

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 [aspectexample](#), 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 [embodimentexamples](#) 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), the targeting sequence of the gNA is complementary to a sequence within or proximal to an exon of the PCSK9 gene. In another [embodimentexample](#), the targeting sequence of the gNA is complementary to a sequence within

or proximal to an intron of the PCSK9 gene. In another [embodimentexample](#), 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 [embodimentexample](#), the targeting sequence of the gNA is complementary to a sequence within or proximal to a regulatory element of the PCSK9 gene. In another [embodimentexample](#), 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 [embodimentexamples](#), the targeting sequence of the gNA consists of 20 nucleotides. In other [embodimentexamples](#), the targeting sequence consists of 19 nucleotides. In other [embodimentexamples](#), the targeting sequence consists of 18 nucleotides. In other [embodimentexamples](#), the targeting sequence consists of 17 nucleotides. In other [embodimentexamples](#), the targeting sequence consists of 16 nucleotides. In other [embodimentexamples](#), the targeting sequence consists of 15 nucleotides.

[0010] In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), a CasX variant exhibits one or more improved characteristics relative to the reference CasX protein. In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#) 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 [embodimentexample](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), the disclosure provides nucleic acids encoding the systems of any of the [embodimentexamples](#) described herein, as well as vectors comprising the nucleic acids. In some [embodimentexamples](#), 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 [embodimentexamples](#), the vector is a virus-like particle (VLP) comprising an RNP of a CasX and gNA of any of the [embodimentexamples](#) 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 embodimentexamples, 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 embodimentexamples disclosed herein comprising a first gNA; b) the nucleic acid of any of the embodimentexamples disclosed herein; c) the vector of any of the embodimentexamples disclosed herein; d) the VLP of any of the embodimentexamples 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 embodimentexamples 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 embodimentexamples 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 embodimentexamples disclosed herein. In some embodimentexamples 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 **embodimentexamples**, 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 **embodimentexamples**, 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 **embodimentexamples**, the cell is an eye cell. In other **embodimentexamples**, 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 **embodimentexamples**, 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 **embodimentexamples** described herein and, optionally, a donor template nucleic acid. In some **embodimentexamples** 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 **embodimentexamples**, 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 **embodimentexamples** 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 [embodimentexamples](#) 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [aspectexample](#), 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 [aspectexample](#), 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 [aspectexample](#), 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 [aspect 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 [aspect 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 Class 2 Type V CRISPRCasX protein and a ~~first~~ 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.

2.13. The system of any one of claims 1-12~~claim 1~~, wherein the PCSK9 gene comprises one or more mutations in a region selected from the group consisting of:

- a. a PCSK9 intron;
- b. a PCSK9 exon;
- c. a PCSK9 intron-exon junction;
- d. a PCSK9 regulatory element; and
- e. an intergenic region.

~~3. The system of any one of claim 1 or claim 2,~~ 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.

4.14. The system of any one of claims 1-13, wherein the mutation is a gain of function mutation.

~~5. The system of claim 3, wherein the one or more mutations comprise amino acid substitutions selected from the group consisting of S127R, D129G, F216L, D374H, and D374Y relative to the sequence of SEQ ID NO: 33.~~

~~6. The system of claims 1-5, wherein the targeting sequence of the gNA is complementary to a target nucleic acid sequence encoding the S127R, D129G, F216L, D374H, or D374Y substitution.~~

~~7. The system of claim 6, wherein the targeting sequence of the gNA comprises a sequence of selected from the group consisting of AGCAGGUGCGCCUCUCAUCUU (SEQ ID NO: 272), CAUCUUCACCAGGAAGCCAG (SEQ ID NO: 273), CCUCUCAUCUUCACCAGGAA (SEQ ID NO: 274), UGGUGAAGAUGAGAGGCGAC (SEQ ID NO: 275), GUGGAGGCGGGUCCCGUCCU (SEQ ID NO: 281), AGCCACUGCAGCACCUGCUU (SEQ ID NO: 287), UUGGUGCCUCCAGCCACUGC (SEQ ID NO: 288), AGCUACUGCAGCACCUGCUU (SEQ ID NO: 289), and UUGGUGCCUCCAGCUACUGC (SEQ ID NO: 290).~~

8.15. The system of any one of claims 1-~~3~~14, wherein the mutation is a loss of function mutation.

~~9. The system of claim 8, wherein the one or more mutations comprise amino acid substitutions selected from the group consisting of R46L, G106R, Y142X, N157K, R237W and C679X relative to the sequence of SEQ ID NO: 33.~~

10.16. The system of any one of claims 1-15~~claims 9~~, wherein the targeting sequence of the gRNA is complementary to a target nucleic acid sequence encoding the R46L, G106R, Y142X, N157K, R237W or C679X substitution sequence of:

- a. a PCSK9 exon;
- b. a PCSK9 exon 1 or exon 2;
- c. 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.

