of the zwitterionic phospholipid. In
10 yet another specific example, the lipid excipients in the gene editing composition may comprise about $10 \pm 5\%$ mol% of the zwitterionic phospholipid. In some specific examples, the lipid excipients in the gene editing composition may comprise about 10 mol% of the zwitterionic phospholipid.

- Alternatively, or in addition, the lipid excipients in the HAO1 gene editing composition
15 may comprise about 38-42 mol% of the cholesterol, for example, about 38-40 mol%, about 39-41 mol%, about 39.5-41.5 mol%, or about 40-42 mol% of the cholesterol, based on total mol of the lipid excipients in the LNP. In some examples, the lipid excipients in the gene editing system may comprise about $40 \pm 10\%$ mol% of the cholesterol. In other examples, the lipid excipients in the gene editing system may comprise about $40 \pm 5\%$ mol% of the cholesterol. In
20 one specific example, the lipid excipients in the gene editing system may comprise about 40 mol% of the cholesterol.

- Alternatively, or in addition, the lipid excipients in the HAO1 gene editing composition may comprise about 2.2-2.8 mol% of the pegylated lipid, for example, about 2.2-2.5 mol%, about 2.4-2.6 mol%, or about 2.6-2.8 mol% of the pegylated lipid, based on total mol of the
25 lipid excipients in the LNP. In some examples, the lipid excipients in the gene editing composition may comprise $2.5 \pm 10\%$ mol% of the pegylated lipid. For example, the lipid excipients in the gene editing compositions may comprise about $2.5 \pm 5\%$ mol% of the pegylated lipid. In some specific examples, the lipid excipients in the gene editing compositions may comprise about 2.5 mol% of the pegylated lipid.

- 30 In one specific example, the lipid excipients contain about 47.5 mol% of the cationic lipid (*e.g.*, bis(2-butyloctyl) 10-(N-(3-(pyrrolidine-1-yl)propyl)nonanamido)nonadecanedioate), about 10 mol% of the zwitterionic phospholipid (*e.g.*, DSPC), about 40 mol% of cholesterol, and about 2.5 mol% of the PEG lipid (*e.g.*, 2-[2-(ω -methoxy (polyethyleneglycol2000) ethoxy]-N,N-ditetradecylacetamide).

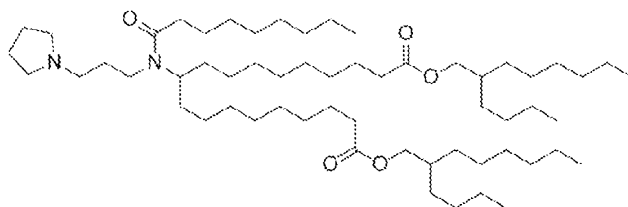
The N/P ratio of the LNPs, where N represents the moles of cationic lipid and P represents the moles of phosphate present as part of the nucleic acid backbone, ranges from 2:1 to 30:1, for example 3:1 to 22:1. In other embodiments, N/P ranges from 6:1 to 20:1, 3:1 to 9:1 or 2:1 to 12:1. Exemplary N/P ranges include about 3:1, about 6:1, about 9:1, about 12:1 and about 22:1.

D. Exemplary HAO1 Gene Editing Compositions

In some embodiments, the HAO1 gene editing composition provided herein comprises a mRNA molecule encoding a Cas12i2 polypeptide as disclosed herein (*e.g.*, SEQ ID NO: 7 or SEQ ID NO: 17), a RNA guide comprising a spacer sequence specific to SEQ ID NO: 10 in the exon 1 of human HAO1 gene and a direct repeat sequence of SEQ ID NO: 12, and lipid excipients comprising a cationic lipid (which may be an amino lipid), a zwitterionic phospholipid, cholesterol, and a pegylated lipid. The lipid excipients form LNPs and the nucleic acid components (mRNA and RNA guide) are attached to or encapsulated by the LNPs.

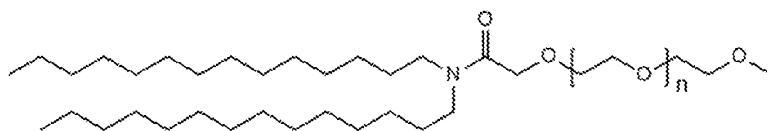
In some examples, the mRNA comprises a nucleotide sequence at least 80% (*e.g.*, at least 85%, at least 90%, at least 95%, at least 98% or above) identical to SEQ ID NO: 8. In one specific example, the mRNA may comprise the nucleotide sequence of SEQ ID NO: 8.

In the lipid excipients, the cationic lipid can have the structure of



, or is a pharmaceutically

acceptable salt or stereoisomer thereof. The pegylated lipid can have the structure of



, in which the

average n is between 45-49, inclusive (*e.g.*, 45, 46, 47, 48, or 49). The zwitterionic phospholipid is 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC).

In the exemplary HAO1 gene editing composition, the LNPs may comprise 45-50 mol% (*e.g.*, about 47.5 mol%) of the cationic lipid, 9-11 mol% (*e.g.*, about 10) mol% of the

zwitterionic phospholipid, 38-42 mol% (*e.g.*, about 40 mol%) of the cholesterol, and 2.2-2.8 (*e.g.*, about 2.5) mol% of the pegylated lipid, based on total mol of the lipid excipients in the LNP. In one specific example, the LNPs may comprise about 47.5 mol% of the cationic lipid, about 10 mol% of the zwitterionic phospholipid, about 40 mol% of the cholesterol, and about 2.5 mol% of the pegylated lipid, based on total mol of the lipid excipients in the LNP.

In some examples, the mRNA comprises UTRs flanking the coding sequence for the Cas12i2 polypeptide, for example, a 5' UTR and a 3' UTR and a poly A tail. In one specific example, the mRNA may comprise (*e.g.*, consists of) the nucleotide sequence of SEQ ID NO: 23. Further, the mRNA may carry a 5' cap, *e.g.*, m7G(5')ppp(5')(2'OMeA)pG, and may contain pseudouridine residues in replacement of uridine residues (*e.g.*, 100%).

In some examples, the RNA guide may comprise a spacer sequence of SEQ ID NO: 11. Such an RNA guide may comprise (*e.g.*, consists of) the nucleotide sequence of SEQ ID NO: 13. The RNA guide may comprise one or more modifications, *e.g.*, phosphorothioate linkage and 2'-O-methylation, which may be located at the 5' and/or 3' nucleotides. In one specific example, the RNA guide is set forth in SEQ ID NO: 14.

In some examples, the mRNA and RNA guide have a ratio of $1 \pm 20\%$ (*e.g.*, 0.8 to 1.2) in the HAO1 gene editing composition. In some specific examples, the ratio is 1:1 (1.0).

In some examples, the HAO1 gene editing composition has a nitrogen:phosphate (N/P) ratio of $6.0 \pm 20\%$ (*e.g.*, $6.0 \pm 15\%$, $6.0 \pm 10\%$, $6.0 \pm 5\%$, $6.0 \pm 2\%$, or $6.0 \pm 1\%$), which represents the relative amount LNPs and nucleic acids in the composition. In other examples, the HAO1 gene editing composition has a nitrogen:phosphate (N/P) ratio of 6.0 ± 1 (*e.g.*, 6.0 ± 0.5). In one example, the N/P ratio in the HAO1 gene editing composition provided herein ranges from 5.18 to 7 (*e.g.*, 5.6 or 6.0).

In general, the N/P ratio may be calculated from the results of two assays, HPLC (for determination of amino lipid mol) and ribogreen (for determination of mol of total RNAs). Details are provided in **Example 1** below.

II. Preparation of Gene Editing Components

The present disclosure provides methods for production of components of the gene editing systems disclosed herein, *e.g.*, the RNA guide, methods for production of the Cas12i polypeptide-coding mRNA, and methods for complexing the RNA guide and Cas12i mRNA with the lipid excipients.

A. RNA Guides and Cas12i2-Encoding mRNAs

In some embodiments, the RNA guide is made by *in vitro* transcription of a DNA template. Thus, for example, in some embodiments, the RNA guide is generated by *in vitro* transcription of a DNA template encoding the RNA guide and/or the mRNA using an upstream promoter sequence (*e.g.*, a T7 polymerase promoter sequence). In some embodiments, the DNA template encodes multiple RNA guides or the *in vitro* transcription reaction includes multiple different DNA templates, each encoding a different RNA guide. In some embodiments, the DNA template encodes both the RNA guide and the mRNA molecule and can produce both *in vitro* as separate RNA molecules.

In some embodiments, the mRNA is made by *in vitro* transcription of a DNA template. In some examples, the mRNA made by *in vitro* transcription may be subject to modifications such as addition of a 5'-cap (*e.g.*, those disclosed herein), following methods known in the art. In some embodiments synthetic capping residues, synthetic 5'-caps (*e.g.*, those disclosed herein) may be used in the *in vitro* transcription so that the mRNA products comprise a synthetic 5'-cap. Alternatively or in addition, modified nucleotide residues (*e.g.*, pseudouridine residues) may be used in the *in vitro* transcription so that the modified nucleotide residues are incorporated into the mRNA products thus produced.

In some embodiments, the RNA guide is made using chemical synthetic methods. In some examples, the RNA guide can be synthesized using one or more modified nucleotide, *e.g.*, as described above.

B. Nucleic Acid-Loaded LNPs

In some embodiments, the HAO1 gene editing compositions disclosed herein comprise lipid excipients (*e.g.*, those disclosed herein), which form LNPs, and the nucleic acid components in the composition (mRNA and gRNA) are attached to or encapsulated by the LNPs, resulting in nucleic acid-loaded LNPs.

A variety of methods are available for preparing LNPs. See, *e.g.*, Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, 4,946,787, PCT Publication No. WO 91/17424, Deamer & Bangham, Biochim. Biophys. Acta 443:629-634 (1976); Fraley, et al., PNAS 76:3348-3352 (1979); Hope et al., Biochim. Biophys. Acta 812:55-65 (1985); Mayer et al., Biochim. Biophys. Acta 858:161-168 (1986); Williams et al., PNAS 85:242-246 (1988); Hope et al., Chem. Phys. Lip. 40:89 (1986); Gregoriadis, Liposome Technology (1984). Such conventional

methods can be used to prepare the LNPs with the lipid excipients disclosed herein. Any of such methods may be performed in the presence of the nucleic acid components of the gene editing composition (mRNA and gRNA) such that the resultant lipid nanoparticles would carry the nucleic acids to produce nucleic acid-loaded LNPs.

5 The lipid nanoparticles prepared following any of the methods known in the art or disclosed herein can be analyzed to determine concentration and/or particle size distribution (*e.g.*, by NTA). Alternatively, or in addition, the lipid nanoparticles can be fractionated and particles having suitable sizes may be collected for use in the fusion method disclosed herein.

10 In addition, the nucleic acid-loaded LNPs may be analyzed to determine the N/P ratio following methods known in the art and/or disclosures provided herein. The N/P ratio is the ratio of moles of cationic lipid (N) to moles of phosphate present as part of the nucleic acid backbone (P).

15 LNPs can be prepared according to methods known in the art, for example as disclosed in WO 2017/004143, which is incorporated herein by reference in its entirety. Briefly, cationic lipid, zwitterionic phospholipid, cholesterol and pegylated are solubilized in ethanol at a molar ratio of 40:50:5-15:35-45:1-5, respectively. LNPs are generally prepared at a total lipid to mRNA weight ratio of approximately 10:1 to 30:1. mRNA is diluted to 0.2 mg/mL in 10 to 50 mM citrate buffer, pH 4. Syringe pumps are used to mix the ethanolic lipid solution with the mRNA aqueous solution at a ratio of about 1:5 to 1:3 (vol/vol) with total flow rates above 15 ml/min. The ethanol is then removed and the external buffer replaced with PBS by dialysis. Finally, the lipid nanoparticles are filtered through a 0.2 µm pore sterile filter.

III. Methods for Genetic Editing of HAO1 Gene and Treatment of Primary Hyperoxaluria

25 Any of the HAO1 gene editing compositions disclosed herein can be used to genetically edit the HAO1 gene in host cells. In some embodiments, the methods comprise introducing the HAO1 gene editing composition into host cells. Such an HAO1 gene editing composition may comprise nucleic acid (mRNA/gRNA)-loaded LNPs as disclosed herein. In some instances, the HAO1 gene editing composition can be introduced into *in vitro*-cultured host cells. In other instances, the HAO1 gene editing composition described herein is delivered to a subject to genetically modify the HAO1 gene in the subject.

30 As used herein, the term “edit” refers to one or more modifications introduced into a target nucleic acid, *e.g.*, within the HAO1 gene. The edit can be one or more substitutions, one or more insertions, one or more deletions, or a combination thereof. As used herein, the term

“substitution” refers to a replacement of a nucleotide or nucleotides with a different nucleotide or nucleotides, relative to a reference sequence. As used herein, the term “insertion” refers to a gain of a nucleotide or nucleotides in a nucleic acid sequence, relative to a reference sequence. As used herein, the term “deletion” refers to a loss of a nucleotide or nucleotides in a nucleic acid sequence, relative to a reference sequence.

Any of the HAO1 gene editing compositions as disclosed herein (*e.g.*, comprising nucleic acid-loaded LNPs) or modified cells generated using such a gene editing system as disclosed herein may be used for treating a disease that would benefit from genetic editing of the HAO1 gene, for example, primary hyperoxaluria (PH). In some embodiments, the PH is PH1, PH2, or PH3. In specific examples, the target disease is PH1.

