

modified to modulate cholesterol levels. Other concerns involve off-target effects, genome instability, or oncogenic modifications that may be caused by genome editing, as well as a lack of safe delivery modalities for gene-editing systems. Thus, there remains a need for improved compositions and methods to regulate PCSK9. Provided herein are compositions and methods for targeting PCSK9, as well as delivery vectors, to the address this need.

SUMMARY

[0007] The present disclosure relates to modified Class 2, Type V CRISPR proteins and guide nucleic acids used in the editing of proprotein convertase subtilisin/kexin Type 9 (PCSK9) gene target nucleic acid sequences having one or more mutations. The Class 2, Type V CRISPR proteins and guide nucleic acids can be modified for passive entry into target cells. The Class 2, Type V CRISPR proteins and guide nucleic acids are useful in a variety of methods for target nucleic acid modification, which methods are also provided.

[0008] In one example, the present disclosure relates to Class 2 Type V CRISPR protein and guide nucleic acid systems (*e.g.* CasX:gNA system) and methods used to alter a target nucleic acid comprising the gene encoding the PCSK9 protein (PCSK9 gene) in cells. In some examples of the disclosure, the CasX:gNA system has utility in knocking-down or knocking-out a PCSK9 gene with one or more mutations, which may be a gain of function mutation, in order to reduce or eliminate expression of the mutant PCSK9 gene product and resulting elevated hypercholesterolemia in subjects having a PCSK9 disorder. In other examples, the CasX:gNA system has utility in correcting the sequence of a PCSK9 gene with a gain of function mutation.

[0008a] In one aspect, the present disclosure refers to a system comprising a CasX protein and a guide ribonucleic acid (gRNA), wherein the gRNA comprises a targeting sequence complementary to a proprotein convertase subtilisin/kexin Type 9 (PCSK9) gene target nucleic acid sequence, wherein the PCSK9 gene comprises one or more mutations.

[0009] In some examples, the Class 2 Type V:gNA system gNA is a gRNA, or a gDNA, or a chimera of RNA and DNA, and may be a single-molecule gNA or a dual-molecule gNA. In other examples, the system gNA has a targeting sequence comprising a sequence having at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%, or 100% sequence identity to a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861. In some examples, the gNA has a targeting sequence consisting of a sequence selected from the group consisting of SEQ ID NOS: 247-303, 315-436, 612-2100, and 2286-13861. In some examples, the targeting sequence of the gNA is complementary to a sequence within or proximal to an exon of the PCSK9 gene. In another example, the targeting sequence of the gNA is complementary to a sequence within

or proximal to an intron of the PCSK9 gene. In another example, the targeting sequence of the gNA is complementary to a sequence within or proximal to an intron-exon junction of the PCSK9 gene. In another example, the targeting sequence of the gNA is complementary to a sequence within or proximal to a regulatory element of the PCSK9 gene. In another example, the targeting sequence of the gNA is complementary to a sequence within or proximal to an intergenic region of the PCSK9 gene. The gNA can comprise a targeting sequence comprising 14 to 30 consecutive nucleotides. In other examples, the targeting sequence of the gNA consists of 20 nucleotides. In other examples, the targeting sequence consists of 19 nucleotides. In other examples, the targeting sequence consists of 18 nucleotides. In other examples, the targeting sequence consists of 17 nucleotides. In other examples, the targeting sequence consists of 16 nucleotides. In other examples, the targeting sequence consists of 15 nucleotides.

[0010] In some examples, the gNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 4-16 as set forth in Table 1, or a sequence having at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity thereto. In other examples, the CasX:gNA system gNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2101-2286 as set forth in Table 2, or a sequence having at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity thereto.

[0011] In some examples, the CasX:gNA systems comprise a reference CasX sequence comprising any one of SEQ ID NOS: 1-3 or a CasX variant comprising a sequence of SEQ ID NOS: 49-160, 439, 441, 443, 445, 447-460, 472, 474, 476, 478, 480, 482, 484, 486, or 488, or 490 as set forth in Tables 3, 5-7 and 9, or a sequence having at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity thereto. In these examples, a CasX variant exhibits one or more improved characteristics relative to the reference CasX protein. In some examples, the CasX protein has binding affinity for a protospacer adjacent motif (PAM) sequence selected from the group consisting of TTC, ATC, GTC, and CTC. In some examples, the CasX protein has binding

affinity for the PAM sequence that is at least 1.5-fold greater compared to the binding affinity of any one of the CasX proteins of SEQ ID NOS: 1-3 for the PAM sequences selected from the group consisting of TTC, ATC, GTC, and CTC.

