CLAIMS

1. A chimericCasX variant, comprising a sequence with at least 90% sequence identity to SEQ ID NO:2, wherein:

a. the chimeric CasX variant comprises a substituition of a non-target strand binding (NTSB) domain of SEQ ID NO: 2 with the NTSB domain from SEQ ID NO: 1, or a sequence with at least 90% sequence identity thereto;

b. the chimeric CasX variant comprises a helical 1 domain comprising amino acids 59-102 of SEQ ID NO: 2 and amino acids 192-332 of SEQ ID NO: 1, or a sequence with at least 90% sequence identity to either of the foregoing;

c. the chimeric CasX variant comprises a helical II domain, an oligonucleotide binding domain (OBD)-I domain, an OBD-II, a target strand loading (TSL) domain, and a RuvC domain of SEQ ID NO: 2 comprising one or more amino acid modifications; and

d. the chimeric CasX variant is capable of forming a ribonucleoprotein complex (RNP) with a guide ribonucleic acid (gNA).

2. The chimeric CasX variant of claim 1, wherein the one or more amino acid modifications are selected from the group consisting of:

a. an amino acid substitution of L379R;

b. an amino acid substitution of F399L;

c. an amino acid substitution of I658V;

d. an amino acid substitution of A708K; and

e. a deletion of amino acid P793.

3. The chimeric CasX variant of claim 1 or claim 2, comprising SEQ ID NO: 333 or SEQ ID NO: 336, or a sequence having at least 90% sequence identity thereto.

4. The chimeric CasX variant of any one of claims 1-3, wherein the chimeric CasX variant has an improved characteristic relative to that of SEQ ID NO:2, wherein the improved characteristic is selected from the group consisting of: improved folding of the chimeric CasX variant; improved binding affinity to a gNA; 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; 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 protein:gNA complex (RNP) stability; improved protein:gNA

complex solubility; improved protein yield; improved protein expression; improved fusion characteristics or a combination thereof.

5. The chimeric CasX variant of claim 4, wherein:

a. one or more of the improved characteristics of the chimeric CasX variant is at least 1.1 to 100-fold or more improved relative to the reference CasX protein of SEQ ID NO: 2;

b. one or more of the improved characteristics of the chimeric CasX variant is at least 1.1, at least 2, at least 10, at least 100-fold or more improved relative to the reference CasX protein of SEQ ID NO: 2;

c. the improved characteristic comprises editing efficiency, and the chimeric CasX variant comprises a 1.1 to 100-fold improvement in editing efficiency compared to the reference CasX protein of SEQ ID NO: 2; and/or

d. the RNP comprising the chimeric CasX variant exhibits greater editing efficiency and/or binding of a target sequence in a target DNA when any one of a PAM sequence of TTC, ATC, GTC, or CTC is located 1 nucleotide 5' to a non-target strand of the protospacer having identity with a targeting sequence of a guide ribonucleic acid (gNA) in a cellular assay system compared to editing efficiency and/or binding of an RNP comprising a reference CasX protein in a comparable assay system.

6. The chimeric CasX variant of any one of claims 1-5, wherein the chimeric CasX variant protein is a catalytically inactive CasX (dCasX) protein, wherein

a. the dCasX protein comprises a mutation at residues:

i) D672, and/or E769, and/or D935 corresponding to the CasX protein of SEQ ID NO:1; or

ii) D659, and/or E756, and/or D922 corresponding to the CasX protein of SEQ ID NO: 2; and

iii) the mutation is a substitution of alanine for the residue, and

b. the dCasX and a guide nucleic acid (gNA) retain the ability to bind to a target DNA.

7. The chimeric CasX variant of any one of claims 1-6, further comprising one or more nuclear localization signals (NLS) wherein:

a. the one or more NLS are selected from the group of sequences consisting of PKKKRKV (SEQ ID NO: 352), KRPAATKKAGQAKKKK (SEQ ID NO: 353), PAAKRVKLD (SEQ ID NO: 354), RQRRNELKRSP (SEQ ID NO: 355), NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 356), RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 357), VSRKRPRP (SEQ ID NO: 358), PPKKARED (SEQ ID NO: 35), PQPKKKPL (SEQ ID NO:

360), SALIKKKKKMAP (SEQ ID NO: 361), DRLRR (SEQ ID NO: 362), PKQKKRK (SEQ ID NO: 363), RKLKKKIKKL (SEQ ID NO: 364), REKKKFLKRR (SEQ ID NO: 365), KRKGDEVDGVDEVAKKKSKK (SEQ ID NO: 366), RKCLQAGMNLEARKTKK (SEQ ID NO: 367), PRPRKIPR (SEQ ID NO: 368), PPRKKRTVV (SEQ ID NO: 369), NLSKKKKRKREK (SEQ ID NO: 370), RRPSRPFRKP (SEQ ID NO: 371), KRPRSPSS (SEQ ID NO: 372), KRGINDRNFWRGENERKTR (SEQ ID NO: 373), PRPPKMARYDN (SEQ ID NO: 374), KRSFSKAF (SEQ ID NO: 375), KLKIKRPVK (SEQ ID NO: 376), PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 377), PKTRRRPRRSQRKRPPT (SEQ ID NO: 378), SRRRKANPTKLSENAKKLAKEVEN (SEQ ID NO: 379), KTRRRPRRSQRKRPPT (SEQ ID NO: 380), RRKKRRPRRKKRR (SEQ ID NO: 381), PKKKSRKPKKKSRK (SEQ ID NO: 382), HKKKHPDASVNFSEFSK (SEQ ID NO: 383), QRPGPYDRPQRPGPYDRP (SEQ ID NO: 384), LSPSLSPLLSPSLSPL (SEQ ID NO: 385), RGKGGKGLGKGGAKRHRK (SEQ ID NO: 386), PKRGRGRPKRGRGR (SEQ ID NO: 387), and PKKKRKVPPPPKKKRKV (SEQ ID NO: 389); and

b. wherein the one or more NLS are positioned:

i) at or near a C-terminus of the chimeric CasX variant;

ii) at or near at an N-terminus of the chimeric CasX variant; or

iii) at least two NLS are positioned at or near the N-terminus and at or near the C-terminus of the chimeric CasX variant.

8. A gene editing pair comprising the chimeric CasX variant of any one of claims 1-7 and a first guide nucleic acid (gNA) comprising a scaffold and a linked targeting sequence, wherein:

a. the chimeric CasX variant and the first gNA are capable of associating together in a ribonucleoprotein complex (RNP); and/or

b. the chimeric CasX variant and the first gNA are associated together in a ribonucleoprotein complex (RNP).

9. The gene editing pair of claim 8, wherein the first gNA comprises a scaffold comprising, relative to SEQ ID NO:5, one or more sequence modifications selected from the group consisting of:

a. a C18G substitution;

b. a G55 insertion;

c. a U1 deletion;

d. a modification of an extended stem loop wherein

i) a 6 nt loop and 13 loop-proximal base pairs are replaced by a Uvsx hairpin; and

ii) a deletion of A99 and a substitution of G64U results in a loop-distal base that is fully base-paired.

10. The gene editing pair of claim 8 or claim 9, wherein the first gNA comprises a scaffold comprising a scaffold stem loop sequence of CCAGCGACUAUGUCGUAGUGG (SEQ ID NO: 245), or a sequence having 1, 2, 3, 4, or 5 mismatches thereto.

11. The gene editing pair of any one of claims 8-10, wherein the scaffold comprises the sequence of SEQ ID NO:2238, or a sequence having 90% sequence identity thereto.

12. The gene editing pair of any one of claims 8-11, wherein the linked targeting sequence:

a. is linked to a 3' end of the scaffold;

b. is complementary to a target nucleic acid sequence in a target strand of a gene; and

c. has 15, 16, 17, 18, 19, 20, 21, or 22 nucleotides.

13. The gene editing pair of any one of claims 8-12, wherein the first gNA is chemically modified.

14. The gene editing pair of any one of claims 8-13, wherein the gene editing pair of the chimeric CasX variant and the first gNA has one or more improved characteristics compared to a gene editing pair comprising a reference CasX protein of SEQ ID NO: 1 or SEQ ID NO: 2, and a reference guide ribonucleic acid of SEQ ID NO: 5, wherein the one or more improved characteristics comprises improved CasX:gNA (RNP) complex stability, improved binding affinity between the chimeric CasX variant and gNA, improved kinetics of RNP complex formation, higher percentage of cleavage-competent RNP, improved RNP binding affinity to a target DNA, ability to utilize an increased spectrum of PAM sequences, improved unwinding of the target DNA, increased editing activity, improved editing efficiency, improved editing specificity, increased nuclease activity, increased target strand loading for double strand cleavage, decreased target strand loading for single strand nicking, decreased off-target cleavage, improved binding of the non-target strand of DNA, or improved resistance to nuclease activity.

15. The gene editing pair of claim 14, wherein:

a. the one or more improved characteristics is at least 1.1 to 100-fold or more improved relative to a gene editing pair of the reference CasX protein and the reference guide nucleic acid;

b. the one or more improved characteristics is at least 1.1, at least 2, at least 10, or at least 100-fold or more improved relative to the gene editing pair of the reference CasX protein and the reference guide nucleic acid; or

c. the one or more improved characteristics comprise a 4- to 9-fold increase in editing activity compared to the gene editing pair of the reference CasX protein and the reference guide nucleic acid.