PH is a rare genetic disorder effecting subjects of all ages from infants to elderly. PH includes three subtypes involving genetic defects that alter the expression of three distinct proteins. PH1 involves alanine-glyoxylate aminotransferase, or AGT/AGT1. PH2 involves glyoxylate/hydroxypyruvate reductase, or GR/HPR, and PH3 involves 4-hydroxy-2-oxoglutarate aldolase, or HOGA.

In PH1, excess oxalate can also combine with calcium to form calcium oxalate in the kidney and other organs. Deposits of calcium oxalate can produce widespread deposition of calcium oxalate (nephrocalcinosis) or formation of kidney and bladder stones (urolithiasis) and lead to kidney damage. Common kidney complications in PH1 include blood in the urine (hematuria), urinary tract infections, kidney damage, and end-stage renal disease (ESRD). Over time, kidneys in patients with PH1 may begin to fail, and levels of oxalate may rise in the blood. Deposition of oxalate in tissues throughout the body, *e.g.*, systemic oxalosis, may occur due to high blood levels of oxalate and can lead to complications in bone, skin, and eye. Patients with PH1 normally have kidney failure at an early age, with renal dialysis or dual kidney/liver organ transplant as the only treatment options.

In some embodiments, provided herein is a method for treating PH such as PH1 in a subject (*e.g.*, a human patient) in need of the treatment. The method may comprise administering to the subject (*e.g.*, the human patient) in need of the treatment any of the HAO1 gene editing composition disclosed herein. The HAO1 gene editing composition may be delivered to a specific tissue or specific type of cells where the gene edit is needed. In some examples, the HAO1 gene editing composition comprises LNPs loaded with the Cas12i2-encoding mRNA and the HAO1-targeting gRNA as also disclosed herein. In specific examples, the exemplary HAO1 gene editing composition disclosed above (see, *e.g.*, Section I, subsection D above) can be used in the treatment method disclosed herein.

The HAO1 gene editing composition disclosed herein may be prepared, packaged, or sold in a formulation for a suitable administration route, for example, intravenous or another route of administration. The composition of the disclosure may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a “unit dose” is discrete amount of the pharmaceutical composition (*e.g.*, the gene editing system or components thereof), which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

In some embodiments, the HAO1 gene editing composition as disclosed herein may be administered to a subject in need thereof, *e.g.*, one who suffers from a liver disease associated with the HAO1 gene. In some instances, the gene editing composition may be delivered to specific cells or tissue (*e.g.*, to liver cells), where the gene editing components could function to genetically modify the HAO1 gene in such cells.

A formulation of the HAO1 gene editing composition suitable for parenteral administration may comprise the active agent (*e.g.*, the mRNA encoding the nuclease and gRNA) combined with a pharmaceutically acceptable excipients (*e.g.*, the lipid excipients such as the LNPs formed thereby) and carrier, such as sterile water or sterile isotonic saline. Such a formulation may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Some injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Some formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Some formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents.

The HAO1 gene editing composition may be in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the cells, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulation may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or saline. Other acceptable diluents and solvents include, but are not limited to, Ringer’s solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which that are useful include those which may comprise the cells in a packaged form, in a liposomal preparation, or as a component of a biodegradable polymer system. Some compositions for sustained release

or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

5 In some embodiments, an effective amount of the HAO1 gene editing composition disclosed herein (*e.g.*, the exemplary HAO1 gene editing composition disclosed in Section I, subsection D above) can be administered to a human patient having PH (*e.g.*, PH1) *via* a suitable route, for example, intravenous infusion, to treating the disease.

As used herein, the term “treating” refers to the application or administration of a composition including one or more active agents (here the nuclease-encoding mRNA and the HAO1-targeting gRNA) to a subject, who has a target disease or disorder (PH such as PH1 in the instant disclosure), a symptom of the disease/disorder, or a predisposition toward the disease/disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disorder, the symptom of the disease, or the predisposition toward the disease or disorder.

15 Alleviating a target disease/disorder includes delaying the development or progression of the disease, or reducing disease severity or prolonging survival. Alleviating the disease or prolonging survival does not necessarily require curative results. As used therein, “delaying” the development of a target disease or disorder means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that “delays” or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

25 “Development” or “progression” of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. “Development” includes occurrence, recurrence, and onset. As used herein “onset” or “occurrence” of a target disease or disorder includes initial onset and/or recurrence.

As used herein, “an effective amount” refers to the amount of each active agent (*e.g.*, the nuclease-encoding mRNA and the HOA1-targeting gRNA) required to confer therapeutic

effect on the subject, either alone or in combination with one or more other active agents. Determination of whether an amount of the HAO1 gene editing composition the therapeutic effect would be evident to one of skill in the art. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment.

Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a target disease/disorder.

In one example, dosages for the HAO1 gene editing composition, specifically the mRNA and gRNA components therein, as described herein may be determined empirically in individuals who have been given one or more administration(s) of the composition. Individuals are given incremental dosages of the agonist. To assess efficacy of the agonist, an indicator of the disease/disorder can be followed.

In some embodiments, a human PH patient can be administered the HAO1 gene editing composition disclosed herein at a dose of about 2.0-5.0 mg of the nucleic acids in the composition (mRNA and gRNA in total) per kg of the patient's body weight.

Treatment efficacy can be evaluated via routine medical practice. In some embodiments, the host cells and/or the subject treated by the HAO1 gene editing composition may be examined for genetic edits in the HAO1 gene, for example, percentage of Indels in the HAO1 gene (*e.g.*, at the target sequence site). Alternatively, or in addition, HAO1 gene product (the GO enzyme) and molecules involved in the pathway catalyzed by the GO enzyme, *e.g.*, glycolate, glyoxylate, and/or oxalate can be measured in the subject before and after treatment for assessing HAO1 gene editing outcome.

V. Kits and Uses Thereof

The present disclosure also provides kits that can be used, for example, to carry out a method described herein for genetical modification of the HAO1 gene. In some embodiments,

the kits include the components of the HAO1 gene editing composition disclosed herein, e.g., the mRNA encoding a Cas12i2 polypeptide, the gRNA targeting the HAO1 gene, and the lipid excipients. In some embodiments, the kit comprises LNPs loaded with the mRNA and gRNA. The kits can additionally include, optionally, a buffer and/or instructions for use of the HAO1 gene editing composition.

In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of administration of the HAO1 gene editing composition disclosed herein to treat, delay the onset, or alleviate the target disease as also described herein (PH such as PH1). The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the target disease.

The instructions relating to the use of the HAO1 gene editing composition generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

The label or package insert indicates that the composition is used for treating, delaying the onset and/or alleviating the target disease. Instructions may be provided for practicing any of the methods described herein.

The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle).

Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiments, the present disclosure provides articles of manufacture comprising contents of the kits described above.

SEQUENCE TABLE

Description	Sequence	SEQ ID NO.
Cas12i2 parent	MSSAIKSYKSVLRPNERKNQLLKSTIQCLEDGSAFFFKMLQGLFGGIT PEIVRFSTEQEKQQQDIALWCAVNWFRPVSQDSLTHTIASDNLVEKFE EYYGGTASDAIKQYFSASIGESYYWDCRQQYYDLCRELGVEVSDLTH DLEILCREKCLAVATESNQNNSSIISVLFGTGEKEDRSVKLRITKKILE AISNLKEIPKNVAPIQEIIILNVAKATKETFRQVYAGNLGAPSTLEKFI AKDGQKEFDLKKLQTDLKKVIRGKSKERDWCCQEELRSYVEQNTIQYD LWAWGEMFNKAHTALKIKSTRNYNFAKQRLEQFKEIQSLNNLLVVKKL NDDFDSEFFSGEETYTICVHHLGGKDL SKLYKAWEDDPADPENAI VVL CDDLKNNFKKEPIRNILRYIFTIRQECQAQDILAAAKYNQQLDRYKSQ KANPSVLGNQGF TWNAVILPEKAQRNDRPNSLDLRIWLYLKL RHPDG RWKKHHIPFYDTRFFQEIIYAAGNSPVDTCQFRTPRFGYHLPKLTQTA IRVNKKHVKA AK TEARIRLAIQQGTLPVSNLKITEISATINSKGQVRIPVKFDVGRQKGT LQIGDRFCGYDQNTASHAYSLWEVVKEGQYHKELGCFVRFISSGDIV SITENRGNQFDQLSYEGLAYPQYADWRKKASKFVSLWQITKKNKKKEI VTVEAKEKFDAICKYQPRLYKFENKEYAYLLRDIVRGKSLVELQQIRQE IFRFIEQDCGVTRLGSLSLSTLETVKAVKGIIYSYFSTALNASKNNPI SDEQRKEFDPELFALLEKLELIRTRKKKQKVERIANSLIQTCLENNIK FIRGEDLSTTNNATKKKANSRSMDWLARGVFNKIRQLAPMHNITLFG CGSLYTS HQDPLVHRNPDKAMKCRWAAIPVKDIGDWVLRKLSQNLRAK NIGTGEYYHQGVKEFLSHYELQDLEEELLKWRSRKSNI PCWVLQNRL AEKLG NKEAVVYIPVRGGRIYFATHKVATGAVSIVFDQKQVWVCNADH VAAANIALTVKGIGEQSSDEENPDGSRIKLQ LTS	1
Cas12i2 A	MSSAIKSYKSVLRPNERKNQLLKSTIQCLEDGSAFFFKMLQGLFGGIT PEIVRFSTEQEKQQQDIALWCAVNWFRPVSQDSLTHTIASDNLVEKFE EYYGGTASDAIKQYFSASIGESYYWDCRQQYYDLCRELGVEVSDLTH DLEILCREKCLAVATESNQNNSSIISVLFGTGEKEDRSVKLRITKKILE AISNLKEIPKNVAPIQEIIILNVAKATKETFRQVYAGNLGAPSTLEKFI AKDGQKEFDLKKLQTDLKKVIRGKSKERDWCCQEELRSYVEQNTIQYD LWAWGEMFNKAHTALKIKSTRNYNFAKQRLEQFKEIQSLNNLLVVKKL NDDFDSEFFSGEETYTICVHHLGGKDL SKLYKAWEDDPADPENAI VVL CDDLKNNFKKEPIRNILRYIFTIRQECQAQDILAAAKYNQQLDRYKSQ KANPSVLGNQGF TWNAVILPEKAQRNDRPNSLDLRIWLYLKL RHPDG RWKKHHIPFYDTRFFQEIIYAAGNSPVDTCQFRTPRFGYHLPKLTQTA IRVNKKHVKA AKTEARIRLAIQQGTLPVSNLKITEISATINSKGQVRI PVKFRVGRQKGT LQIGDRFCGYDQNTASHAYSLWEVVKEGQYHKELG CFVRFISSGDIVSITENRGNQFDQLSYEGLAYPQYADWRKKASKFVSL WQITKKNKKKEIVTVEAKEKFDAICKYQPRLYKFENKEYAYLLRDIVRG KSLVELQQIRQEIFRFIEQDCGVTRLGSLSLSTLETVKAVKGIIYSYF STALNASKNNPISDEQRKEFDPELFALLEKLELIRTRKKKQKVERIAN SLIQTCLENNIKFIRGEDLSTTNNATKKKANSRSMDWLARGVFNKIR QLAPMHNITLFGCGSLYTS HQDPLVHRNPDKAMKCRWAAIPVKDIGDW VLRKLSQNLRAKNRGTGEYYHQGVKEFLSHYELQDLEEELLKWRSRDK SNIPCWVLQNRLAEKLG NKEAVVYIPVRGGRIYFATHKVATGAVSIVF DQKQVWVCNADHVAAANIALTGKGIGEQSSDEENPDGSRIKLQ LTS	2
Cas12i2 B	MSSAIKSYKSVLRPNERKNQLLKSTIQCLEDGSAFFFKMLQGLFGGIT PEIVRFSTEQEKQQQDIALWCAVNWFRPVSQDSLTHTIASDNLVEKFE EYYGGTASDAIKQYFSASIGESYYWDCRQQYYDLCRELGVEVSDLTH DLEILCREKCLAVATESNQNNSSIISVLFGTGEKEDRSVKLRITKKILE AISNLKEIPKNVAPIQEIIILNVAKATKETFRQVYAGNLGAPSTLEKFI	3