[0012] In some examples of the Class 2 Type V CRISPR:gNA system of the disclosure, the CRISPR molecule and the gNA molecule are associated together in a ribonuclear protein complex (RNP). In a particular example, the RNP comprising a CasX variant and the gNA variant exhibits greater editing efficiency and/or binding of a target sequence in the target DNA when any one of the PAM sequences TTC, ATC, GTC, or CTC is located 1 nucleotide 5' to the non-target strand sequence having identity with the targeting sequence of the gNA in a cellular assay system compared to the editing efficiency and/or binding of an RNP comprising a reference CasX protein and a reference gNA in a comparable assay system.

[0013] In some examples, the system further comprises a donor template comprising a nucleic acid comprising at least a portion of a gene encoding a PCSK9 protein or RNA sequence, a PCSK9 regulatory region, or both the encoding and the regulatory regions, and wherein the PCSK9 encoding gene portion is selected from the group consisting of a PCSK9 exon, a PCSK9 intron, and a PCSK9 intron-exon junction, wherein the donor template is used to knock down or knock out the PCSK9 gene or is used to correct the mutation in the PCSK9 gene. In some examples, the system further comprises a donor template comprising a nucleic acid comprising a sequence encoding at least a portion of SEQ ID NO: 33. In other examples, the system further comprises a donor template comprising a nucleic acid comprising a nucleic acid sequence having one or more mutations relative to the wild-type PCSK9 gene sequence of SEQ ID NO: 33. In some cases the donor sequence is a single-stranded DNA template or a single stranded RNA template. In other cases, the donor template is a double-stranded DNA template.

[0013a] In one aspect, the present disclosure refers to a nucleic acid comprising a sequence that encodes the CasX protein disclosed herein.

[0013b] In one aspect, the present disclosure refers to a nucleic acid comprising a sequence that encodes the gRNA disclosed herein.

[0014] In other examples, the disclosure provides nucleic acids encoding the systems of any of the examples described herein, as well as vectors comprising the nucleic acids. In some examples, the vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a plasmid, a minicircle, a nanoplasmid, and an RNA vector. In other examples, the vector is a virus-like particle (VLP) comprising an RNP of a CasX and gNA of any of the examples described herein and, optionally, a donor template nucleic acid.

[0014a] In one aspect, the present disclosure refers to a vector comprising: a. the gRNA disclosed herein, b. the CasX protein disclosed herein, c. the nucleic acid disclosed herein; d. the nucleic acid disclosed herein; or e. a combination of two or more of (a)-(d).

[0015] In other examples, the disclosure provides a method of modifying a PCSK9 target nucleic acid sequence of a cell wherein the PCSK9 gene comprises one or more mutations, wherein said method comprises introducing into the cell: a) a composition comprising the Class 2 Type V:gNA system of any of the examples disclosed herein comprising a first gNA; b) the nucleic acid of any of the examples disclosed herein; c) the vector of any of the examples disclosed herein; d) the VLP of any of the examples disclosed herein; or e) a combination of two or more of the foregoing wherein the PCSK9 target nucleic acid sequence of the cells targeted by the first gNA is modified by the Class 2 Type V CRISPR protein (*e.g.* CasX). In some examples of the method, the method comprises introducing into the cells of the population a second gNA or a nucleic acid encoding the second gNA, wherein the second gNA has a targeting sequence complementary to a different or overlapping portion of the PCSK9 target nucleic acid compared to the first gNA, resulting in an additional break in the PCSK9 target nucleic acid of the cells of the population. In some examples of the method, the modifying comprises introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the target nucleic acid sequence as compared to the wild-type sequence. In some cases, the method further comprises contacting the target nucleic acid with a donor template nucleic acid of any of the examples disclosed herein. In some examples of the method, the donor template comprises a nucleic acid comprising at least a portion of a PCSK9 gene for correcting (by knocking in) the mutation of the PCSK9 gene, or comprises a sequence comprising a mutation or heterologous sequence for knocking out the mutant PCSK9. In those cases where the modification results in a knock-down of the PCSK9 gene, expression of the non-functional PCSK9 protein is reduced by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% in comparison to cells that have not been modified. In other cases, wherein the modification results in a knock-out of the PCSK9 gene, the target nucleic acid of the cells of the population is modified such that at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% of the cells do not express a detectable level of non-functional PCSK9 protein. Expression of PCSK9 protein can be measured by flow cytometry, ELISA, cell-based assays, Western blot or other methods known in the art or as described in the Examples.