16. The gene editing pair of any one of claims 8-15 further comprising a second gene editing pair comprising:

a. the chimeric CasX variant of any one of claims 1-6; and

b. a second gNA, wherein the second gNA has a targeting sequence, complementary to a different or overlapping portion of a target DNA compared to a targeting sequence of the first gNA.

17. A method of editing a target DNA in vitro outside of a cell, in vitro inside of a cell or ex vivo, comprising contacting the target DNA with a gene editing pair of any one of claims 8-16, wherein the contacting results in editing or modification of the target DNA, comprising:

a. introducing a mutation, an insertion, or a deletion in the target DNA;

b. introducing a single-stranded break in the target DNA; or

c. introducing a double-stranded break in the target DNA.

18. The method of claim 17, comprising contacting the target DNA with a plurality of gNAs comprising targeting sequences complementary to different or overlapping regions of the target DNA, wherein the contacting results in multiple breaks in the target nucleic acid.

19. The method of claim 17 or claim 18, wherein the cell is a eukaryotic cell, preferably wherein the eukaryotic cell is selected from the group consisting of a plant cell, a fungal cell, a protist cell, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, and a non-human primate cell, preferably wherein the eukaryotic cell is a human cell, preferably wherein the cell is an embryonic stem cell, an induced pluripotent stem cell, a germ cell, a fibroblast, an oligodendrocyte, a glial cell, a hematopoietic stem cell, a neuron progenitor cell, a neuron, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell, a retinal cell, a cancer cell, a T-cell, a B-cell, an NK cell, a fetal cardiomyocyte, a myofibroblast, a mesenchymal stem cell, an autotransplated expanded cardiomyocyte, an adipocyte, a totipotent cell, a pluripotent cell, a blood stem cell, a myoblast, an adult stem cell, a bone marrow cell, a mesenchymal cell, a parenchymal cell, an epithelial cell, an endothelial cell, a mesothelial cell, fibroblasts, osteoblasts, chondrocytes, exogenous cell, endogenous cell, stem cell, hematopoietic stem cell, bone-marrow derived progenitor cell, myocardial cell, skeletal cell, fetal cell, undifferentiated cell, multi-potent progenitor cell, unipotent progenitor cell, a monocyte, a cardiac myoblast, a skeletal myoblast, a macrophage, a capillary endothelial cell, a xenogenic cell, an allogenic cell, or a post-natal stem cell.

20. The method of any one of claims 17-19, wherein greater editing of a target sequence in the target DNA is achieved in a cellular assay system comprising an RNP comprising the chimeric CasX variant when any one of a PAM sequence of TTC, ATC, GTC, or CTC is located 1 nucleotide 5' to a non-target strand of a protospacer having identity with a targeting sequence of the gNA in the cellular assay system, compared to the editing efficiency of an RNP comprising a reference CasX protein and a reference guide ribonucleic acid in a comparable assay system.

21. The method of any one of claims 17-20, wherein the method comprises contacting the cell with a vector encoding or comprising the chimeric CasX variant and the gNAof the gene editing pair.

22. The method of claim 21, wherein the vector;

a. is an Adeno-Associated Viral (AAV) vector, wherein the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-Rh74, or AAVRh10;

b. is a lentiviral vector;

c. is a lipid nanoparticle; or

d. is a virus-like particle (VLP)

wherein the vector is administered at a dose of at least 1 x $10^9$ vector genomes (vg), at least 1 x $10^{10}$ vg, at least 1 x $10^{11}$ vg, at least 1 x $10^{12}$ vg, at least 1 x $10^{13}$ vg, at least 1 x $10^{14}$ vg, at least 1 x $10^{15}$ vg, or at least 1 x $10^{16}$ vg.

23. A polynucleotide encoding the chimeric CasX variant of any one of claims 1-7.

24. A vector comprising:

a. the polynucleotide of claim 23; or

b. the chimeric CasX variant of any one of claims 1-7.

25. The vector of claim 24, wherein the vector

a. is an Adeno-Associated Viral (AAV) vector, wherein the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-Rh74, or AAVRh10;

b. is a lentiviral vector;

c. is a lipid nanoparticle; or

d. is a virus-like particle (VLP).

26. A kit, comprising the chimeric CasX variant of any one of claims 1-7 and one or more of a container, a buffer, a nuclease inhibitor, a protease inhibitor, a liposome, a therapeautic agent, a label. A label visualization reagent, or any combination of the foregoing.

27. A kit, comprising the gene editing pair of any one of claims 8-16 and one or more of a container, a buffer, a nuclease inhibitor, a protease inhibitor, a liposome, a therapeutic agent, a label, a label visualization reagent, or any combination of the foregoing.

28. The chimeric CasX variant of any one of claims 1-7 for use as a medicament.

29. The gene editing pair of any one of claims 8-16 for use as a medicament.