	AKDGQKEFDLKKLQTDLKKVIRGKSKERDWCCQEELRSYVEQNTIQYD LWAWGEMFNKAHTALKIKSTRNYNFAKQRLQFKEIQSLNNLLVVKKL NDFFDSEFFSGEETYTICVHHLGGKDLKLYKAWEDDPADPENAIIVVL CDDLKNNFKKEPIRNILRYIFTIRQECSAQDILAAAKYNQQLDRYKSQ KANPSVLGNQGFWTNAVILPEKAQRNDRPNSLDLRIWLYLKLRRHPDG RWKKHHIPFYDTRFFQEIYAAGNSPVDTCQFRTPRFGYHLPKLTQDTA IRVNKKHVKAATEARIRLAIQQGTLPVSNLKITEISATINSKGQVRI PVKFRVGRQKGTLQIGDRFCGYDQNTASHAYSLEWVVKEGQYHKELR CRVRFISSGDIVSITENRGNQFDQLSYEGLAYPQYADWRKKASKFVSL WQITKKNKKKEIVTVEAKEKFDAICKYQPRLYKFNKEYAYLLRDIVRG KSLVELQQIRQEIFRFIEQDCGVTRLGSLSLSTLETVKAVKGIISYF STALNASKNNPISDEQRKEFDPELFALEKLELIRTRKKKQKVERIAN SLIQTCLENNIKFIRGEGDLSTNNATKKKANSRSMDWLARGVFNKIR QLATMHNITLFGCGSLYTSHQDPLVHRNPDKAMKCRWAAIPVKDIGDW VLRKLSQNLRAKNRGTGEYYHQGVKEFLSHYELQDLEEEELLKWRSDRK SNIPCWVLQNLRAEKLGNKEAVVYIPVRGGRIYFATHKVATGAVSIVF DQKQVWVCNADHVAAANIALTGKIGRQSSDEENPDGGRIKLQITS	
Cas12i2 B Coding Sequence	AUGAGCUCCGCCAUCAAGUCCUACAAGUCUGUGUGCGGCCAAACGAG AGAAAGAAUCAGCUGCUGAAGUCCACCAUCCAGUGCCUGGAGGACGGC UCCGCCUUCUUUUCAAGAUGCUGCAGGGCCUGUUUGCGGCCAUACAC CCCGAGAUCCUGAGAUUCAGCACAGAGCAGGAGAAGCAGCAGCAGGAU AUCGCCCUGUGGUGUGCCGUGAAUUGGUUCAGGCCUGUGAGCCAGGAC UCCUGACCCACACAAUCGCCUCCGAUAACCUGUGGAGAAGUUUGAG GAGUACUAUGGCGGCACAGCCAGCGACGCCAUCAAGCAGUACUUCAGC GCCUCCAUCCGGCAGUCCUACUAUUGGAAUGACUGCCGCCAGCAGUAC UAUGAUCUGUGUGCGGAGCUGGGCGUGGAGGUGUCUGACCUACCCAC GAUCUGGAGAUCUGUGCCGGGAGAAGUGUCUGGCCGUGGCCACAGAG AGCAACCAGAACAAUUCUAUCAUCAGCGUGCUGUUUGGCACCGGCGAG AAGGAGGAUAGGUCUGUGAAGCUGCGCAUCACAAAGAAGAUCCUGGAG GCCAUCAAGCAACCUGAAGGAGAUCCCAAAGAAUGUGGCCCCCAUCCAG GAGAUCAUCCUGAAUGUGGCCAAGGCCACCAAGGAGACAUUCAGACAG GUGUACGCGAGGAACCUGGGAGCACCACCCUGGAGAAGUUUAUC GCCAAGGACGCGCCAGAAGGAGUUCGAUCUGAAGAAGCUGCAGACAGAC CUGAAGAAAGUGAUCCGGGGCAAGUCUAAGGAGAGAGAUUGGUGCUGU CAGGAGGAGCUGAGGAGCUACGUGGAGCAGAAUACCAUCCAGUAGAC CUGUGGGCCUGGGGCGAGAUGUUAACAAGGCCACACCGCCUGAAG AUCAAGUCCACAAGAAACUACAAUUUUGCCAAGCAGAGGCUGGAGCAG UUCAAGGAGAUCCAGUCUCUGAACAAUCUGCUGUGGUGAAGAAGCUG AACGACUUUUUCGAUAGCGAGUUUUUCUCCGGCGAGGAGACCUACACA AUCUGCGUGCACCACCUGGGCGGCAAGGACCUGUCCAAGCUGUAUAAG GCCUGGGAGGACGAUCCCGCCGAUCCUGAGAAUGCCAUCGUGGUGCUG UGCGACGAUCUGAAGAACAAUUUUAAGAAGGAGCCUAUCAGGAACAUC CUGCGCUACAUCUACCAUCCGCCAGGAGUGUAGCGCACAGGACAUC CUGGCAGCAGCAAAGUACAAUCAGCAGCUGGAUCGGUAUAAGAGCCAG AAGGCCAACCCAUCCGUGCUGGGCAAUCAGGGCUUUUACCGGACAAAC GCCGUGAUCCUGCCAGAGAAGGCCAGCGGAACGACAGACCCAAUUCU CUGGAUCUGCGCAUCUGGCUGUACCUGAAGCUGCGGCACCCUGACGGC AGAUGGAAGAAGCACCACAUCCCAUUCUACGAUACCCGGUUUUUCCAG GAGAUUCUAUGCCGCCGGCAAUAGCCUGUGGACACCUGUCAGUUUAGG ACACCCCGCUUCGGCUAUCACCUGCCUAAGCUGACCGAUCAGACAGCC AUCCCGGUGAACAAAGAAGCACGUGAAGGCAGCAAAGACCGAGGCACGG AUCAGACUGGCCAUCCAGCAGGGCACACUGCCAGUGUCCAUCUGAAG AUCACCGAGAUCCCGCCACAAUCAACUCUAAGGGCCAGGUGCGCAUC CCCGUGAAGUUUCGGGUGGGAAGGCAGAAGGGAACCCUGCAGAUCCGGC GACCGGUUCUGCGGCUACGAUCAGAACCAGACAGCCUCUCACGCCUAAU	4

	AGCCUGUGGGAGGUGGUGAAGGAGGGCCAGUACCACAAGGAGCUGCGG UGUCGGGUGCGCUUCAUCUCUAGCGGCGACAUCGUGUCCAUCACCGAG AACCGGGGCAAUCAGUUUGAUCAGCUGUCUUUAUGAGGGCCUGGCCUAC CCCCAGUAUGCCGACUGGAGAAAGAAGGCCUCCAAGUUCGUGUCUCUG UGGCAGAUACCAAGAAGAACAAGAAGAAGGAGAUUCGUGACAGUGGAG GCCAAGGAGAAGUUUGACGCCAUCUGCAAGUACCAGCCUAGGCCUGUAU AAGUUCAACAAGGAGUACGCCUAUCUGCUGCGGGGAUAUCGUGAGAGGC AAGAGCCUGGUGGAGCUGCAGCAGAUACAGGCAGGAGAUCUUUCGCUUC AUCGAGCAGGACUGUGGAGUGACCCGCCUGGGAUUCUCUGAGCCUGUCC ACCCUGGAGACAGUGAAGGCCGUGAAGGGCAUCAUCUACUCCUAUUUU UCUACAGCCCUGAAUGCCUCUAAGAACAUCCCAUCAGCGACGAGCAG CGGAAGGAGUUUGAUCCUGAGCUGUUCGCCUGCUGGAGAAGCUGGAG CUGAUACAGGACUCGGAAGAAGAAGCAGAAGGUGGAGAGAAUCGCCAAU AGCCUGAUCCAGACAUGCCUGGAGAACAUAUAAGUUAUCAGGGGC GAGGGCGACCUGUCCACCACAAACAAUGCCACCAAGAAGAAGGCCAAC UCUAGGAGCAUGGAUUGGCUGGCCAGAGGCGUGUUUAUAAGAUCCGG CAGCUGGCCACCAUGCACAACAUCACCCUGUUCGGCUGCGGACGCCUG UACACAUCCCAAGGAGCCUCUGGUGCAGAGAAACCCAGAUAAAGGCC AUGAAGUGUAGAUGGGCAGCAUCCAGUGAAGGACAUCGGCGAUUGG GUGCUGAGAAAGCUGUCCCAGAACUGAGGGCCAAGAAUCGGGGCACC GGCGAGUACUAUCACCAGGGCGUGAAGGAGUUCUGUCUCACUAUGAG CUGCAGGACCUGGAGGAGGAGCUGCUGAAGUGGCGGUCUGAUAGAAAG AGCAACAUCCCUUGCUGGGUGCUGCAGAAUAGACUGGCCGAGAAGCUG GGCAACAAGGAGGCGUGGUGUACAUCCAGUGAGGGGCGCGCAUC UAUUUUGCAACCCACAAGGUGGCAACAGGAGCCGUGAGCAUCGUGUUC GACCAGAAGCAAGUGUGGGUGUGAAUGCAGAUACGUGGCAGCAGCA AACAUCCGACUGACCGGCAAGGGCAUCGGCCGCGAGUCCUCUGACGAG GAGAACCCCGAUGGCGGCAGGAUCAAGCUGCAGCUGACAUCU	
Nuclear Localization Signal (NLS)	KRPAATKKAGQAKKKK	5
Peptide Linker	SAGGGGSGGGGSGGGGSG	6
Cas12i2 Polypeptide B (with Cas12i2 B)	<u>MRPAATKKAGQAKKKK</u> SAGGGGSGGGGSGGGGSGMSSAIKSYKSVLR PNERKNQLLKSTIQCLEDGSAFFFKMLQGLFGGITPEIVRFSTEQEKQ QQDIALWCAVNWFRPVSQDSLTHTIASDNLVEKFEEYYGGTASDAIKQ YFSASIGESYYWDCRQQYYDLCRELGVEVSDLTHDLEILCREKCLAV ATESNQNNSSIISVLFGTGEKEDRSVKLRITKKILEAISNLKEIPKNVA PIQEIIILNVAKATKETFRQVYAGNLGAPSTLEKFIKDGQKEFDLKKL QTDLKKVIRGKSKERDWCCQEELRSYVEQNTIQYDLWAWGEMFNKAHT ALKIKSTRNYNFAQRLEQFKEIQSLNLLVVKLNDFFDSEFFSGEE TYTICVHHLGGKDLKLYKAWEDDPADPENAIIVLCCDLKNNFKKEPI RNILRYIFTIRQECSAQDILAAAKYNQQLDRYKSQKANPSVLGNQGF WTNAVILPEKAQRNDRPNSLDLRIWLYLKLRLHPDGRWKKHHIPFYDTR FFQEIIYAAGNSPVDTCQFRTPRFGYHLPKLTQTAIRVNKKHVKA EARIRLAIQQGTLPVSNLKITEISATINSKGQVRIPVKFRVGRQKGT QIGDRFCGYDQNTASHAYSLWEVVKEGQYHKELRCRVRFISSGDIVS ITENRGNQFDQLSYEGLAYPQYADWRKKASKFVSLWQITKNNKKKEIV TVEAKEKFDAICKYQPRLYKFNKEYAYLLRDIVRGKSLVELQQIRQEI FRFIEQDCGVTRLGSLSLSTLETVKAVKGIISYFSTALNASKNNPIS DEQRKEFDPELFALLEKLELIRTRKKKQKVERIANSLIQTCLENNIK IRGEDLSTTNNATKKKANSRMDWLARGVFNKIRQLATMHNITLFGC GSLYTSHQDPLVHRNPDKAMKCRWAAIPVKDIGDWLRLKLSQNLRAKN RGTGEYYHQGVKEFLSHYELQDLEELLLKWSRDRKSNIPCWVLQNLRLA	7