~~11. The system of any one of claims 1-10, wherein the PCSK9 gene encodes a non-functional PCSK9 protein.~~

~~12. The system of any one of claims 1-11, wherein the gNA is a guide RNA (gRNA).~~

~~13. The system of any one of claims 1-11, wherein the gNA is a guide DNA (gDNA).~~

~~14. The system of any one of claims 1-11, wherein the gNA is a chimera comprising DNA and RNA.~~

~~15. The system of any one of claims 1-14, wherein the gNA is a single-molecule gNA (sgNA).~~

16. The system of any one of claims 1-14, wherein the gNA is a dual-molecule gNA (dgNA).

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.

~~18.—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.~~

~~19.—The system of any one of claims 1-16, wherein the targeting sequence of the gRNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with a single nucleotide removed from the 3' end of the sequence.~~

~~20.—The system of any one of claims 1-16, wherein the targeting sequence of the gRNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with two nucleotides removed from the 3' end of the sequence.~~

~~21.—The system of any one of claims 1-16, wherein the targeting sequence of the gRNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with three nucleotides removed from the 3' end of the sequence.~~

~~22.—The system of any one of claims 1-16, wherein the targeting sequence of the gRNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with four nucleotides removed from the 3' end of the sequence.~~

~~23.—The system of any one of claims 1-16, wherein the targeting sequence of the gRNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with five nucleotides removed from the 3' end of the sequence.~~

~~24.—The system of any one of claims 17-23, wherein the targeting sequence of the gRNA comprises a sequence having one or more single nucleotide polymorphisms (SNP) relative to a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861.~~

~~25.—The system of any one of claims 1-24, wherein the targeting sequence of the gRNA is complementary to a sequence of a PCSK9 exon.~~

- ~~26. — The system of any one of claims 1-24, wherein the targeting sequence of the gNA is complementary to a sequence of PCSK9 exon 1 or exon 2.~~
- ~~27. — The system of any one of claims 1-24, wherein the targeting sequence of the gNA is complementary to a sequence of a PCSK9 intron.~~
- ~~28. — The system of any one of claims 1-24, wherein the targeting sequence of the gNA is complementary to a sequence of a PCSK9 intron-exon junction.~~
- ~~29. — The system of any one of claims 1-24, wherein the targeting sequence of the gNA is complementary to a sequence of a PCSK9 regulatory element.~~
- ~~30. — The system of any one of claims 1-23, wherein the targeting sequence of the gNA is complementary to a sequence comprising one or more single nucleotide polymorphisms (SNPs) of the PCSK9 gene.~~
- ~~31. — The system of any one of claims 1-24, wherein the targeting sequence of the gNA is complementary to a sequence of an intergenic region of the PCSK9 gene.~~
- ~~32. — The system of any one of claims 1-31, further comprising a 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 targeting sequence of the first gNA.~~
- ~~33. — The system of claim 32, wherein the second gNA has a targeting sequence complementary to the same exon targeted by the first gNA.~~
- ~~34. — The system of claim 32, wherein the second gNA has a targeting sequence complementary to a different exon targeted by the first gNA.~~
- ~~35. — The system of claim 32, wherein the second gNA has a targeting sequence complementary to an intron 3' to the exon targeted by the first gNA.~~
- ~~36. — The system of any one of claims 32-35, wherein the targeting sequence of the second gNA 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.~~
- ~~37. — The system of any one of claims 32-35, wherein the targeting sequence of the second gNA comprises a sequence selected from the group consisting of the sequences of SEQ ID NOS: 247-303, 315-436, 612-2100, and 2286-13861.~~
- ~~38. — The system of any one of claims 32-35, wherein the targeting sequence of the second gNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with a single nucleotide removed from the 3' end of the sequence.~~

- ~~39. The system of any one of claims 32-35, wherein the targeting sequence of the second gNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with two nucleotides removed from the 3' end of the sequence.~~
- ~~40. The system of any one of claims 32-35, wherein the targeting sequence of the second gNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with three nucleotides removed from the 3' end of the sequence.~~
- ~~41. The system of any one of claims 32-35, wherein the targeting sequence of the second gNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with four nucleotides removed from the 3' end of the sequence.~~
- ~~42. The system of any one of claims 32-35, wherein the targeting sequence of the second gNA comprises a sequence of SEQ ID NOS: 247-303, 315-436, 612-2100, or 2286-13861 with five nucleotides removed from the 3' end of the sequence.~~
- ~~43. The system of any one of claims 1-42, wherein the first and/or second gNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2201-2285, 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.~~
- ~~44. The system of any one of claims 1-42, wherein the first and/or second gNA has a scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 2201-2285.~~
- ~~45. The system of any one of claims 1-42, wherein the first and/or second gNA has a scaffold consisting of a sequence selected from the group consisting of SEQ ID NOS: 2201-2285.~~
- 46.20. The system of any one of claims 1-~~745~~, 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 first and/or second gNA scaffold comprises a sequence having at least one modification relative to a reference gRNA comprising a sequence of any one selected from the group consisting of SEQ ID NOS: 4-16.
- ~~47. The system of claim 46, wherein the at least one modification of the reference gNA comprises at least one substitution, deletion, or substitution of a nucleotide of the reference gNA sequence.~~
- ~~48. The system of any one of claims 1-47, wherein the first and/or second gNA is chemically modified.~~