[0015a] In one aspect, the present disclosure refers to a method of modifying a PCSK9 target nucleic acid sequence in a population of cells, wherein the PCSK9 target nucleic acid comprises one or more mutations, the method comprising introducing into cells of the population: a. the system disclosed herein; b. the nucleic acid disclosed herein; c. the vector disclosed herein; or d. combinations of two or more of (a)-(c), wherein the PCSK9 target nucleic acid sequence of the cells targeted by the first gNA is modified by the Class 2 Type V protein.

[0016] In some cases, the modifying of the target nucleic acid sequence in a cell occurs *in vivo*. In some examples, the cell is a eukaryotic cell selected from the group consisting of a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell. In some examples, the cell is a hepatocyte, or a cell of the intestine, the kidney, the central nervous system, a smooth muscle cell, a macrophage, a retinal cell, or a cell of arterial walls such as the endothelium. In some examples, the cell is an eye cell. In other examples, the disclosure provides methods of modifying a target nucleic acid sequence wherein the target cells are contacted using vectors encoding the CasX protein and one or more gNAs, and optionally further comprising a donor template. In some cases, the vector is an Adeno-Associated Viral (AAV) vector selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV44.9, AAV-Rh74, or AAVRh10. In other cases, the vector is a lentiviral vector. In other examples, the disclosure provides methods wherein the target cells are contacted using a vector wherein the vector is a virus-like particle comprising an RNP of a CasX and gNA of any of the examples described herein and, optionally, a donor template nucleic acid. In some examples of the method, the vector is administered to a subject at a therapeutically effective dose. The subject can be a mouse, rat, pig, non-human primate, or a human. The dose can be administered by a route of administration selected from the group consisting of intravenous, intraportal vein injection, intraperitoneal, intramuscular, subcutaneous, intraocular, and oral routes.

[0017] In other examples, the disclosure provides a method of treating a PCSK9 or related disorder in a subject in need thereof, comprising modifying a gene encoding PCSK9 gene in a cell of the subject, the modifying comprising contacting said cells with a therapeutically effective dose of: i) a composition comprising a CasX and gNA of any of the examples disclosed herein comprising a first gNA, and, optionally, a donor template; ii) a nucleic acid encoding the composition of (i); a vector selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a plasmid, a minicircle, a nanoplasmid, a DNA vector, and an RNA vector, and comprising a nucleic acid of (ii); iii) a VLP comprising the composition of (i); or iv) combinations of two or more of (i)-(iii), wherein the PCSK9 gene of the cells targeted by the first gNA is modified by the CasX protein (and, optionally, the donor template) such that the mutation of the PCSK9 gene is corrected or compensated for and a functional PCSK9 protein

is expressed. In other examples of the foregoing method of treating a PCSK9-related disease in a subject, the PCSK9 gene is knocked-down or knocked-out such that the expression of non-functional PCSK9 protein is reduced or eliminated. In some examples, the subject is selected from the group consisting of a rodent, a mouse, a rat, a non-human primate, and a human. In the foregoing, the vector or VLP is administered to the subject by a route of administration selected from intravenous, intraportal vein injection, intraperitoneal, intramuscular, subcutaneous, intraocular, or oral routes. In some examples, the PCSK9-related disorder is selected from the group consisting of autosomal dominant hypercholesterolemia (ADH), hypercholesterolemia, elevated total cholesterol levels, hyperlipidemia, elevated low-density lipoprotein (LDL) levels, elevated LDL-cholesterol levels, reduced high-density lipoprotein levels, liver steatosis, coronary heart disease, ischemia, stroke, peripheral vascular disease, thrombosis, type 2 diabetes, high elevated blood pressure, atherosclerosis, obesity, Alzheimer's disease, neurodegeneration, age-related macular degeneration (AMD) or a combination thereof.

[0017a] In one aspect, the present disclosure refers to use of: a. the system disclosed herein; b. the nucleic acid disclosed herein; c. the vector disclosed herein; or d. combinations of two or more of (a)-(c), in the manufacture of a medicament for modifying a PCSK9 gene in cells of a subject having a PCSK9-related disease.