	EKLGNKEAVVYIPVRGGRIYFATHKVATGAVSIVFDQKQVWVCNADHV AAANIALTGKIGRQSSDEENPDGGRIKLQLTSSAGGGGSGGGGSGGG GSG KRPAATKKAGQAKKKK	
Cas12i2 Polypeptide B mRNA	<i>aggagagacccaagcugucuaagacgccacccgccacc</i> AUGAAAAGGCC GGCGGCCACGAAAAAGGCCGGCCAGGCCAAAAAGAAAAAGAGCGCUGG AGGAGGUGGAAGCGGAGGAGGAGGAAGCGGAGGAGGAGGUAGCGGAAU GAGCUCCGCCAUCAAGUCCUACAAGUCUGUGCUGCGGCCAAACGAGAG AAAGAAUCAGCUGCUGAAGUCCACCAUCCAGUGCCUGGAGGACGGCUC CGCCUUCUUUUCAAGAUGCUGCAGGGCCUGUUUGGCGGCAUACCCCC CGAGAUCCGUGAGAUUCAGCACAGAGCAGGAGAAGCAGCAGCAGGAUUAU CGCCUUGUGGUGUGCCGUGAAUUGGUUCAGGCCUGUGAGCCAGGACUC CCUGACCCACACAAUCGCCUCCGAUAACCUGGUGGAGAAGUUUGAGGA GUACUAUGGCGGCACAGCCAGCGACGCCAUCAAGCAGUACUUCAGCGC CUCCAUCCGCGAGUCCUACUAUUGGAAUGACUGCCGCCAGCAGUACUA UGAUCUGUGUCGGGAGCUGGGCGUGGAGGUGUCUGACCUGACCCACGA UCUGGAGAUCCUGUGCCGGGAGAAGUGUCUGGCCGUGGCCACAGAGAG CAACCAGAACAAUUCUAUCAUCAGCGUGCUGUUUGGCACCGCGGAGAA GGAGGAUAGGUCUGUGAAGCUGCGCAUCACAAAGAAGAUCCUGGAGGC CAUCAGCAACCUGAAGGAGAUGCCAAAGAUGUGGCCCCCAUCCAGGA GAUCAUCCUGAAUGUGGCCAAGGCCACCAAGGAGACAUUCAGACAGGU GUACGCAGGAAACCUGGGAGCACCAUCCACCCUGGAGAAGUUUAUCGC CAAGGACGGCCAGAAGGAGUUCGAUCUGAAGAAGCUGCAGACAGACCU GAAGAAAGUGAUCCGGGGCAAGUCUAAGGAGAGAGAUUGGUGCUGUCA GGAGGAGCUGAGGAGCUACGUGGAGCAGAAUACCAUCCAGUAUGACCU GUGGGCCUGGGGCGAGAUUUCAACAAGGCCACACCGCCUGAAGAU CAAGUCCACAAGAAACUACAUAUUUGCCAAGCAGAGGCUGGAGCAGUU CAAGGAGAUCCAGUCUCUGAACAAUCUGCUGGUGGUGAAGAAGCUGAA CGACUUUUUCGAUAGCGAGUUUUUCUCCGGCGAGGAGACCUACACAAU CUGCGUGCACCACUUGGGCGGCAAGGACCUGUCCAAGCUGUAUAAGGC CUGGGAGGACGAUCCCGCCGAUCCUGAGAAUGCCAUCGUGGUGCUGUG CGACGAUCUGAAGAACAUAUUUAAGAAGGAGCCUAUCAGGAACAUCCU GCGCUACAUCUUCACCAUCCGCCAGGAGUGUAGCGCACAGGACUCCU GGCAGCAGCAAAGUACAUAUCAGCAGCUGGAUCGGUAUAAGAGCCAGAA GGCCAAACCAUCCGUGCUGGGCAAUCAGGGCUUUACCUGGACAAACGC CGUGAUCCUGCCAGAGAAGGCCACGCGGAACGACAGACCCAAUUCUCU GGAUCUGCGCAUCUGGCUGUACCUGAAGCUGCGGCACCCUGACGGCAG AUGGAAGAAGCACCAUAUCCAUUCUACGAUACCCGGUUUUUCCAGGA GAUCUAUGCCGCGGCAUAAGCCUGUGGACACCUAGUAGUUUAGGAC ACCCCGCUUCGGCUAUCACCUGCCUAAGCUGACCGAUCAGACAGCCAU CCGCGUGAACAAAGAAGCACGUGAAGGCAGCAAAGACCGAGGCACGGAU CAGACUGGCCAUCCAGCAGGGCACACUGCCAGUGUCCAUCUGAAGAU CACCGAGAUUCUCCGCCACAUAACUCUAAGGGCCAGGUGCGCAUCCC CGUGAAGUUUCGGGUGGGAAGGCAGAAGGGAACCCUGCAGAUCCGGCA CCGGUUCUGCGGCUACGAUCAGAACCAAGACAGCCUCUCACGCCUAUAG CCUGUGGGAGGUGGUGAAGGAGGGCCAGUACCACAAGGAGCUGCGGUG UCGGGUGCGCUUCAUCUAGCGGCGACAUCGUGUCCAUCACCGAGAA CCGGGGCAAUCAGUUUGAUCAGCUGUCUUAUGAGGGCCUGGCCUACCC CCAGUAUGCCGACUGGAGAAAGAAGGCCUCCAAGUUCGUGUCUCUGUG GCAGAUCCAAGAAGAACAAGAAGAAGGAGAUUCGUGACAGUGGAGGC CAAGGAGAAGUUUGACGCCAUUCUGCAAGUACCAGCCUAGGCUGUAUAA GUUCAACAAGGAGUACGCCUAUCUGCUGCGGGAUAUCGUGAGAGGCCAA GAGCCUGGUGGAGCUGCAGCAGAUACGGCAGGAGAUUUUCGUUCAU CGAGCAGGACUGUGGAGUGACCCGCCUGGGAUCUCUGAGCCUGUCCAC CCUGGAGACAGUGAAGGCCGUGAAGGGCAUCAUCUACUCCUAUUUUUC UACAGCCCUGAAUGCCUUAAGAACAUAUCCAUACGCGACGAGCAGCG	8

	GAAGGAGUUUGAUCCUGAGCUGUUCGCCUGCUGGAGAAGCUGGAGCU GAUCAGGACUCGGAAGAAGAAGCAGAAGGUGGAGAGAAUCGCCAAUAG CCUGAUCCAGACAUGCCUGGAGAACAAUAUCAAGUUAUCAGGGGCGA GGGCGACCUGUCCACCACAAACAAUGCCACCAAGAAGAAGGCCAACUC UAGGAGCAUGGAUUGGUGGCCAGAGGCGUGUUUAAUAAGAUCGGCA GCUGGCCACCAUGCACACAUCACCCUGUUCGGCUGCGGCAGCCUGUA CACAUCCCACCAGGACCCUCUGGUGCACAGAAACCAGAUAAAGGCCAU GAAGUGUAGAUGGGCAGCAAUCCAGUGAAGGACAUCGGCGAUUGGGU GCUGAGAAAGCUGUCCAGAACCCUGAGGGCCAAGAAUCGGGGCACC GG CGAGUACUAUCACCAGGGCGUGAAGGAGUUCUGUCUCACUAUGAGCU GCAGGACCUGGAGGAGGAGCUGCUGAAGUGGCGGUCUGAUAGAAAGAG CAACAUCCCUUGCUGGGUGCUGCAGAAUAGACUGGCCGAGAAGCUGGG CAACAAGGAGGCCGUGGUGUACAUCCAGUGAGGGGCGGCCGCAUCUA UUUUGCAACCCACAAGGUGGCAACAGGAGCCGUGAGCAUCGUGUUCGA CCAGAAGCAAGUGUGGGUGUGUAAUGCAGAUACGUGGCAGCAGCAAA CAUCGCACUGACCGCAAGGGCAUCGGCCGGCAGUCCUCUGACGAGGA GAACCCCGAUGGCGGCAGGAUCAAGCUGCAGCUGACAUCUAGCGCUGG AGGAGGUGGAAGCGGAGGAGGAGGAAGCGGAGGAGGAGGUAGCGGAAA AAGGCCGGCGGCCACGAAAAGGCCGGCCAGGCAGGCAAAAAGAAAAGUA <i>Auaagcggccgc</i>	
HA01 Exon 1	AACGAACTCCATCTGGGATAGCAATAACCTGTGAAAATGCTCCCCCGG CTAATTTGTATCAATGATTATGAACAACATGCTAAATCAGTACTTCCA AAGTCTATATATGACTATTACAGGTCTGGGGCAAATGATGAAGAACT TTGGCTGATAATATTGCAGCATTTTCCAGGTAAGAAAATTTATTTTTT AAAATCATGTTTTAAATTTACACAAAGACCG	9
HA01 target sequence 1	CAAAGTCTATATATGACTAT	10
gRNA Spacer 1	CAAAGUCUAUAUAUGACUAAU	11
Direct Repeat	AGAAAUCCGUCUUUCAUUGACGG	12
gRNA 1	AGAAAUCCGUCUUUCAUUGACGGCAAAGUCUAUAUAUGACUAAU	13
gRNA 1 with modifications	mA*mG*mA*AAUCCGUCUUUCAUUGACGGCAAAGUCUAUAUAUGAC*m U*mA*mU	14
gRNA 2	AGAAAUCCGUCUUUCAUUGACGGCGGAGCAUCCUUGGAUACAG	15
gRNA 2 with modifications	mA*mG*mA*AAUCCGUCUUUCAUUGACGGCGGAGCAUCCUUGGAUA*mC*mA *mG	16
Cas12i2 Polypeptide A (with Cas12i2 A)	MSSAIKSYKSVLRPNERKNQLLKSTIQCLEDGSAFFFKMLQGLFGGIT PEIVRFSTEQEKQQQDIALWCAVNWFRPVSQDSLTHTIASDNLVEKFE EYYGGTASDAIKQYFSASIGESYYWDCRQQYYDLCRELGVEVSDLTH DLEILCREKCLAVATESNQNNSTISVLFGTGEKEDRSVKLRITKKILE AISNLKEIPKNVAPIQEII LNVA KATKETFRQVYAGNLGAPSTLEKFI AKDGQKEFDLKKLQTDLKKVIRGKSKERDWCCQEELRSYVEQNTIQYD LWAWGEMFNKAHTALKIKSTRNYNFAKQRLEQFKEIQSLNNLLVVKKL NDFDSEFFSGEETYTCVHHLGGKDL SKLYKAWEDDPADPENAIIVVL CDDLKNNFKKEPIRNILRYIFTIRQECSAQDILAAAKYNQQLD RYKSQ KANPSVLGNQGFWTNAVILPEKAQRNDRPNSLDLRIWLYLKL RHPDG RWKKHHIPFYDTRFFQEIIYAAGNSPVDTCQFRTPRFGYHLPKLT DQTA IRVNKKHVKA AKTEARIRLAIQQGTL PVS NLKI TEISATINSKGQVRI PVKFRVGRQKGT LQIGDRFCGYDQNTASHAYSLWEVVKEGQYHKELG CFVRFISSGDIVSITENRGNQFDQLSYEGLAYPQYADWRKKASKFVSL WQITKKNKKKEIVTVEAKEKFDAICKYQPRLYKFNKEYAYLLRDIVRG KSLVELQQIRQEIFRFIEQDCGVTRLGSLSLSTLETVKAVKGI IYSYF	17

	STALNASKNNPISDEQRKEFDPELFALLEKLELIRTRKKKQKVERIAN SLIQTCLENNIKFIERGEGLSTTNNATKKKANSRSMDWLARGVFNKIR QLAPMHNITLFGCGSLYTSHQDPLVHRNPDKAMKCRWAAIPVKDIGDW VLRKLSQNLRAKNRGTGEYYHQGVKEFLSHYELQDLEEEELLKWRSDRK SNIPCWVLQNLRLAEKLGNEAVVYIPVRGGRIYFATHKVATGAVSIVF DQKQVWVCNADHVAAANIALTGKGIGEQQSDEENPDGSRIKLQLTSKR PAATKKAGQAKKK	
Cas12i2 Polypeptide A coding sequence	AUGAGCUCGCGCAUCAAGUCCUACAAGUCUGUGCUGCGGCCAAACGAGAGAA AGAAUCAGCUGCUGAAGUCCACCAUCCAGUGCCUGGAGGACGGCUCGCCCUU CUUUUUCAAGAUGCUGCAGGGCCUGUUUGCGGCAUACCCCCGAGAUCCUG AGAUUCAGCACAGAGCAGGAGAGCAGCAGGAAUUCGCCUGUGGUGUG CCGUGAAUUGGUUCAGGCCUGUGAGCCAGGACUCCUGACCCACACAAUCGC CUCCGAUAAUCUGUGGAGAAGUUUGAGGAGUACUAUGCGCGCACAGCCAGC GACGCCAUCAAGCAGUACUUCAGCGCCUCCAUCCGCGAGUCCUACUUAUGGA AUGACUGCCGCGCAGCAGUACUAUGAUCUGUGCGGAGCUGGGCCUGGAGGU GUCUGACCUGACCCACGAUCUGGAGAUCCUGUGCCGGGAGAAUGUCUGGCC GUGGCCACAGAGAGCAACCAGAAACAAUUCUAUCUACGCGUGCUGUUUGGCA CCGGCGAGAAGGAGGAUAGGUUCUGAAGCUGCGCAUCACAAAGAAGAUCCU GGAGGCCAUCAAGCAACCUAGAAGGAGAUCCAAAGAAUUGUGGCCCCACG GAGAUCAUCCUGAAUUGUGGCCAAGGCCACCAAGGAGACAUUCAGACAGGUGU ACGAGGAAACCUUGGGAGCACCAUCCACCCUGGAGAAAGUUUUCGCCAAGGA CGGCCAGAAGGAGUUCGAUCUGAAGAAGCUGCAGACAGACCUGAAGAAAGUG AUCCGGGGCAAGCUAAGGAGAGAGAUUGGUGCUGUACAGGAGGACUGGAGGA GCUACGUGGAGCAGAAUACCAUCCAGUAUGACCUGUGGGCCUGGGGCGAGAU GUUACAACAGGCCACACCGCCUGAAGAUCAAGUCCACAGAAACUACAAU UUUGCCAAGCAGAGGCUGGAGCAGUUCAGGAGAUCCAGUCUCUGAACAUC UGCUGUGUGGUGAAGAGCUGAACGACUUUUUCGAGAGCAGUUUUUCGCCG CGAGGAGACCUACACAUCUGCGUGCACCACCUGGGCGGCAAGGACCUGUCC AAGCUGUAUAAGGCCUGGAGGAGCAUCCGCGCAUCUGAGAAUGCCAUCCG UGGUGCUGUGCGACGAUCUGAAGAACAUAUUUAAGAAGGAGCCUACAGGAA CAUCCUGCGCUACAUUCACCAUCCGCGCAGGAGUGUAGCGCACAGGACAUCC CUGGCAGCAGCAAGUACAUAUCAGCAGCUGGAUCCGUAUAAGAGCCAGAAGG CCAACCCAUCCUGCUGGGCAAUCAGGGCUUUACUGGACAAACGCCGUGAU CCUGCCAGAGAAGGCCAGCGGAACGACAGACCCAAUUCUCUGGAUCUGCGC AUCUGGCUGUAUCUGAAGCUGCGGCACCCUGACGGCAGAUUGGAAGAGCACC ACAUCCCAUUCUACGAUACCCGGUUUUUCAGGAGAUCAUGCCGCCGGCAA UAGCCUGUGGACACCUGUCAGUUUAGGACACCCCGCUUCGGCUAUCACCUG CCUAAGCUGACCGAUACAGACGCAUCCGCGUGAACAAGAGCAGGUGAAGG CAGCAAAGACCGAGGCACGAUCAGACUGGCCAUCCAGCAGGGCACACUGCC AGUGUCCAAUUCGAGAUACCGAGAUUCGCCCAUAUACAUCUAAGGGC CAGGUGCGCAUCCCGUGAAGUUUCGGUGGGAAGGCAGAAAGGGAAACCCUGC AGAUCGGCGACCGGUUCUGCGGCUACGAUCAGAACAGACAGCCUCUACGC CUAUAGCCUGUGGAGGUGGUGAAGGAGGGCCAGUACCACAAGGAGCUGGGC UGUUUUGUGCGCUUACUUCUAGCGGCAGAUUCGUGUCCAUACCGGAAACC GGGGCAUUCAGUUUGAUCAGCUGUCUUAUGAGGGCCUGGCCUACCCCCAGUA UGCCGACUGGAGAAAGAAGGCCUCCAAGUUCUGUCUCUGUGGCAGAUACCC AAGAAGACAAGAAGAGAGAUCCUGACAGUGGAGGCCAAGGAGAAGUUUG ACGCCAUUCGCAAGUACCGCCUAGGCUGUAUAAGUUCAACAAGGAGUACGC CUAUCUGCUGCGGGAUUCUGGAGAGGCCAAGAGCCUGGUGGAGCUGCAGCAG AUCAGGCAGGAGAUUCUUCGCUUACUAGCAGGAGCUGUGGAGUGACCCGCC UGGGAUCUCUGAGCCUGUCCACCCUGGAGACAGUGAAGGCCUGAAGGGCAU CAUCUACUCCUAUUUUUCUACAGCCUGAAGCCUUAAGAACAUAUCCCAUC AGCGACGAGCAGCGGAAGGAGUUUGAUCCUGAGCUGUUCGCCUCUGGAGGA AGCUGGAGCUGAUACGAGCUCGGAAGAAGAGCAGAAGGUGGAGAGAAUCCG CAAUAGCCUGAUCCAGACAUGCCUGGAGAACAAUAUCAAGUUAUCAGGGGC GAGGGCGACCUUGUCCACCACAAACAAGCCACCAAGAAGAGGCCAACUCUA GGAGCAUGGAUUGGUGGCGCAGAGCGUGUUUAUAAGAUCCGGCAGCUGGC CCCAAUGCACAACAUACCCUGUUCGGCUGCGGCAGCCUGUACACAUCCCA CAGGACCCUCUGGUGCAGAGAAACCCAGAUAAAGGCCAUGAAGUGUAGAUGGG CAGCAUCCCAUGUAGGAGACUCCGCGAUUGGGUGCUGAGAAAGCUGUCCCA GAACCUGAGGGCCAAGAAUCCGGGACCGCGAGUACUAUACACAGGGCGUG	18