- ~~49. — The system of any one of claims 1-48, wherein the Class 2 Type V CRISPR protein is a reference CasX protein having a sequence of any one of SEQ ID NOS: 1-3, a CasX variant protein having a sequence of SEQ ID NOS: 49-160, 329, 441, 443, 445, 447-460, 472, 474, 476, 478, 480, 482, 484, 486, 488, or 490, or a sequence having at least about 50%, at least about 60%, 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.~~
- ~~50. — The system of any one of claims 1-49, wherein the Class 2 Type V CRISPR protein is a CasX variant protein having a sequence of SEQ ID NOS: 49-160, 329, 441, 443, 445, 447-460, 472, 474, 476, 478, 480, 482, 484, 486, 488, or 490.~~
- ~~51. — The system of 49, wherein the CasX variant protein comprises at least one modification relative to a reference CasX protein having a sequence selected from SEQ ID NOS: 1-3.~~
- ~~52. — The system of claim 51, wherein the at least one modification comprises at least one amino acid substitution, deletion, or substitution in a domain of the CasX variant protein relative to the reference CasX protein.~~
- ~~53. — The system of claim 52, wherein the domain is selected from the group consisting of a non-target strand binding (NTSB) domain, a target strand loading (TSL) domain, a helical I domain, a helical II domain, an oligonucleotide binding domain (OBD), and a RuvC DNA cleavage domain.~~
- ~~54. — The system of any one of claims 49-53, wherein the CasX protein further comprises one or more nuclear localization signals (NLS).~~
- ~~55. — The system of claim 54, wherein the one or more NLS are selected from the group of sequences consisting of SEQ ID NOS: 161-194, 217 and 223-224.~~
- ~~56. — The system of claim 54 or claim 55, wherein the one or more NLS are expressed at or near the C-terminus of the CasX protein.~~
- ~~57. — The system of claim 54 or claim 55, wherein the one or more NLS are expressed at or near the N-terminus of the CasX protein.~~
- ~~58. — The system of claim 54 or claim 55, comprising one or more NLS located at or near the N-terminus and at or near the C-terminus of the CasX protein.~~
- ~~59. — The system of any one of claims 49-58, wherein the Class 2 Type V CRISPR protein is capable of forming a ribonuclear protein complex (RNP) with the gNA.~~

~~60. The system of any one of claims 49-58, wherein the CasX variant is capable of forming a ribonuclear protein complex (RNP) with the gNA.~~

~~61. The system of any one of claims 49-58, wherein the CasX variant and the gNA are complexed as an RNP.~~

~~62.21.~~ The system of claim ~~2061~~, wherein an RNP comprising the CasX variant protein and the gRNA comprising at least one modification exhibits ~~at least~~ one or more improved characteristics as compared to an RNP comprising ~~the 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.

~~63.22.~~ The system of claim ~~2162~~, wherein the improved characteristic is selected from one or more of the group consisting of improved folding of the CasX ~~variantprotein~~; improved binding affinity of the CasX protein to a ~~guide-nucleic acid (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 target strand loading for single strand nicking~~; decreased off-target cleavage; improved binding of non-target DNA strand; improved protein stability; improved protein solubility; improved ~~ribonuclear protein complex (RNP)~~ formation; higher percentage of cleavage-competent RNP; improved ~~protein:gNA complex (RNP)~~ stability; ~~improved protein:gNA complex solubility~~; ~~improved protein yield~~; ~~improved protein expression~~; and improved RNP solubility~~fusion characteristics~~.

~~64.23.~~ The system of claim ~~62 or claim 63~~~~22~~, wherein the improved characteristic of the RNP ~~of the CasX variant protein and the gNA variant~~ 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.