[0018] In some cases, the method results in improvement in at least one clinically-relevant endpoint selected from the group consisting of percent change from baseline in LDL-cholesterol, decrease in plaque atheroma volume, reduction in in coronary plaque, reduction in atherosclerotic cardiovascular disease (ASCVD), cardiovascular death, nonfatal myocardial infarction, ischemic stroke, nonfatal stroke, coronary revascularization, visual acuity, and unstable angina. In other cases, the method results in improvement in at least two clinically-relevant endpoints.

[0019] In another example, the present disclosure provides pharmaceutical compositions and kits comprising the nucleic acids, vectors, Class 2, Type V CRISPR proteins, gNAs and gene editing pairs described herein.

[0019a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising: a. the system disclosed herein; b. the nucleic acid disclosed herein; or c. the vector disclosed herein, and one or more pharmaceutically suitable excipients.

[0019b] In one aspect, the present disclosure refers to a kit comprising: a. the system disclosed herein; b. the nucleic acid disclosed herein; c. the vector disclosed herein; d. the pharmaceutical composition disclosed herein; or e. combinations of two or more of (a)-(e), and a suitable container.

[0020] In another example, provided herein are compositions comprising gene editing pairs, or compositions of vectors comprising or encoding gene editing pairs for use as a medicament for the treatment of a subject having a PCSK9-related disease.

[0021] In another example, provided herein are Class 2, Type V CRISPR:gNA systems, compositions comprising Class 2, Type V CRISPR:gNA systems, vectors comprising or encoding Class 2, Type V CRISPR:gNA systems, VLP comprising Class 2, Type V

CRISPR:gNA systems, or populations of cells edited using the Class 2, Type V CRISPR:gNA systems for use as a medicament for the treatment of a PCSK9-related disease.

[0022] In another example, provided herein are Class 2, Type V CRISPR:gNA systems, composition comprising Class 2, Type V CRISPR:gNA systems, or vectors comprising or encoding Class 2, Type V CRISPR:gNA systems, VLP comprising Class 2, Type V CRISPR:gNA systems, populations of cells edited using the Class 2, Type V CRISPR:gNA systems, for use in a method of treatment of a PCSK9-related disease in a subject in need thereof..

[0023] In another example, provided herein are Class 2, Type V CRISPR:gNA systems, composition comprising Class 2, Type V CRISPR:gNA systems, or vectors comprising or encoding Class 2, Type V CRISPR:gNA systems, VLP comprising Class 2, Type V CRISPR:gNA systems, populations of cells edited using the Class 2, Type V CRISPR:gNA systems, for use in the manufacture of a medicament for the treatment of a PCSK9-related disease in a subject in need thereof.

INCORPORATION BY REFERENCE

[0024] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. The contents of PCT/US2020/036505, filed on June 5, 2020, and the contents of U.S. Provisional Patent Application No. 63/121,196, filed on December 3, 2020, both which disclose CasX variants and gNA variants, are hereby incorporated by reference in their entireties.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0026] FIG. 1 shows an SDS-PAGE gel of StX2 purification fractions visualized by colloidal Coomassie staining, as described in Example 1.

CLAIMS

What is claimed is:

1. A system comprising a CasX protein and a guide ribonucleic acid (gRNA), wherein the gRNA comprises a targeting sequence complementary to a proprotein convertase subtilisin/kexin Type 9 (PCSK9) gene target nucleic acid sequence, wherein the PCSK9 gene comprises one or more mutations.
2. The system of claim 1, wherein the CasX protein is a chimeric CasX protein comprising protein domains from two or more different CasX proteins.
3. The system of claim 1 or claim 2, wherein the CasX protein comprises a non-target strand binding (NTSB) domain comprising the amino acids of positions 101-191 of SEQ ID NO: 1, or a sequence with at least 70% sequence identity thereto, and a RuvC DNA cleavage domain comprising amino acids of positions 648-812 and 922-978 of SEQ ID NO: 2, or sequences with at least 70% sequence identity thereto.
4. The system of any one of claims 1-3, wherein the CasX protein comprises at least one chimeric domain.
5. The system of claim 1, wherein the CasX protein comprises a RuvC cleavage domain, wherein the RuvC cleavage domain comprises the sequence of amino acids 648-812 of SEQ ID NO: 2 with one or more amino acid modifications relative to said RuvC cleavage domain sequence, and wherein the CasX protein exhibits one or more improved characteristics compared to SEQ ID NO: 2 selected from the group consisting of improved editing of target DNA, improved target DNA cleavage rate, increased formation of cleavage-competent ribonucleoprotein (RNP) complexes, improved protospacer adjacent motif (PAM) utilization, and improved solubility.
6. The system of claim 1, wherein the CasX protein comprises a sequence selected from the group consisting of SEQ ID NOS: 138-160, or a sequence having at least about 70%, at least about 80%, at least about 90%, or at least about 95%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity thereto.
7. The system of claim 6, wherein the CasX protein comprises a sequence of SEQ ID NO: 138, or a sequence having at least about 90% thereto.