	AAGGAGUCCUGUCACUAUGAGCUGCAGGACCUGGAGGAGGAGCUGCUGA AGUGGCGGUCUGAUAGAAAGAGCAACAUCCUUGCUGGGUGCUGCAGAAUAG ACUGGCGGAGAGCUGGGCAACAAGGAGGCCUGGUGUACAUCCAGUGAGG GGCGGCCGCAUCUAUUUUGCAACCCACAAGGUGGCAACAGGAGCCGUGAGCA UCGUGUUCGACCAGAAGCAAGUGUGGGUGUGUAAUGCAGAUACAGUGGCAGC AGCAAACAUCCGACUGACCGGCAAGGGCAUCGGCGAGCAGUCCUCUGACGAG GAGAACCCCGAUGGCUCCAGGAUCAAGCUGCAGCUGACAUCAAAGGCCCGG CGGCCACGAAAAAGGCCGCGCCAGGCCAAAAAGAAAAAGUAA	
HAO1 target sequence 2	CGGAGCATCCTTGGATACAG	19
gRNA Spacer 2	CGGAGCAUCCUUGGAUACAG	20
Mouse HAO1 gRNA	mA*mG*mA*AAUCCGUCUUUAUUGACGGUGAAGCACUGAUCGGAC*mA*mU *mG	21
Cas12i2 Polypeptide B coding sequence	AUGAAAAGGCCGCGGCCACGAAAAAGGCCGCGCCAGGCCAAAAAGAAA AAGAGCGCUGGAGGAGGUGGAAGCGGAGGAGGGAAGCGGAGGAGGA GGUAGCGGAUAGAGCUCCGCCAUCAAGUCCUACAAGUCUGUGCUGCGG CCAAACGAGAGAAAGAAUCAGCUGCUGAAGUCCACCAUCCAGUGCCUG GAGGACGGCUCGCUUUUUUCAAAGAUGCUGCAGGGCCUGUUUGGC GGCAUACCCCCGAGAUUCGUGAGAUUCAGCACAGAGCAGGAGAAGCAG CAGCAGGAUAUCGCCUGUGGUGUGCCGUGAAUUGGUUCAGGCCUGUG AGCCAGGACUCCUGACCCACACAUCGCCUCCGAUAACCUGGUGGAG AAGUUUAGGAGAUACUAUGGCGGCACAGCCAGCGACGCCAUCAAGCAG UACUUCAGCGCCUCCAUCCGCGAGUCCUACUAUUGGAAUGACUGCCGC CAGCAGUACUAUGAUCUGUGUCGGGAGCUGGGCGUGGAGGUGUCUGAC CUGACCCACGAUCUGGAGAUCUUGUGCCGGGAGAAGUGUCUGGCCUG GCCACAGAGAGCAACCAGAACAUAUUAUUAUCAGCGUGCUGUUUGGC ACCGCGGAGAAGGAGGAUAGGUCUGUGAAGCUGCGCAUCACAAAGAAG AUCCUGGAGGCCAUAGCAACCUGAAGGAGAUCCAAAGAAUGUGGCC CCCAUCCAGGAGAUCAUCCUGAAUGUGGCCAAGGCCACCAAGGAGACA UUCAGACAGGUGUACGCAGGAACCUUGGGAGCACCAUCCACCCUGGAG AAGUUUAUCGCCAAGGACGGCCAGAAGGAGUUCGAUCUGAAGAAGCUG CAGACAGACCUGAAGAAAGUGAUCCGGGGCAAGUCUAAGGAGAGAGAU UGGUGCUGUCAGGAGGAGCUGAGGAGCUACUGGAGCAGAAUACCAUC CAGUAUGACCUGUGGGCCUGGGGCGAGAUUUCAACAAGGCCACACC GCCCUGAAGAUCAAGUCCACAAGAAACUACAUAUUUUGCCAAGCAGAGG CUGGAGCAGUUAAGGAGAUCAGUCUCUGAACAUCUGCUGGUGGUG AAGAAGCUGAACGACUUUUUCGAUAGCGAGUUUUUCCCGCGGAGAG ACCUACACAAUCUGCGUGCACCACCUGGGCGGCAAGGACCUGUCCAAG CUGUAUAAGGCCUGGGAGGACGAUCCCGCCGAUCCUGAGAAUGCCAUC GUGGUGCUGUGCGACGAUCUGAAGAACAAUUUUAAGAAGGAGCCUAC AGGAACAUCUGCGCUACAUCUACCAUCCGCCAGGAGUGUAGCGCA CAGGACAUCUGGCAGCAGCAAAGUACAAUCAGCAGCUGGAUCGGUAU AAGAGCCAGAAGGCCAACCAUCCGUGCUGGGCAUACAGGGCUUUACC UGGACAAACGCCGUGAUCCUGCCAGAGAAGGCCAGCGGAACGACAGA CCCAAUUCUCUGGAUCUGCGCAUCUGGCUGUACCUGAAGCUGCGGCAC CCUGACGGCAGAUUGAAGAAGCACCAUCCCAUUCUACGAUACCCGG UUUUUCCAGGAGAUCAUGCCGCGGCAUAGCCUGUGGACACCUGU CAGUUUAGGACACCCCGCUUCGGCUAUCACCUGCCUAAGCUGACCGAU CAGACAGCCAUCCCGUGAACAAGAAGCACGUGAAGGCAGCAAGAGACC GAGGCACGGAUCAGACUGGCCAUCCAGCAGGGCACACUGCCAGUGUCC AAUCUGAAGAUAACCGAGAUCUCCGCCACAUAACUCUAAGGGCCAG GUGCGCAUCCCGUGAAGUUUCGGGUGGGAAGGCAGAAGGGAACCCUG CAGAUCCGCGACCCGUUCUGCGGCUACGAUCAGAACCAGACGCCUCU CACGCCUAUAGCCUGUGGGAGGUGUGAAGGAGGGCCAGUACCACAAG	22

	GAGCUGCGGUGUCGGGUGCGCUUCAUCUCUAGCGGCGACAUCGUGUCC AUCACCGAGAACCGGGGCAAUCAGUUUGAUCAGCUGUCUUAUGAGGGC CUGGCCUACCCCCAGUAUGCCGACUGGAGAAAAGAGCCUCCAAGUUC GUGUCUCUGUGGCAGAUACCAAGAAGAACAAGAAGAAGGAGAUUCGUG ACAGUGGAGGCCAAGGAGAAGUUUGACGCCAUCUGCAAGUACCGAGCCU AGGCUGUAUAAGUUCACAAAGGAGUACGCCUUAUCUGCUGCGGGAUAUC GUGAGAGGCAAGAGCCUGGUGGAGCUGCAGCAGAUACAGGCAGGAGAU UUUCGCUUCAUCGAGCAGGACUGUGGAGUGACCCGCCUGGGAUCUCUG AGCCUGUCCACCCUGGAGACAGUGAAGGCCGUGAAGGGCAUCAUCUAC UCCUAUUUUUCUACAGCCCUGAAUGCCUCUAAGAACAUAUCCCAUCAGC GACGAGCAGCGGAAGGAGUUUGAUCCUGAGCUGUUCGCCCUGCUGGAG AAGCUGGAGCUGAUACAGGACUCGGAAGAAGAAGCAGAAGGUGGAGAGA AUCGCCAAUAGCCUGAUCCAGACAUGCCUGGAGAACAUAUCAAGUUC AUCAGGGGCGAGGGCGACCUGUCCACCACAAACAUGCCACCAAGAAG AAGGCCAACUCUAGGAGCAUGGAUUGGCUGGCCAGAGGCGUGUUAAU AAGAUCCGGCAGCUGGCCACCAUGCACAACAUACCCUGUUCGGCUGC GGCAGCCUGUACACAUCCACCAGGACCCUCUGGUGCAGACGAAACCCA GAUAAGGCCAUGAAGUGUAAGAUGGGCAGCAAUCCAGUGAAGGACAUC GGCGAUUGGGUGCUGAGAAAGCUGUCCAGAACCUGAGGGCCAAGAAU CGGGGCACCGGCAGUACUAUCACCAGGGCGUGAAGGAGUUCUGUCU CACUAUGAGCUGCAGGACCUGGAGGAGGAGCUGCUGAAGUGGCGGUCU GAUAGAAAGAGCAACAUCCCUUGCUGGGUGCUGCAGAAUAGACUGGCC GAGAAGCUGGGCAACAAGGAGGCCUGGUGUACAUCCAGUGAGGGGC GGCCGCAUCUAUUUUGCAACCCACAAGGUGGCAACAGGAGCCUGAGC AUCGUGUUCGACCAGAAGCAAGUGUGGGUGUGUAAUGCAGAUACGUG GCAGCAGCAAACAUGCACUGACCGGCAAGGGCAUCGGCCGGCAGUCC UCUGACGAGGAGAACCCCGAUGGCGGCAGGAUCAAGCUGCAGCUGACA UCUAGCGCUGGAGGAGGUGGAAGCGGAGGAGGAGGAAGCGGAGGAGGA GGUAGCGGAAAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAA AAGAAAAAGUAA	
Cas12i2 Polypeptide B mRNA with poly A tail	<i>aggagagacccaagcugucuaagacgccacccgccacc</i>AUGAAAAGGCC GGCGGCCACGAAAAAGGCCGGCCAGGCCAAAAAGAAAAAGAGCGCUGG AGGAGGUGGAAGCGGAGGAGGAGGAAGCGGAGGAGGAGGUAGCGGAAU GAGCUCGCGCAUCAAGUCCUACAAGUCUGUGCUGCGGCCAAACGAGAG AAAGAAUCAGCUGCUGAAGUCCACCAUCCAGUGCCUGGAGGACGGCUC CGCCUUCUUUUUCAAGAUGCUGCAGGGCCUGUUUGGCGGCAUACCCCC CGAGAUCCUGAGAUUCAGCACAGAGCAGGAGAAGCAGCAGCAGGAUAU CGCCUGUGGUGUGCCGUGAAUUGGUUCAGGCCUGUGAGCCAGGACUC CCUGACCCACACAUCGCCUCCGAUAACCGUGGUGGAGAAGUUUGAGGA GUACUAUGGCGGCACAGCCAGCGACGCCAUCAAGCAGUACUUCAGCGC CUCCAUCGGCGAGUCCUACUAUUGGAAUGACUGCCGCCAGCAGUACUA UGAUCUGUGUCGGGAGCUGGGCGUGGAGGUGUCUGACCUGACCCACGA UCUGGAGAUCCUGUGCCGGGAGAAGUGUCUGGCCCGUGGCCACAGAGAG CAACCAGAACAAUUCUAUCAUCAGCGUGCUGUUUGGCACCGGCAGAGAA GGAGGAUAGGUCUGUGAAGCUGCGCAUCACAAAGAAGAUAUCCUGGAGGC CAUCAGCAACCUGAAGGAGAUCCCAAAGAAUGUGGCCCCCAUCCAGGA GAUCAUCCUGAAUGUGGCCAAGGCCACCAAGGAGACAUUCAGACAGGU GUACGCAGGAAACCGUGGAGCACCAUCCACCCUGGAGAAGUUUAUCGC CAAGGACGGCCAGAAGGAGUUCGAUCUGAAGAAGCUGCAGACAGACCU GAAGAAAGUGAUCCGGGGCAAGUCUAAGGAGAGAGAUUGGUGCUGUCA GGAGGAGCUGAGGAGCUACGUGGAGCAGAAUACCAUCCAGUAUGACCU GUGGGCCUGGGGCGAGAUUUCAACAAGGCCACACCGCCUGAAGAU CAAGUCCACAAGAAACUACAUAUUUGCCAAGCAGAGGCUGGAGCAGUU CAAGGAGAUCCAGUCUCUGAACAUUCUGCUGGUGGUGAAGAAGCUGAA CGACUUUUUCGAUAGCGAGUUUUUCUCCGGCGAGGAGACCUACACAAU	23