~~65. The system of claim 62 or claim 63, wherein the improved characteristic of the CasX variant protein is at least about 1.1, at least about 2, at least about 10, at least about 100 fold or more improved relative to the reference CasX protein of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 and the gNA comprising a sequence of any one of SEQ ID NOS: 4-16.~~

~~66. The system of claim 64 or claim 65, wherein the improved characteristic is improved binding affinity to the target nucleic acid sequence.~~

- ~~67. — The system of any one of claims 61–66, wherein the RNP comprising the CasX variant and the gNA variant exhibits greater editing efficiency and/or binding of a target sequence in the target nucleic acid 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.~~
- ~~68. — The system of claim 67, wherein the PAM sequence is TTC.~~
- ~~69. — The system of claim 68, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOS: 3184–7251.~~
- ~~70. — The system of claim 67, wherein the PAM sequence is ATC.~~
- ~~71. — The system of claim 70, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOS: 315–436, 612–2100 and 2286–3183.~~
- ~~72. — The system of claim 67, wherein the PAM sequence is CTC.~~
- ~~73. — The system of claim 72, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOS: 7252–11521.~~
- ~~74. — The system of claim 67, wherein the PAM sequence is GTC.~~
- ~~75. — The system of claim 74, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOS: 11522–13861.~~
- ~~76. — The system of any one of claims 61–75, wherein the RNP has at least a 5%, at least a 10%, at least a 15%, or at least a 20% higher percentage of cleavage-competent RNP compared to an RNP of the reference CasX proteins of SEQ ID NOS: 1–3 and the gNA of SEQ ID NOS: 4–16.~~
- ~~77. — The system of any one of claims 60–75, wherein the RNP has at least a 5-fold, at least a 10-fold, or at least a 30-fold increased cleavage rate in an *in vitro* assay compared to an RNP of the reference CasX proteins of SEQ ID NOS: 1–3.~~
- ~~78. — The system of any one of claims 49–77, wherein the CasX variant protein comprises a RuvC DNA cleavage domain having nickase activity.~~
- ~~79. — The system of any one of claims 49–77, wherein the CasX variant protein comprises a RuvC DNA cleavage domain having double-stranded cleavage activity.~~

80.24. The system of any one of claims ~~491-7523~~, 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.

81.25. The system of claim ~~2480~~, 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.

~~82.—The system of claim 81, wherein the mutation is a substitution of alanine for the residue.~~

~~83.—The system of any one of claims 1-79, further comprising a donor template nucleic acid.~~

~~84.—The system of claim 83, wherein the donor template comprises a nucleic acid comprising at least a portion of a PCSK9 gene selected from the group consisting of a PCSK9 exon, a PCSK9 intron, a PCSK9 intron-exon junction, and a PCSK9 regulatory element, or a combination thereof.~~

~~85.—The system of claim 84, wherein the donor template comprises a wild-type nucleic acid sequence.~~

~~86.—The system of claim 84, wherein the donor template comprises a nucleic acid sequence having one or more mutations relative to the wild-type PCSK9 gene sequence.~~

~~87.—The system of any one of claims 83-86, wherein the donor template ranges in size from 10-15,000 nucleotides.~~

~~88.—The system of any one of claims 83-87, wherein the donor template is a single-stranded DNA template or a single-stranded RNA template.~~

~~89.—The system of any one of claims 83-87, wherein the donor template is a double-stranded DNA template.~~

~~90.—The system of any one of claims 83-89, wherein the donor template comprises homologous arms at or near the 5' and 3' ends of the donor template that are complementary to sequences flanking cleavage sites in the PCSK9 target nucleic acid introduced by the Class 2 Type V CRISPR protein.~~

~~91.—The system of any one of claims 1-90, wherein the target nucleic acid sequence is complementary to a non-target strand sequence located 1 nucleotide 3' of a protospacer adjacent motif (PAM) sequence.~~

~~92.—The system of claim 91, wherein the PAM sequence comprises a TC motif.~~

~~93.—The system of claim 91, wherein the PAM sequence comprises ATC, GTC, CTC or TTC.~~

- ~~94.~~—The system of any one of claims 91–93, wherein the Class 2 Type V CRISPR protein comprises a RuvC domain.
- ~~95.~~—The system of claim 94, wherein the RuvC domain generates a staggered double-stranded break in the target nucleic acid sequence.
- ~~96.~~—The system of any one of claims 91–95, wherein the Class 2 Type V CRISPR protein does not comprise an HNH nuclease domain.
- ~~97.~~ A nucleic acid comprising the donor template of any one of claims 83–90.
- ~~98.26.~~ A nucleic acid comprising a sequence that encodes the CasX protein of any one of claims ~~149–2582~~.
- ~~99.~~ The nucleic acid of claim 98, wherein the sequence that encodes the CasX protein is codon-optimized for expression in a eukaryotic cell.
- ~~100.27.~~ A nucleic acid comprising a sequence that encodes the gRNA of any one of claims 1–~~2548~~.
- ~~101.28.~~ 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) the gNA of any one of claims 1–48, the CasX protein of any one of claims 49–82, or the nucleic acid of any one of claims 97–100.
- ~~102.~~ The vector of claim 101, wherein the vector further comprises a promoter.
- ~~103.29.~~ The vector of claim ~~101~~ or claim ~~102~~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.
- ~~104.~~—The vector of claim 103, wherein the vector is an AAV vector.
- ~~105.~~—The vector of claim 104, wherein the AAV vector is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV44.9, AAV-Rh74, or AAVRh10.
- ~~106.~~—The vector of claim 105, wherein the AAV vector is selected from AAV1, AAV2, AAV5, AAV8, or AAV9.
- ~~107.~~—The vector of claim 103, wherein the vector is a retroviral vector.