8. The system of any one of claims 1-7, wherein the CasX protein further comprises one or more nuclear localization signals (NLS), wherein the one or more NLS are located at or near the N-terminus and/or at or near the C-terminus of the CasX protein.
9. The system of any one of claims 1-8, wherein the gRNA has a scaffold comprising a stem loop sequence of CCAGCGACUAUGUCGUAGUGG (SEQ ID NO: 32), or a sequence of SEQ ID NO: 32 with at least 1, 2, 3, 4, or 5 mismatches thereto.
10. The system of any one of claims 1-9, wherein the gRNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2201-2285, or a sequence having at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity thereto.
11. The system of any one of claims 1-10, wherein the gRNA has a scaffold comprising a sequence of SEQ ID NO: 2238, or a sequence having at least about 65%, at least about 75%, at least about 85%, or at least about 95% identity thereto.
12. The system of any one of claims 1-11, wherein the gRNA is chemically modified.
13. The system of any one of claims 1-12, wherein the PCSK9 gene comprises one or more mutations in a region selected from the group consisting of:
- a PCSK9 intron;
 - a PCSK9 exon;
 - a PCSK9 intron-exon junction;
 - a PCSK9 regulatory element; and
 - an intergenic region,
- wherein the mutation is an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides as compared to the wild-type PCSK9 gene sequence.
14. The system of any one of claims 1-13, wherein the mutation is a gain of function mutation.
15. The system of any one of claims 1-14, wherein the mutation is a loss of function mutation.
16. The system of any one of claims 1-15, wherein the targeting sequence of the gRNA is complementary to a sequence of:
- a PCSK9 exon;
 - a PCSK9 exon 1 or exon 2;
 - a PCSK9 intron;

- d.** a PCSK9 intron-exon junction;
 - e.** a PCSK9 regulatory element;
 - f.** one or more single nucleotide polymorphisms (SNPs) of the PCSK9 gene; or
 - g.** an intergenic region of the PCSK9 gene.
- 17.** The system of any one of claims 1-16, wherein the targeting sequence of the gRNA comprises a sequence selected from the group consisting of the sequences of SEQ ID NOS: 247-303, 315-436, 612-2100, and 2286-13861, or a sequence having at least about 65%, at least about 75%, at least about 85%, or at least about 95% identity thereto.
- 18.** The system of any one of claims 1-17, wherein the CasX protein forms a ribonuclear protein complex (RNP) with the gRNA.
- 19.** The system of claim 18, wherein the RNP is capable of binding and modifying the target nucleic acid, wherein the modifying comprises a knock-down or knock-out of expression of the PCSK9 protein.
- 20.** The system of any one of claims 1-7, wherein the CasX protein is a variant of a reference CasX protein of SEQ ID NOS: 1, 2, or 3, and/or the gRNA comprises at least one modification relative to a reference gRNA comprising a sequence of any one of SEQ ID NOS: 4-16.
- 21.** The system of claim 20, wherein an RNP comprising the CasX variant protein and the gRNA comprising at least one modification exhibits one or more improved characteristics as compared to an RNP comprising a reference CasX protein of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 and a gRNA comprising a sequence of any one of SEQ ID NOS: 4-16.
- 22.** The system of claim 21, wherein the improved characteristic is selected from one or more of the group consisting of improved folding of the CasX protein; improved binding affinity of the CasX protein to a gRNA; improved binding affinity to a target DNA; improved ability to utilize a greater spectrum of one or more PAM sequences, including ATC, CTC, GTC, or TTC, in the editing of target DNA; improved unwinding of the target DNA; increased editing activity; improved editing efficiency; improved editing specificity; increased nuclease activity; improved target nucleic acid sequence cleavage rate; increased target strand loading for double strand cleavage; decreased off-target cleavage; improved binding of non-target DNA strand; improved protein stability; improved protein solubility; improved RNP formation; higher percentage of cleavage-competent RNP; improved RNP stability; and improved RNP solubility.
- 23.** The system of claim 22, wherein the improved characteristic of the RNP is at least about 1.1 to about 100-fold or more improved relative to the RNP of the reference CasX protein of

SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 and the gRNA comprising a sequence of any one of SEQ ID NOS: 4-16.