	CUGCGUGCACCACCUGGGCGGCAAGGACCUGUCCAAGCUGUAUAAGGC CUGGGAGGACGAUCCCGCCGAUCCUGAGAAUGCCAUCGUGGUGCUGUG CGACGAUCUGAAGAACAUAUUUAAGAAGGAGCCUAUCAGGAACAUCU GCGCUACAUCUUCACCAUCCGCCAGGAGUGUAGCGCACAGGACAUCU GGCAGCAGCAAAGUACAAUCAGCAGCUGGAUCGGUAUAAGAGCCAGAA GGCCAACCCAUCCGUGCUGGGCAUACAGGGCUUUACCUGGACAAACGC CGUGAUCCUGCCAGAGAAGGCCAGCGGAACGACAGACCCAAUUCUCU GGAUCUGCGCAUCUGGCUGUACCUGAAGCUGCGGCACCCUGACGGCAG AUGGAAGAAGCACCACAUCCCAUUCUACGAUACCCGGUUUUUCCAGGA GAUCUAUGCCGCCGGCAAUAGCCUGUGGACACCUGUCAGUUUAGGAC ACCCGCUUCGGCUAUCACCUGCCUAAGCUGACCGAUCAGACAGCCAU CCGCGUGAACAAGAAGCACGUGAAGGCAGCAAAGACCGAGGCACGGAU CAGACUGGCCAUCCAGCAGGGCACACUGCCAGUGUCCAUCUGAAGAU CACCGAGAUUCGCCACAUAACUCUAAGGGCCAGGUGCGCAUCCC CGUGAAGUUUCGGUGGGGAAGGCAGAAGGGAACCCUGCAGAUCCGGCGA CCGGUUCUGCGGCUACGAUCAGAACCAGACAGCCUCACGCCUAUAG CCUGUGGGAGGUGUGAAGGAGGGCCAGUACCACAAGGAGCUGCGGUG UCGGGUGCGCUUCAUCUCUAGCGGCGACAUCGUGUCCAUCACCGAGAA CCGGGGCAAUCAGUUUGAUCAGCUGUCUUAUGAGGGCCUGGCCUACCC CCAGUAUGCCGACUGGAGAAAGAAGGCCUCCAAGUUCGUGUCUCUGUG GCAGAUCCACCAAGAAGAACAAGAAGAAGGAGAUUCGUGACAGUGGAGGC CAAGGAGAAGUUUGACGCCAUUCUGCAAGUACCAGCCUAGGCUGUAUAA GUUCAACAAGGAGUACGCCUAUCUGCUGCGGGAUAUCGUGAGAGGC GAGCCUGGUGGAGCUGCAGCAGAUACAGGCAGGAGAUUUUCGUUCAU CGAGCAGGACUGUGGAGUGACCCGCCUGGGAUCUCUGAGCCUGUCCAC CCUGGAGACAGUGAAGGCCGUGAAGGGCAUCAUCUACUCCUAUUUUUC UACAGCCCUGAAUGCCUCUAAGAACAUCCCAUCAGCGACGAGCAGCG GAAGGAGUUUGAUCCUGAGCUGUUCGCCUGCUGGAGAAGCUGGAGCU GAUCAGGACUCGGAAGAAGAAGCAGAAGGUGGAGAGAAUCGCCAAUAG CCUGAUCCAGACAUGCCUGGAGAACAAUAUCAAGUUCAUCAGGGCGA GGGCGACCCUGUCCACCACAAACAUGCCACCAAGAAGAAGGCCAACUC UAGGAGCAUGGAUUGGCUGGCCAGAGGCGUGUUUAUAAGAUCGGCA GCUGGCCACCAUGCACAACAUCACCCUGUUCGGCUGCGGCAGCCUGUA CACAUCCCACCAGGACCCUCUGGUGCACAGAAACCCAGAUAAAGGCCAU GAAGUGUAGAUGGGCAGCAAUCCAGUGAAGGACAUCGGCGAUUGGGU GCUGAGAAAGCUGUCCAGAACCCUGAGGGCCAAGAAUCGGGGCACC CGAGUACUAUCACCAGGGCGUGAAGGAGUUCUGUCUACUAUGAGCU GCAGGACCUGGAGGAGGAGCUGCUGAAGUGGCGGUCUGAUAGAAAGAG CAACAUCCCUUGCUGGGUGCUGCAGAAUAGACUGGCCGAGAAAGCUGGG CAACAAGGAGGCCUGGUGUAUCCAGUGAGGGGCGGCCGCAUCUA UUUUGCAACCCACAAGGUGGCAACAGGAGCCUGAGCAUCGUGUCCGA CCAGAAGCAAGUGUGGGUGUGUAAUGCAGAUACGUGGCAGCAGCAAA CAUCGCACUGACCGCAAGGGCAUCGGCCGGCAGUCCUCUGACGAGGA GAACCCGCAUGGCGGCAGGAUCAAGCUGCAGCUGACAUCUAGCGCUGG AGGAGUGGAAGCGGAGGAGGAGGAAGCGGAGGAGGAGGUAGCGGAAA AAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAGUA <u>Auaagcgcgccgcaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa</u> <u>aa</u> <u>nn</u> <u>nnn</u>	
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- 2'-O-methyl phosphorothioate modifications indicated: *, phosphorothioate; m, 2' O-methyl
- Underlined and italicized nucleotide sequences refer to 5' and 3' untranslated regions (UTRs)
- "n" refers to adenosine (a) residues or is absent.

General techniques

The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; *Oligonucleotide Synthesis* (M. J. Gait, ed. 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J. E. Cellis, ed., 1989) Academic Press; *Animal Cell Culture* (R. I. Freshney, ed. 1987); *Introduction to Cell and Tissue Culture* (J. P. Mather and P. E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J. B. Griffiths, and D. G. Newell, eds. 1993-8) J. Wiley and Sons; *Methods in Enzymology* (Academic Press, Inc.); *Handbook of Experimental Immunology* (D. M. Weir and C. C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J. M. Miller and M. P. Calos, eds., 1987); *Current Protocols in Molecular Biology* (F. M. Ausubel, et al. eds. 1987); *PCR: The Polymerase Chain Reaction*, (Mullis, et al., eds. 1994); *Current Protocols in Immunology* (J. E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C. A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: a practice approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal antibodies: a practical approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using antibodies: a laboratory manual* (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds. Harwood Academic Publishers, 1995); *DNA Cloning: A practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds.(1985»; *Transcription and Translation* (B.D. Hames & S.J. Higgins, eds. (1984»; *Animal Cell Culture* (R.I. Freshney, ed. (1986»; *Immobilized Cells and Enzymes* (IRL Press, (1986»; and B. Perbal, *A practical Guide To Molecular Cloning* (1984); F.M. Ausubel *et al.* (eds.).

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLES

The following examples are provided to further illustrate some embodiments of the present invention but are not intended to limit the scope of the invention; it will be understood by their exemplary nature that other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLE 1: EFFICACY AND TOLERABILITY OF HAO1 GENE EDITING SYSTEM *IN VIVO*

This example evaluates the efficacy and tolerability of a gene editing system comprising a Cas12i2 mRNA and an HAO1 guide RNA. mRNAs expressing a Cas12i2 protein and gRNA targeting HAO1 were loaded into lipid nanoparticles.

Composition and Treatment Groups

Composition of the specific treatment groups is described in Table 1.

Table 1. Composition Characteristics and Dosing Concentrations of Treatment Groups

Group	Cas12i2- Encoding mRNA* (SEQ ID NO)	gRNA (modified) (SEQ ID NO)	LNP	Dose (mg total RNA/body weight)	N/P	gRNA/mRNA
1	18	16	A	2.0 mg/kg	6.0	1:1
2	18	14	A	2.0 mg/kg	6.0	1:1
3	18	14	B	2.5 mg/kg	6.0	1:1
4	18	14	A	2.5 mg/kg	6.0	1:1
5	18	14	A	1.0 mg/kg	6.0	1:1
6	18	14	A	2.0 mg/kg	6.0	1:1
7	18	14	A	2.5 mg/kg	6.0	1:1
8	8	14	A	2.5 mg/kg	4.5	0.33:1
9	8	14	A	2.5 mg/kg	4.5	1:1
10	8	14	A	2.5 mg/kg	6.0	1:1

*: the mRNA contains a 5' cap and pseudouridine modifications.

LNP A contains 47.5% cationic lipid bis(2-butyloctyl) 10-(N-(3-(pyrrolidine-1-yl)propyl)nonanamido)nonadecanedioate, 10.0% phospholipid DSPC, 40.0% cholesterol, and 2.5% PEG lipid 2-[2-(ω-methoxy (polyethyleneglycol2000) ethoxy)]-N,N-ditetradecylacetamide. LNP A was prepared according to the general procedures of WO 2017/004143, which is incorporated herein by reference for the subject matter and purpose

referenced herein.

LNP B was a comparative LNP containing different lipid excipients than LNP A.

The nitrogen:phosphate (N/P) ratio is calculated from the results of HPLC (for determination of amino lipid mol) and ribogreen (for determination of mol of total RNAs), as

5 indicated by the formula below:

$$\frac{\text{mol aminolipid (HPLC)}}{\text{mol total RNA (ribogreen)}}$$

Animal Treatment Protocol

The test compositions comprising nucleic acid loaded LNPs were administered by 1-
10 hour intravenous infusion to cynomolgus monkeys (NHPs). Prior to study start, animals were acclimated to the laboratory housing conditions over a minimum period of 14 days. Premedication drugs comprising dexamethasone at 1 mg/kg, Famotidine at 0.5 mg/kg, and diphenhydramine at 5 mg/kg were administered via intramuscular injection into the quadriceps muscle at one day prior to administration and at 60-90 minutes prior to administration.

15 The composition was diluted to the appropriate test dose concentration (mg total RNA/mL) using 0.9% sodium chloride as the vehicle to achieve the appropriate dose level (mg/kg/dose) at an infusion rate of 5 mL/kg/hr. The dose formulations were administered by IV infusion over 1 hour (± 5 minutes). Dose formulations were administered using a temporary catheter inserted into a peripheral vein connected to an infusion line. The appropriate volume
20 was delivered by an infusion pump.

In some animals, additional doses of dexamethasone were administered *via* intramuscular injection into the quadriceps at 1 mg/kg at 6 hours post- administration and approximately 24 hours post- administration.

25 Following administration, animals were monitored at least twice daily for morbidity, appetite level/food consumption, and other cage side observations such as overall appearance and activity level. Body weight was also monitored at 24-, 72-, and 168-hours post dosing, then once weekly thereafter throughout the study. Blood samples were also collected for serum chemistry and coagulation assays at different time points after the infusion. Exemplary biomarkers for examination are listed in **Table 2** below.

30

Table 2. Biomarkers for Examination

Serum Chemistry Parameters		
Albumin	Creatine Kinase	Potassium
Alkaline Phosphatase	Creatinine	Chloride
Alanine Aminotransferase	Glucose	Globulin
Aspartate Aminotransferase	Inorganic Phosphorus	Albumin/Globulin Ratio
Total Bilirubin	Total Protein	Blood Urea Nitrogen
Calcium	Triglyceride	Gamma
Total Cholesterol	Sodium	Glutamyltransferase
Disposition	Remaining samples will be stored in a freezer set to maintain -80°C and discarded prior to study finalization	
Coagulation Parameters		
Activated Partial Thromboplastin Time	Fibrinogen	Prothrombin Time
Disposition	Remaining samples will be discarded after analysis	

The in-life portion of the NHP studies concluded at 3-4 weeks post- administration. Animals were fasted overnight prior to termination. Following terminal blood collection, animals were sedated, weighed, and euthanized by an overdose of euthanasia solution. Animals undergo full body perfusion with phosphate buffered saline and subjected to a complete gross examination and tissue collection.