~~108.~~—The vector of claim 103, wherein the vector is a VLP vector comprising one or more components of a gag polyprotein.

~~109.~~—The vector of claim 108, wherein the one or more components of the Gag polyprotein are selected from the group consisting of matrix protein (MA), nucleocapsid protein (NC), capsid protein (CA), p1-p6 protein, a PP21/24 peptide, a P12/P3/P8 peptide, a p2 peptide, a P10 peptide, a p68 Gag polypeptide, a p3 Gag polypeptide, and a protease cleavage site.

~~110.~~—The vector of claim 108 or claim 109, comprising the CasX protein and the gNA.

~~111.~~—The vector of claim 110, wherein the CasX protein and the gNA are associated together in an RNP.

~~112.~~—The vector of any one of claims 108-111, further comprising the donor template.

~~113.~~—The vector of any one of claims 108-112, further comprising a pseudotyping viral envelope glycoprotein or antibody fragment that provides for binding and fusion of the VLP to a target cell.

~~114.~~—A host cell comprising the vector of any one of claims 101-113.

~~115.~~ The host cell of claim 114, wherein the host cell is selected from the group consisting of BHK, HEK293, HEK293T, NS0, SP2/0, YO myeloma cells, P3X63 mouse myeloma cells, PER, PER.C6, NIH3T3, COS, HeLa, CHO, and yeast cells.

~~116.~~30.A pharmaceutical composition comprising:

- a. the system of any one of claims 1-~~25~~96;
- b. the nucleic acid of ~~any one of claims 97-100~~claim 26 or claim 27; or
- c. the vector of claim 28 or claim 29~~any one of claims 101-113~~,

and one or more pharmaceutically suitable excipients.

~~117.~~—The pharmaceutical composition of claim 116, wherein the pharmaceutical composition is formulated for a route of administration selected from the group consisting of intravenous, intraportal vein injection, intraperitoneal, intramuscular, subcutaneous, intraocular, and oral routes.

~~118.~~—The pharmaceutical composition of claim 116, wherein the pharmaceutical composition is in a liquid form or a frozen form.

~~119.~~ The pharmaceutical composition of any one of claims 116-118, wherein the pharmaceutical composition is in a pre-filled syringe for a single injection.

120.31. ~~_____~~ 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~~96;
- b.** the nucleic acid of ~~any one of claims 97-100~~claim 26 or claim 27;
- c.** the vector of ~~claim 28 or claim 29~~any one of claims 101-113; or
- d.** ~~the pharmaceutical composition of any one of claims 116-119; or~~
- e.d.** combinations of two or more of (a)-(cd),

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

~~121.—The method of claim 120, wherein the modifying comprises introducing a single-stranded break in the PCSK9 target nucleic acid sequence of the cells of the population.~~

~~122.—The method of claim 120, wherein the modifying comprises introducing a double-stranded break in the PCSK9 target nucleic acid sequence of the cells of the population.~~

~~123. The method of any one of claims 120-122, further comprising 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.~~

~~124.—The method of any one of claims 120-123, wherein the modifying comprises introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the PCSK9 target nucleic acid of the cells of the population.~~

~~125. The method of any one of claims 120-124, wherein the PCSK9 target nucleic acid of at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, or at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60% or more of the cells of the population is modified.~~

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.

126.33. ~~_____~~ The use of claim 31 ~~method of any one of claims 120-124~~, wherein the modifying results in a knocking down or knocking out ~~of~~ the PCSK9 gene in the modified cells of the population-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 cell-subject where the PCSK9 gene has not been modified.

127. ~~— The method of any one of claims 120-126, wherein the PCSK9 gene 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 modified cells do not express a detectable level of non-functional PCSK9 protein.~~

128. ~~— The method of any one of claims 120-124, wherein the modifying results in a correction or compensation of the mutation of the PCSK9 gene in the cells of the population such that functional PCSK9 protein is expressed by the cells.~~

129. ~~— The method of any one of claims 120-124 and 128, wherein expression of the functional PCSK9 protein by the cells of the population is increased 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 cell where the PCSK9 gene has not been modified.~~

130. ~~— The method of any one of claims 120-123, wherein the method comprises insertion of a sequence of the donor template into the break site(s) of the PCSK9 gene target nucleic acid sequence of the cells of the population.~~

131. ~~— The method of claim 130, wherein the insertion of the sequence of the donor template is mediated by homology-directed repair (HDR) or homology-independent targeted integration (HITI).~~

132. ~~— The method of claim 130 or claim 131, wherein insertion of the sequence of the donor template results in a correction or compensation of the PCSK9 gene in the cells of the population such that functional PCSK9 protein is expressed by the cells.~~

133. ~~— The method of any one of claims 130-132, wherein expression of the functional PCSK9 protein by the cells of the population is increased 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 cell where the PCSK9 gene has not been modified.