- 24.** The system of any one of claims 1-23, wherein the CasX protein is a catalytically inactive CasX (dCasX) protein, and wherein an RNP of the dCasX and the gRNA retain the ability to bind to the PCSK9 target nucleic acid.
- 25.** The system of claim 24, wherein the dCasX comprises a mutation at residues:

 - a.** D672, E769, and/or D935 corresponding to the CasX protein of SEQ ID NO:1; or
 - b.** D659, E756 and/or D922 corresponding to the CasX protein of SEQ ID NO: 2.
- 26.** A nucleic acid comprising a sequence that encodes the CasX protein of any one of claims 1-25.
- 27.** A nucleic acid comprising a sequence that encodes the gRNA of any one of claims 1-25.
- 28.** A vector comprising:

 - a.** the gRNA of any one of claims 1-25,
 - b.** the CasX protein of any one of claims 1-25,
 - c.** the nucleic acid of claim 26;
 - d.** the nucleic acid of claim 27; or
 - e.** a combination of two or more of (a)-(d).
- 29.** The vector of claim 28, wherein the vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a virus-like particle (VLP), a plasmid, a minicircle, a nanoplasmid, a DNA vector, and an RNA vector.
- 30.** A pharmaceutical composition comprising:

 - a.** the system of any one of claims 1-25;
 - b.** the nucleic acid of claim 26 or claim 27; or
 - c.** the vector of claim 28 or claim 29,

and one or more pharmaceutically suitable excipients.
- 31.** A method of modifying a PCSK9 target nucleic acid sequence in a population of cells, wherein the PCSK9 target nucleic acid comprises one or more mutations, the method comprising introducing into cells of the population:

 - a.** the system of any one of claims 1-25;
 - b.** the nucleic acid of claim 26 or claim 27;
 - c.** the vector of claim 28 or claim 29; or

d. combinations of two or more of (a)-(c),

wherein the PCSK9 target nucleic acid sequence of the cells targeted by the first gNA is modified by the Class 2 Type V protein.

32. Use of:

a. the system of any one of claims 1-25;

b. the nucleic acid of claim 26 or claim 27;

c. the vector of claim 28 or claim 29; or

d. combinations of two or more of (a)-(c),

in the manufacture of a medicament for modifying a PCSK9 gene in cells of a subject having a PCSK9-related disease.

33. The use of claim 31, wherein the modifying results in a knocking down or knocking out the PCSK9 gene in the modified cells of the subject such that expression of non-functional PCSK9 protein in the subject is decreased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% in comparison to a subject where the PCSK9 gene has not been modified.

34. The use of claim 31 or claim 33, wherein the subject is selected from the group consisting of a rodent, a mouse, a rat, a non-human primate, and a human.

35. The use of any one of claims 29-31, wherein the PCSK9-related disease is autosomal dominant hypercholesterolemia (ADH), hypercholesterolemia, elevated total cholesterol levels, hyperlipidemia, elevated low-density lipoprotein (LDL) levels, elevated LDL-cholesterol levels, reduced high-density lipoprotein levels, liver steatosis, coronary heart disease, ischemia, stroke, peripheral vascular disease, thrombosis, type 2 diabetes, high elevated blood pressure, atherosclerosis, obesity, Alzheimer's disease, neurodegeneration, age-related macular degeneration (AMD), or a combination thereof.

36. The use of any one of claims 31-35, wherein the modification results in improvement in at least one clinically-relevant endpoint selected from the group consisting of change from baseline in LDL-cholesterol, decrease in plaque atheroma volume, reduction in in coronary plaque, reduction in atherosclerotic cardiovascular disease (ASCVD), cardiovascular death, nonfatal myocardial infarction, ischemic stroke, nonfatal stroke, coronary revascularization, unstable angina, or visual acuity.

- 37.** A kit comprising:
- a.** the system of any one of claims 1-25;
 - b.** the nucleic acid of claim 26 or claim 27;
 - c.** the vector of claim 28 or claim 29;
 - d.** the pharmaceutical composition of claim 30; or
 - e.** combinations of two or more of (a)-(e),
- and a suitable container.