HAOI Gene Editing: % Indel determination

Liver tissue was collected at study termination to assess indel levels. Approximately 250 mg were collected from the caudal, left, medial, & right liver lobes, flash frozen in liquid nitrogen, and stored at -80C. To extract DNA for indel analysis, 4 x 20 mg liver samples from each of the 4 lobes were placed on ice in 700 uL Quick Extract solution and homogenized under cold conditions using the Qiagen TissueLyser II.

Next Generation Sequencing (NGS) samples were prepared by two rounds of PCR. The first round (PCR1) was used to amplify specific genomic regions depending on the target. Round 2 PCR (PCR2) was performed to add Illumina adapters and indices. Reactions were then pooled and purified by column purification. Sequencing runs were performed using a 150 Cycle NextSeq 500/550 Mid or High Output v2.5 Kit or a 200 Cycle NovaSeq 6000 SP or S1 Reagent Kit v1.5.

For NGS analysis, the indel mapping function used a sample's fastq file, the amplicon reference sequence, and the forward primer sequence. For each read, a kmer-scanning algorithm was used to calculate the edit operations (match, mismatch, insertion, deletion)

between the read and the reference sequence. In order to remove small amounts of primer dimer present in some samples, the first 30 nt of each read was required to match the reference and reads where over half of the mapping nucleotides are mismatches were filtered out as well. Up to 50,000 reads passing those filters were used for analysis, and reads were counted as an indel read if they contained an insertion or deletion. The QC standard for the minimum number of reads passing filters was 10,000.

For each target, indel ratios, referring to the percentage of NGS reads comprising indels, were calculated for each sample and its cognate no template control.

10 ***GO enzyme Activity and Glycolate Measurement***

GO activity was measured by an HPLC-based assay which measures glyoxylate levels after the addition of sodium glycolate substrate to liver tissue homogenates. In this assay, 25-50 mgs of tissue is sonicated in a lysis buffer containing HEPES and 10% Triton-X. Liver lysate solution is incubated with sodium glycolate master mix (100mM Tris HCL, 5mM Na glycolate) for 15 minutes at 37deg C. After incubation, 5M PCA is added, samples are centrifuged for 5 minutes, and the supernants are run on HPLC to detect glyoxylate levels. HAO1-edited samples are expected to have lower glyoxylate levels as GO (the enzyme encoded by HAO1 gene) is responsible for the conversion of glycolate to glyoxylate.

Ion chromatography mass spectrometry (ICMS) was used to measure glycolate levels in serum. Plasma was diluted ~10x in 13C2-glycolate. Plasma macromolecules were removed by centrifugal filtration with 0.4ml Amicon Ultra 10 kD nominal molecular mass limit filters (Millipore Sigma). Ion Chromatography was completed using Integrion Reagent Free HPIC with AS-AP refrigerated autosampler (Thermo Fisher) and Mass Spectrometry was completed using the ISQ-EC (Thermo Fisher).

25

Summary

These results obtained from the studies noted above demonstrate the impact of optimizing guide, LNP, guide:mRNA ratios, N/P ratios, and nuclease variant on *in vivo* editing levels in NHP liver. The treatment resulting in the highest indel levels (60% average) was LNP-A loaded with mRNA of SEQ ID NO: 23 and HAO1 E1T2 guide RNA at a 1:1 ratio, and overall N/P ratio of ~ 6.0 (*e.g.*, 5.64). See **FIG. 1**.

Further, animals in the treatment group that achieved >55% editing on average are shown as individual animals for editing (**FIG. 2C**), GO activity (**FIG. 2A**), and glycolate biomarker levels (**FIG. 2B**). Two of 3 animals exceeded indels levels >55% (dots represent

individual liver lobes). All animals showed >75% decrease in GO activity as compared to a treatment group that yielded low editing (<5%). All animals showed increased glycolate levels.

5 These results demonstrate that treatment with LNP-A loaded with Cas12i2 B-encoding mRNA (SEQ ID NO: 23) and HAO1 gRNA 1 a 1:1 ratio, and overall N/P ratio of ~6.0 can achieve a target indel rate of >55% and the concomitant reduction of GO activity and increases glycolate needed for a physiologically meaningful outcome.

EXAMPLE 2: Effects of *HAO1* Gene Editing in Wildtype Mice

10 This example illustrates effects of a mouse counterpart of the HAO1 gene editing composition provided herein in wild-type mice.

Animal Treatment Protocol

15 A short-term PK study was conducted in male C57BL/6 mice aged 6-8 weeks. Three days prior to dosing, mice were weighed. After a 30-min room temperature incubation period, whole blood was centrifuged for 10 min at 2,000 x g. Plasma was pipetted into an Eppendorf collection tube and stored at -80° C.

20 On the day of dosing, LNPs (LNPA) loaded with Cas12i2 mRNA A (SEQ ID NO: 18) (encoding Cas12i2 polypeptide A of SEQ ID NO: 17) and mouse HAO1 guide (mA*mG*mA*AAUCCGUCUUUCAUUGACGGUGAAGCACUGAUCGGAC*mA*mU*mG, SEQ ID NO: 19) were delivered via tail vein injection, retro orbital injection can also be used. The mRNA/gRNA-loaded LNPs were dosed at 2.5 mg RNA/kg body weight. Necropsy and tissue collections occurred at various time points after the treatment as indicated in **Table 3** below.

25 An insulin syringe with attached needle was used to collect whole blood via cardiac puncture to enable maximal blood collection. Tissue was perfused with PBS using via the vena cava using a 10 ml syringe with 23-gauge needle. Once perfused, the spleen and 2 individual liver lobes were collected flash frozen on dry ice. For histology, place a third liver lobe was collected in 10% NBF for 24-48hr, followed by 70% Ethanol. Whole blood was processed as described above.

30

Table 3. Schedule of Study

	Study Day, Date													
	Day													
Day	-3	0								1	2	3	5	7
Timepoint	-3	0	15m	30m	1h	2h	4h	6h	12h	24h	48h	72hr	120hr	168hr
Body Weight (Groups)	All													
Phlebotomy	All													
Dosing		All												
Necropsy, Tissue Collection		1	2	3	4	5	6	7	8	9	10	11	12	13

This study demonstrated that editing the *Hao1* gene at >55% Indels leads to a >90% reduction in GO protein. The reduction in GO protein correlates with an 8x increase in plasma glycolate. These studies suggest that 55% editing of the *Hao1* gene can have a physiologically beneficial effect. See **FIG. 3**.

EXAMPLE 3 - STUDY OF *HAOI* GENE EDITING IN AGXT ^{-/-} MICE: A MODEL FOR PH1.

To understand the *in vivo* pharmacologic effects of HAO1 editing, mRNA/gRNA-loaded LNPs described above were administered to alanine-glyoxylate aminotransferase deficient (AGXT^{-/-}) mice, a model of PH1 disease. Salido et al., *Proc. Natl. Acad. Sci. U.S.A.*, (2006) 103(48):18249-18254, the relevant disclosures of which are incorporated by reference for the subject matter and purposes references herein. AGXT^{-/-} mice exhibit complete loss of expression of both AGXT mRNA and protein and exhibit hyperoxaluria (3-4 fold elevation in urinary oxalate) relative to control littermates. Male AGXT^{-/-} mice animals aged 15-20 weeks were housed in a regular cage with low oxalate diet on day 1-7. On day 8-11, mice were acclimated to metabolic cages. Baseline urines and serum were collected on days 15-18. Animals were dosed on day 19-20 with one of two doses (Low: 1.5 mg/kg; High: 3.0 mg/kg). Serum and urine were collected weekly thereafter, and the study was terminated on day 40. At necropsy, terminal blood collection was done via cardiac puncture, and PBS perfusion was performed for tissue collection.

HAOI protein measurement

GO protein levels were measured using the Jess Simple Western (Protein Simple). Approximately 50mg of liver tissue was added to 400uL RIPA buffer with 1X protease inhibitor and homogenized using the Qiagen tissue homogenizer. Samples were spun down and the supernatant was collected. BCA assay was used to determine the concentration. Samples were normalized to 1mg/mL and mixed with a fluorescent master mix (Protein Simple, PS-ST01EZ-8). Samples were denatured by incubating at 65 degrees C for 5 minutes. Luminol-S/Peroxide, Primary and Secondary antibodies were prepared for the detection of GO protein and Vinculin as a loading control. Samples were loaded into the Jess Simple Western for protein measurement.

HAOI Gene Editing: Indel% Determination

Liver tissue was collected at study termination to assess indel levels. Approximately 250 mg samples were collected from the caudal, left, medial, & right liver lobes, flash frozen in liquid nitrogen, and stored at -80C. To extract DNA for indel analysis, approximately 20 mg liver samples from each of the 4 lobes were placed on ice in 700 uL Quick Extract solution and homogenized under cold conditions using the Qiagen TissueLyser II. NGS was performed as described previously.

Next Generation Sequencing (NGS) samples were prepared by two rounds of PCR. The first round (PCR1) was used to amplify specific genomic regions depending on the target. Round 2 PCR (PCR2) was performed to add Illumina adapters and indices. Reactions were then pooled and purified by column purification. Sequencing runs were performed using a 150 Cycle NextSeq 500/550 Mid or High Output v2.5 Kit or a 200 Cycle NovaSeq 6000 SP or S1 Reagent Kit v1.5.

For NGS analysis, the indel mapping function used a sample's fastq file, the amplicon reference sequence, and the forward primer sequence. For each read, a kmer-scanning algorithm was used to calculate the edit operations (match, mismatch, insertion, deletion) between the read and the reference sequence. In order to remove small amounts of primer dimer present in some samples, the first 30 nt of each read was required to match the reference and reads where over half of the mapping nucleotides are mismatches were filtered out as well. Up to 50,000 reads passing those filters were used for analysis, and reads were counted as an indel read if they contained an insertion or deletion. The QC standard for the minimum number of reads passing filters was 10,000.

For each target, indel ratios, referring to the percentage of NGS reads comprising indels, were calculated for each sample and its cognate no template control.

Biological Assays

5 GO Enzyme activity and Glycolate levels were assayed as described above. Urinary Oxalate levels were measured by intact cell mass spectrometry (ICMS). In brief, urine is acidified to 300mM HCL, centrifuged to remove any particulate matter, and diluted, typically >20x, in ¹³C2-oxalate internal standard (final concentration 5 micromolar). Ion Chromatography was completed using ICS-5000+ with AS-AP refrigerated autosampler
10 (Thermo Fisher) and Mass Spectrometry is completed using the ISQ-EC (Thermo Fisher).

Results

The results demonstrate that *in vivo* editing of the mouse *Hao1* gene in the liver is dose dependent (**FIG. 4A**). In addition, efficient editing of *Hao1* *in vivo* leads to >8-fold induction
15 of glycolate (**FIG. 4B**) and ablation of GO enzymatic activity in liver (**FIG. 4C**). Further, editing the *Hao1* gene in mice leads to a ~40% reduction of urinary oxalate levels, correlating with a reduction of urinary oxalate to therapeutic range (**FIG. 4D**).

20 **EXAMPLE 4 - LONG-TERM DURABILITY OF HAO1 GENE EDITING, GLYCOLATE INDUCTION AND URINARY OXALATE LEVELS IN AGXT^{-/-} MICE**

This example explores the durability of *Hao1* indel formation and the resulting pharmacodynamic effects of the composition comprising the Cas12i2 B mRNA (SEQ ID NO: 23) and a mouse-specific *Hao1* gRNA loaded into lipid nanoparticles utilized at a single dose
25 of 1.5 mg/kg in C57BL/6 alanine glyoxylate aminotransferase knockout (*Agxt*^{-/-}) mice.

Alanine glyoxylate aminotransferase is a hepatic peroxisomal enzyme that catalyzes transamination between L-alanine and glyoxylate to produce pyruvate and glycine and is important for glyoxylate detoxification. The deficiency of this enzyme causes primary hyperoxaluria type I. Pey et al., 2013. Biomed Res Int. 687658. *Agxt*^{-/-} mice exhibit complete
30 loss of expression of both *Agxt* mRNA and protein resulting in hyperoxaluria (3- to 4-fold elevation in urinary oxalate) relative to control littermates (Salido et al., Proc. Natl. Acad. Sci. U.S.A., (2006) 103(48):18249-18254), which enables detection of reductions in urinary oxalate elicited by hepatic *Hao1* editing.

Due to species specificity of the gRNA in the test composition, mouse *in vivo* studies
35 used a murine surrogate, which consists of the same LNP A and Cas12i2 B mRNA but utilizes

a different gRNA that targets a 20-nucleotide sequence of the mouse *Hao1* gene that target exon 1 of *Hao1*.

Study Design and Methods

5 Male *Agxt*^{-/-} mice were housed following the same conditions disclosed in **Example 3** above. Baseline urines and serum were collected on days 15-18. To assess the durability of hepatic *Hao1* editing and oxalate reduction, the test composition at 1.5 mg/kg or saline was administered intravenously to *Agxt*^{-/-} mice and *Hao1* editing and urinary oxalate were measured 3 or 9 months later. Male *Agxt*^{-/-} mice aged 14 to 17 weeks old (at the time of dosing) were
10 treated with a single bolus dose via retro-orbital injection of the test composition and monitored over 12 months.

Hao1 gene editing and urinary oxalate levels were analyzed following the methods disclosed herein. See, e.g., **Example 3** above.