134.—The method of any one of claims 130–132, wherein the PCSK9 gene of the cells of the population is modified such that at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the modified cells express a detectable level of functional PCSK9.

135.—The method of claim 130 or claim 131, wherein insertion of the sequence of the donor template results in a knocking down or knocking out the PCSK9 gene in the cells of the population such that expression of a non-functional PCSK9 protein 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 cell where the PCSK9 gene has not been modified.

136.—The method of claim 130 or claim 131, wherein the PCSK9 gene 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 modified cells do not express a detectable level of non-functional PCSK9 protein.

137. The method of any one of claims 120–136, wherein the cells are eukaryotic.

138.34. The method-use of claim 13731 or claim 33, wherein the subject iseukaryotic cells are selected from the group consisting of a rodent-cells, a mouse-cells, a rat-cells, anda non-human primate-cells, and a human.

139.—The method of claim 137, wherein the eukaryotic cells are human cells.

140.—The method of claim 137–139, wherein the eukaryotic cells are selected from the group consisting of a hepatocyte, a cell of the intestine, a cell of the kidney, a cell of the central nervous system, a smooth muscle cell, a macrophage, a cell of the retina, and an arterial endothelial cell.

141.—The method of any one of claim 120–140, wherein the modifying of the PCSK9 gene target nucleic acid sequence of the population of cells occurs *in vitro* or *ex vivo*.

142.—The method of claims 120–140, wherein the modifying of the PCSK9 gene target nucleic acid sequence of the population of cells occurs *in vivo* in a subject.

143.—The method of claim 142, wherein the subject is selected from the group consisting of a rodent, a mouse, a rat, and a non-human primate.

144.—The method of claim 142, wherein the subject is a human.

145.—The method of any one of claims 142–144, wherein the method comprises administering a therapeutically effective dose of an AAV vector to the subject.

146.—The method of claim 145, wherein the AAV vector is administered to the subject at a dose of at least about 1×10^5 vector genomes/kg (vg/kg body weight), at least about 1×10^6 vg/kg, at least about 1×10^7 vg/kg, at least about 1×10^8 vg/kg, at least about 1×10^9 vg/kg, at least about 1×10^{10} vg/kg, at least about 1×10^{11} vg/kg, at least about 1×10^{12} vg/kg, at least about 1×10^{13} vg/kg, at least about 1×10^{14} vg/kg, at least about 1×10^{15} vg/kg, or at least about 1×10^{16} vg/kg.

147.—The method of claim 145, wherein the AAV vector is administered to the subject at a dose of at least about 1×10^5 vg/kg to about 1×10^{16} vg/kg, at least about 1×10^6 vg/kg to about 1×10^{15} vg/kg, at least about 1×10^7 vg/kg to about 1×10^{14} vg/kg, at least about 1×10^8 vg/kg to about 1×10^{13} vg/kg, at least about 1×10^9 vg/kg to about 1×10^{12} vg/kg, or at least about 1×10^{10} vg/kg to about 1×10^{11} vg/kg.

148.—The method of any one of claims 142–144, wherein the method comprises administering a therapeutically effective dose of a VLP to the subject.

149.—The method of claim 148, wherein the VLP is administered to the subject at a dose of at least about 1×10^5 particles/kg body weight (particles/kg), at least about 1×10^6 particles/kg, at least about 1×10^7 particles/kg, at least about 1×10^8 particles/kg, at least about 1×10^9 particles/kg, at least about 1×10^{10} particles/kg, at least about 1×10^{11} particles/kg, at least about 1×10^{12} particles/kg, at least about 1×10^{13} particles/kg, at least about 1×10^{14} particles/kg, at least about 1×10^{15} particles/kg, at least about 1×10^{16} particles/kg.

150.—The method of claim 148, wherein the VLP is administered to the subject at a dose of at least about 1×10^5 particles/kg to about 1×10^{16} particles/kg, at least about 1×10^6 particles/kg to about 1×10^{15} particles/kg, at least about 1×10^7 particles/kg to about 1×10^{14} particles/kg, at least about 1×10^8 particles/kg to about 1×10^{13} particles/kg, at least about 1×10^9 particles/kg to about 1×10^{12} particles/kg, at least about 1×10^{10} particles/kg to about 1×10^{11} particles/kg.

151.—The method of any one of claims 142–150, wherein the vector or VLP is administered to the subject by a route of administration selected from the group consisting of intravenous,

~~intraportal vein injection, intraperitoneal, intramuscular, subcutaneous, intraocular, and oral routes.~~

~~152.—The method of any one of claims 142–151, further comprising contacting the PCSK9 target nucleic acid sequence of the population of cells with:~~

- ~~a.—an additional CRISPR nuclease and a gNA targeting a different or overlapping portion of the PCSK9 target nucleic acid compared to the first gNA;~~
- ~~b.—one or more polynucleotides encoding the additional CRISPR nuclease and the gNA of (a);~~
- ~~c.—a vector comprising the polynucleotide(s) of (b); or~~
- ~~d.—a VLP comprising the additional CRISPR nuclease and the gNA of (a);~~

~~wherein the contacting results in modification of the PCSK9 gene at a different location in the sequence compared to the sequence targeted by the first gNA.~~

~~153.—The method of claim 152, wherein the additional CRISPR nuclease is a CasX protein having a sequence different from the CasX protein of any of the preceding claims.~~

~~154.—The method of claim 152, wherein the additional CRISPR nuclease is not a CasX protein.~~

~~155.—The method of claim 154, wherein the additional CRISPR nuclease is selected from the group consisting of Cas9, Cas12a, Cas12b, Cas12c, Cas12d (CasY), Cas12J, Cas13a, Cas13b, Cas13c, Cas13d, CasX, CasY, CasZ, Cas14, Cpf1, C2c1, Csn2, Cas Phi, and sequence variants thereof.~~

~~156.—A population of cells modified by the method of any one of claims 142–155, wherein the cells have been modified such that at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the modified cells do not express a detectable level of non-functional PCSK9 protein.~~

~~157.—A population of cells modified by the method of any one of claims 142–156, wherein the mutation of the PCSK9 target nucleic acid is corrected or compensated for in the modified cells of the population, resulting in expression of a functional PCSK9 protein by the modified cells.~~

~~158.—The population of cells of claim 157, wherein the cells have been modified such that expression of a functional PCSK9 protein is increased 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 cell where the PCSK9 gene has not been modified.~~

~~159.—The population of cells of any one of claim 156-158, wherein the cells are selected from the group consisting of a hepatocyte, a cell of the intestine, a cell of the kidney, a cell of the central nervous system, a smooth muscle cell, a macrophage, a retinal cell, and an arterial endothelial cell.~~

~~160. A method of treating a PCSK9-related disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the cells of any one of claims 156-159.~~

~~161.35. The method-use of any one of claims 29-31~~~~claim 160~~, 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.

~~162.—The method of claim 160 or claim 161, wherein the subject is selected from the group consisting of a rodent, a mouse, a rat, and a non-human primate.~~

~~163.—The method of any one of claims 160-162, wherein the subject is a human.~~

~~164.—The method of any one of claims 160-163, wherein the cells are autologous with respect to the subject to be administered the cells.~~

~~165.—The method of any one of claims 160-163 wherein the cells are allogeneic with respect to the subject to be administered the cells.~~

~~166.—The method of any one of claims 160-165, wherein the cells are administered by a route of administration selected from the group consisting of intravenous, intraportal vein injection, intraperitoneal, intramuscular, subcutaneous, intraocular, and oral routes.~~

~~167.—A method of treating a PCSK9-related disease in a subject in need thereof, comprising modifying a PCSK9 gene having one or more mutations in cells of the subject, the modifying comprising contacting said cells with a therapeutically effective dose of:~~

- ~~a.—the system of any one of claims 1-96;~~
- ~~b.—the nucleic acid of any one of claims 97-100;~~
- ~~c.—the vector of any one of claims 101-107;~~
- ~~d.—the VLP of any one of claims 108-113;~~
- ~~e.—the pharmaceutical composition of any one of claims 116-119; or~~

f.—combinations of two or more of (a)–(e),
 wherein the PCSK9 gene of the cells targeted by the first gNA is modified by the CasX protein.

168.—The method of claim 167, wherein the modifying comprises introducing a single-stranded break in the PCSK9 gene of the cells.

169.—The method of claim 167, wherein the modifying comprises introducing a double-stranded break in the PCSK9 gene of the cells.

170.—The method of any one of claims 167–169, further comprising introducing into the cells of the subject 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 target nucleic acid compared to the first gNA, resulting in an additional break in the PCSK9 target nucleic acid of the cells of the subject.

171.—The method of any one of claims 167–169, wherein the modifying comprises introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the PCSK9 gene of the cells.

172.—The method of any one of claims 167–170, wherein the modifying comprises insertion of a sequence of the donor template into the break site(s) of the PCSK9 gene target nucleic acid sequence of the cells.