Results

A. Whole Liver Indels

Significant hepatic *Hao1* editing as measured by NGS was observed 3 months after dosing with the test composition at the 1.5 mg/kg dose in *Agxt*^{-/-} mice and this level of editing persisted in the animals assessed at the 6-month time point (**FIG. 5A**).

B. Urinary Oxalate

Urinary oxalate in the saline treated *Agxt*^{-/-} mice did not change from baseline at 3 or 6 months (**FIG. 5B**). Urinary oxalate in the group treated with the test composition at 1.5 mg/kg dose was reduced at 3 months, and no further drop in the urinary oxalate levels was observed from 3 to 6 months following administration (**FIG. 5B**). The reduction in urinary oxalate was
25 statistically significant at both timepoints compared to the saline control.

EXAMPLE 5 – DISEASE PREVENTION IN JUVENILE SUBJECTS

This example assesses the durability of hepatic *Hao1* editing and prevention of urinary oxalate buildup as the juvenile animals progress into adulthood.

30

Study Design and Methods

To assess the durability of hepatic *Hao1* editing and reduction of urinary oxalate in juvenile *Agxt*^{-/-} mice as they age into adulthood, 2.0 mg/kg of the test composition was administered intravenously (IV) to 3-week-old juvenile *Agxt*^{-/-} mice and the resulting

pharmacodynamic (PD) effects were assessed. *Hao1* editing was assessed 1, 5, and 9 weeks postdosing, while urinary oxalate was measured only at the 9-week timepoint in both the test composition and saline treated mice. Only 9-week post dosing (12-week old) urinary oxalate was measured because a suitable amount of urine cannot be collected at baseline from the juvenile, 3-week old animals prior to dosing and disease onset prior to 12-weeks is not fully characterized.

For determination of *Hao1* gene editing by insertion/ deletion analysis, genomic DNA was extracted from primary tissue, a library was prepared by 2 rounds of polymerase chain reaction (PCR) after which the samples were pooled, column purified, and then sequenced by Illumina. See **Examples** above. To detect low levels of editing above background, a windowed analysis using only 20 nucleotides centered around the Cas12i2 cut site. In the windowed analysis, indels identified through NGS were removed if they were 1 nt in length or if they did not at least partially overlap with a 20-nt window centered on the predicted nontarget strand cut site. These filtering steps increased the sensitivity of the analysis by lowering background signal from regions outside of the cut site or signal likely to be caused by sequencing error. Urinary Oxalate levels were measured by intact cell mass spectrometry (ICMS).

Results

A. Whole Liver Indels

A high level of *Hao1* editing was observed in the *Agxt*^{-/-} mice administered with the test composition at 2.0 mg/kg dose after 1 week, and this level of editing was durably maintained through 5- and 9-week following the administration (**FIG. 6A**). There was no statistical difference in *Hao1* editing observed across the 1-, 5-, and 9-week dosed groups (**FIG. 6A**).

B. Urinary Oxalate

3 months after administration, a statistically significant 55% reduction in urinary oxalate was observed in the group dosed with 2.0 mg/kg of the test composition compared to the saline-treated *Agxt*^{-/-} mice (**FIG. 4B**).

Together, these studies demonstrate durability of hepatic *Hao1* editing and reduction in urinary oxalates in *Agxt*^{-/-} mice upon administration of 2.0 mg/kg mice. The results of this study indicate that the treatment of the test composition to juvenile *Agxt*^{-/-} mice, a mouse model that is representative of PH1 disease biology, results in persistent hepatic *Hao1* editing and prevention of urinary oxalate buildup as the animals progress into adulthood.

EXAMPLE 6 – DURABILITY OF HAO1 EDITING AFTER PARTIAL HEPATECTOMY IN MICE

This example explores durability of *Hao1* editing mediated by the test composition comprising the Cas12i2 B mRNA and the mouse-specific *Hao1* gRNA disclosed above loaded into lipid nanoparticles utilized at a single dose of 2.0 mg/kg in male C57BL/6 WT mice after deliberate injury to the liver.

Study Design and Methods

Male C57BL/6 mice aged 6 to 8 weeks old were treated with a single bolus dose via retro-orbital injection of the test composition at the 2 mg/kg dose and underwent a 30 to 70% hepatectomy or a sham surgery 10 days later. Liver biopsies were collected during the surgery and 28 days later at the study endpoint. GO protein expression were assessed in both liver biopsies to detect the durability of the test composition-mediated indels and GO protein knockdown following a deliberate liver resection. Serum albumin, a protein synthesized by the hepatocytes, was measured before surgery and at the end of the study as a marker for liver size.

GO enzyme activity were evaluated in liver samples collected at necropsy using an HPLC-based method. This assay which measures glyoxylate levels after the addition of sodium glycolate substrate to liver tissue homogenates. 25-50 mg of tissue was sonicated in a lysis buffer containing HEPES and 10% Triton-X. Liver lysate solution is incubated with sodium glycolate master mix (100mM Tris HCL, 5mM Na glycolate) for 15 minutes at 37 °C. After incubation, 5M PCA is added, samples are centrifuged for 5 minutes, and the supernatants are run on HPLC to detect glyoxylate levels. *Hao1*-edited samples are expected to have lower glyoxylate levels as GO (the enzyme encoded by *Hao1* gene) is responsible for the conversion of glycolate to glyoxylate.

Results

GO protein expression in the liver was significantly decreased by approximately 88% in both the resected liver biopsy collected during hepatectomy and the endpoint livers collected 28 days after surgery compared to the negative control animals (**FIG. 7**). There was no difference in GO protein expression between the resected and endpoint livers of animals that underwent the hepatectomy.

This study shows the persistence of hepatic *Hao1* editing and GO protein knockdown elicited by the HAO1 gene editing composition following a 30 to 70% hepatectomy in wide-type mice. Significant gene editing was detected in livers collected during surgery and 28 days after surgery at levels greater than 50%. Both biopsies showed approximately 88% GO protein

expression compared to the negative control mice. Moreover, similar levels of serum albumin were detected at both the pre-surgery and endpoint timepoints in the hepatectomy and sham surgery groups.

In conclusion, the data indicate that the treatment in WT mice with the HAO1 gene editing composition results in significant *Hao1* indels and GO protein knockdown that persist 28-days following a deliberate liver resection.

EXAMPLE 7 – HAO1 GERMLINE TRANSMISSION ASSESSMENT OF PROGENY OF FEMALE MICE TREATED WITH HAO1 EDITING COMPOSITION

This example explores potential of germline transmission of the test composition comprising the Cas12i2 B mRNA and the mouse-specific *Hao1* gRNA disclosed above loaded into lipid nanoparticles and determine *Hao1* indel frequencies in the offspring of the dosed female C57BL/6 WT mice.

Study Design and Methods

Female C57BL/6 mice (n= 38) were administered a single IV dose of 5 mg/kg of the test composition comprising the mouse-*Hao1* gRNA surrogate. This dose is 5 times the saturating dose for on-target *Hao1* editing in the livers of mice. Dosed females were paired with untreated males 2 weeks after dosing (2 females per male). Tail biopsies were collected from each pup and genotyped to evaluate editing of *Hao1*. Pharmacologic activity of the test composition was confirmed in dams, with mean *Hao1* editing in dam livers of 56.3%. A total of 583 pups were analyzed across 36 dosed dams (2-3 litters/dam) for the presence of an inherited *Hao1* indel (expected to be 50% indel frequency, as this level indicates that the animal is heterozygous for *Hao1* knockdown).

Hao1 gene editing and urinary oxalate levels were analyzed following the methods disclosed herein. See, e.g., **Example 3** above. To detect low levels of editing above background, a windowed analysis using only 20 nucleotides centered around the Cas12i2 cut site. In the windowed analysis, indels identified through NGS were removed if they were 1 nt in length or if they did not at least partially overlap with a 20-nt window centered on the predicted nontarget strand cut site. These filtering steps increased the sensitivity of the analysis by lowering background signal from regions outside of the cut site or signal likely to be caused by sequencing error.

Results

Of the 583 offspring, 0 were positive for germline transmission from the mothers, and all animals showed editing < 1%. A summary of germline transmission from the test composition dosed mothers to progeny is provided in **Table 4**. This study demonstrates that while *Hao1* indels were present in the livers of the mothers at the levels of approximately 56.31% on average, germline transmission of the *Hao1* indel present in the livers of the dosed female mice is unlikely to occur in their progeny. Based on the power analysis conducted, this number of offspring assessed provides over 99% power to detect an *Hao1* germline transmission frequency of 1.0%, and over 80% power to detect a germline transmission frequency of 0.3%.

Table 4. Summary of germline transmission from the test composition-edited mothers to progeny

Test Condition	Test Composition
Liver indels of mothers	56.31%
Total offspring analyzed	583
Total offspring with inherited <i>Hao1</i> indel	0

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or

configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are

5 presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or

10 methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

15 All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

20 The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, *i.e.*, elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, *i.e.*, “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements

25 specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A

30 and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, *i.e.*, the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional

unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (*i.e.*, “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within an acceptable standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to $\pm 20\%$, preferably up to $\pm 10\%$, more preferably up to $\pm 5\%$, and more preferably still up to $\pm 1\%$ of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated, the term “about” is implicit and in this context means within an acceptable error range for the particular value. In some embodiments, the hinge domain is a hinge domain of a naturally occurring protein.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

WHAT IS CLAIMED IS:

1. A composition for genetic editing of a hydroxyacid oxidase 1 (HAO1) gene, comprising:

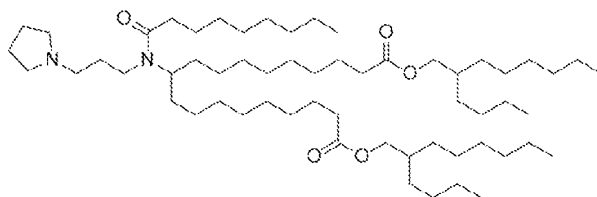
(a) a messenger RNA (mRNA) comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 8, wherein the mRNA encodes a Cas12i2 polypeptide set forth as SEQ ID NO: 7;

(b) a guide RNA (gRNA) comprising the nucleotide sequence of SEQ ID NO: 13; and

(c) lipid excipients comprising 45-50 mol% of a cationic lipid, 9-11 mol% of a zwitterionic phospholipid, 38-42 mol% of cholesterol, and 2.2-2.8 mol% of a pegylated lipid;

wherein:

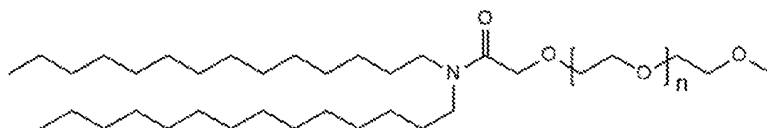
the cationic lipid has the structure of



, or is a pharmaceutically

acceptable salt or stereoisomer thereof.

the pegylated lipid has the structure of



, in which the

average n is 45-49, inclusive; and

the zwitterionic phospholipid is 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC).

2. The composition of claim 1, wherein the mRNA comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 8.

3. The composition of claim 1, wherein the mRNA comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 8.

4. The composition of any one of claims 1-3, wherein the lipid excipients comprise 47.5 mol% of the cationic lipid, 10 mol% of the zwitterionic phospholipid, 40 mol% of cholesterol, and 2.5 mol% of the pegylated lipid.

5. The composition of any one of claims 1-4, wherein the mRNA comprises a 5' cap moiety and a 3' polyadenylation tail.

6. The composition of any one of claims 1-5, wherein the mRNA comprises one or more modified nucleotides, which comprise pseudouridine; optionally wherein the mRNA comprises pseudouridine residues at 75% or more of the uridine positions in the mRNA.

7. The composition of claim 6, wherein the mRNA comprises pseudouridine residues at all uridine positions in the mRNA.

8. The composition of any one of claims 1-7, wherein the mRNA comprises the nucleotide sequence of SEQ ID NO: 23.

9. The composition of claim 8, wherein the mRNA comprises a 5' cap moiety and pseudouridine residues at all uridine positions in the mRNA.

10. The composition of any one of claims 1-9, wherein the gRNA comprises one or more modifications.

11. The composition of claim 10, wherein the gRNA comprises phosphorothioate linkage and 2'-O-methylation.

12. The composition of claim 11, wherein the modifications are located at the 5' and/or 3' nucleotides.

13. The composition of claim 1, wherein the gRNA comprises the nucleotide sequence of SEQ ID NO: 14.

14. The composition of any one of claims 1-13, wherein the nitrogen:phosphate (N/P) ratio in the composition ranges from 3 to 9.
15. The composition of claim 14, wherein the N/P ratio in the composition is 6.0 ± 1.0 .
16. The composition of claim 15, wherein the N/P ratio of the composition is 6.0 ± 0.5 .
17. The composition of claim 15, wherein the N/P ratio of the composition is about 6.0.
18. The composition of any one of claims 1-17, wherein the mRNA:gRNA ratio is $1.0 \pm 20\%$.
19. The composition of claim 18, wherein the mRNA:gRNA ratio is about 1.0.
20. A method for genetic editing a hydroxyacid oxidase 1 (HAO1) gene in a subject, the method comprising: administering to a subject in need thereof an effective amount of a composition for genetic editing the HAO1 gene set forth in any one of claims 1-19.
21. The method of claim 20, wherein the subject is a human subject having primary hyperoxaluria (PH).
22. The method of claim 21, wherein the human subject has PH1.