173.—The method of claim 172, wherein the insertion of the sequence of the donor template is mediated by homology directed repair (HDR) or homology independent targeted integration (HITI).

174.—The method of any one of claims 167–173, wherein the modifying results in a correction of or compensation for the mutation(s) in the PCSK9 gene in the modified cells of the subject.

175.—The method of claim 174, wherein correction of the mutation results in expression of functional PCSK9 protein by the modified cells of the subject.

176.—The method of claim 174 or claim 175, wherein the PCSK9 gene of the modified cells express increased levels of a functional PCSK9 protein, and wherein the increase is 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 cell with a PCSK9 gene that has not been modified.

177.—The method of any one of claims 167–173, wherein the modifying results in a knocking down or knocking out the PCSK9 gene in the modified cells of the subject 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 modified cells do not express a detectable level of non-functional PCSK9 protein.

~~178.—The method of any one of claims 167-173, 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.~~

~~179.—The method of any one of claims 167-178, wherein the subject is selected from the group consisting of rodent, mouse, rat, and non-human primate.~~

~~180.—The method of any one of claims 167-178, wherein the subject is a human.~~

~~181.—The method of any one of claims 167-180, wherein the cells that are modified are selected from the group consisting of a hepatocyte, a cell of the intestine, a cell of the kidney, a cell of the central nervous system, a smooth muscle cell, a macrophage, a cell of the retina, and an arterial endothelial cell.~~

~~182.—The method of any one of claims 167-181, 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.~~

~~183.—The method of any one of claims 167-182, wherein the vector is administered to the subject at a therapeutically effective dose.~~

~~184.—The method of any one of claims 167-183, wherein the vector is an AAV, and is administered to the subject at a dose of at least about 1×10^5 vector genomes (vg)/kg, at least about 1×10^6 vg/kg, at least about 1×10^7 vg/kg, at least about 1×10^8 vg/kg, at least about 1×10^9 vg/kg, at least about 1×10^{10} vg/kg, at least about 1×10^{11} vg/kg, at least about 1×10^{12} vg/kg, at least about 1×10^{13} vg/kg, at least about 1×10^{14} vg/kg, at least about 1×10^{15} vg/kg, or at least about 1×10^{16} vg/kg.~~

~~185.—The method of any one of claims 167-183, wherein the vector is an AAV, and is administered to the subject at a dose of at least about 1×10^5 vg/kg to about 1×10^{16} vg/kg, at~~

least about 1×10^6 vg/kg to about 1×10^{15} vg/kg, at least about 1×10^7 vg/kg to about 1×10^{14} vg/kg, at least about 1×10^8 vg/kg to about 1×10^{13} vg/kg, at least about 1×10^9 vg/kg to about 1×10^{12} vg/kg, or at least about 1×10^{10} vg/kg to about 1×10^{11} vg/kg.

186.—The method of any one of claims 167–182, wherein the VLP is administered to the subject at a therapeutically effective dose.

187.—The method of claim 186, wherein the VLP is administered to the subject at a dose of at least about 1×10^5 particles/kg, at least about 1×10^6 particles/kg, at least about 1×10^7 particles/kg, at least about 1×10^8 particles/kg, at least about 1×10^9 particles/kg, at least about 1×10^{10} particles/kg, at least about 1×10^{11} particles/kg, at least about 1×10^{12} particles/kg, at least about 1×10^{13} particles/kg, at least about 1×10^{14} particles/kg, at least about 1×10^{15} particles/kg, at least about 1×10^{16} particles/kg.

188.—The method of claim 186, wherein the VLP is administered to the subject at a dose of at least about 1×10^5 particles/kg to about 1×10^{16} particles/kg, at least about 1×10^6 particles/kg to about 1×10^{15} particles/kg, at least about 1×10^7 particles/kg to about 1×10^{14} particles/kg, at least about 1×10^8 particles/kg to about 1×10^{13} particles/kg, at least about 1×10^9 particles/kg to about 1×10^{12} particles/kg, at least about 1×10^{10} particles/kg to about 1×10^{11} particles/kg.

189.—The method of any one of claims 183–188, wherein the vector or VLP is administered by a route of administration selected from the group consisting of intravenous, intraportal vein injection, intraperitoneal, intramuscular, subcutaneous, intraocular, and oral routes.

190. The method of any one of claims 167–189, wherein the method 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.

191.36. The method-use of any one of claims 31–167–35–189, wherein the method modification results in improvement in at least two-one clinically-relevant endpoints 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.

~~192. The system of any one of claims 1-96; the nucleic acid of any one of claims 97-100; the vector of any one of claims 101-107; the VLP of any one of claims 108-113; the pharmaceutical composition of any one of claims 116-119; or combinations thereof, for use as a medicament for the treatment of a PCSK9-related disease.~~

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)-(c),
- and a suitable container.