CLAIMS

1. A variant of a reference CasX protein (CasX variant), wherein:

a. the CasX variant comprises at least one modification in the reference CasX protein, wherein the reference CasX protein comprises the sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3; and

b. the CasX variant exhibits at least one improved characteristic as compared to the reference CasX protein.

2. The CasX variant of claim 1, wherein the improved characteristic of the CasX variant is selected from the group consisting of: improved folding of the CasX variant; improved binding affinity to a guide nucleic acid (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.

3. The Cas X variant of claim 1 or 2, wherein the at least one modification comprises:

a. at least one amino acid substitution in a domain of the CasX variant;

b. at least one amino acid deletion in a domain of the CasX variant;

c. at least one amino acid insertion in a domain of the CasX variant;

d. a substitution of all or a portion of a domain from a different CasX;

e. a deletion of all or a portion of a domain of the CasX variant; or

f. any combination of (a)-(e).

~~4. The CasX variant of any one of claims 1-3, wherein the reference CasX protein comprises the sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.~~

45. The CasX variant of any one of claims 1-34, wherein the at least one modification is in a domain selected from:

a. a non-target strand binding (NTSB) domain;

b. a target strand loading (TSL) domain;

c. a helical I domain;

d. a helical II domain;

e. an oligonucleotide binding domain (OBD); or

f. a RuvC DNA cleavage domain.

65. The CasX variant of claim 45, ~~comprising at least one modification in the NTSB domain.~~

~~7. The CasX variant of claim 5, comprising at least one modification in the TSL domain.~~

~~8. The CasX variant of claim 7~~, wherein:

the at least one modification in the TSL domain comprises an amino acid substitution of one or more of amino acids Y857, S890, or S932 of SEQ ID NO: 2~~.~~;

~~9. The CasX variant of claim 5, comprising at least one modification in the helical I domain.~~

~~10. The CasX variant of claim 9, wherein~~ the at least one modification in the helical I domain comprises an amino acid substitution of one or more of amino acids S219, L249, E259, Q252, E292, L307, or D318 of SEQ ID NO: 2~~.~~;

~~11. The CasX variant of any one of claims 5-10, comprising at least one modification in the helical II domain.~~

~~12. The CasX variant of claim 11, wherein~~ the at least one modification in the helical II domain comprises an amino acid substitution of one or more of amino acids D361, L379, E385, E386, D387, F399, L404, R458, C477, or D489 of SEQ ID NO: 2~~.~~;

~~13. The CasX variant of claim 5, comprising at least one modification in the OBD domain.~~

~~14. The CasX variant of claim 13, wherein~~ the at least one modification in the OBD comprises an amino acid substitution of one or more of amino acids F536, E552, T620, or I658 of SEQ ID NO: 2~~.~~; and/or

~~15. The CasX variant of claim 5, comprising at least one modification in the RuvC DNA cleavage domain.~~

~~16. The CasX variant of claim 15, wherein~~ the at least one modification in the RuvC DNA cleavage domain comprises an amino acid substitution of one or more of amino acids K682, G695, A708, V711, D732, A739, D733, L742, V747, F755, M771, M779, W782, A788, G791, L792, P793, Y797, M799, Q804, S819, or Y857 or a deletion of amino acid P793 of SEQ ID NO: 2.

~~17~~6. The CasX variant of ~~any one of claims~~claim 5~~-16~~, wherein the modification results in an increased ability to edit the target DNA.

~~18~~7. The CasX variant of any one of the preceding claims~~ 1 to 17~~, wherein the CasX variant is capable of forming a ribonuclear protein complex (RNP) with a guide nucleic acid (gNA).

~~19. The CasX variant of any one of claims 1 to 18, wherein the at least one modification comprises:~~

~~a. a substitution of 1 to 100 consecutive or non-consecutive amino acids in the CasX variant;~~

~~b. a deletion of 1 to 100 consecutive or non-consecutive amino acids in the CasX variant;~~

~~c. an insertion of 1 to 100 consecutive or non-consecutive amino acids in the CasX; or~~

~~d. any combination of (a) (c).~~

~~20. The CasX variant of claim 19, wherein the at least one modification comprises:~~

~~a. a substitution of 5-10 consecutive or non-consecutive amino acids in the CasX variant;~~

~~b. a deletion of 1-5 consecutive or non-consecutive amino acids in the CasX variant;~~

~~c. an insertion of 1-5 consecutive or non-consecutive amino acids in the CasX; or~~

~~d. any combination of (a) (c).~~

8~~21~~. The CasX variant of any one of ~~claims 1 to 20~~the preceding claims, wherein the CasX variant comprises two or more modifications in one domain~~.~~

~~22. The CasX variant of any one claims 1 to 21,~~, or wherein the CasX variant comprises modifications in two or more domains.

~~23. The CasX variant of any one of claims 1-20, comprising at least one modification of a region of non-contiguous amino acid residues of the CasX variant that form a channel in which gNA:target DNA complexing with the CasX variant occurs.~~

~~24. The CasX variant of any one of claims 1-20, comprising at least one modification of a region of non-contiguous amino acid residues of the CasX variant that form an interface which binds with the gNA.~~

~~25. The CasX variant of any one of claims 1-20, comprising at least one modification of a region of non-contiguous amino acid residues of the CasX variant that form a channel which binds with the non-target strand DNA.~~

~~26. The CasX variant of any one of claims 1-20, comprising at least one modification of a region of non-contiguous amino acid residues of the CasX variant that form an interface which binds with the protospacer adjacent motif (PAM) of the target DNA.~~

~~27. The CasX variant of any one of claims 1-20, comprising at least one modification of a region of non-contiguous surface-exposed amino acid residues of the CasX variant.~~

~~28. The CasX variant of any one of claims 1-20, comprising at least one modification of a region of non-contiguous amino acid residues that form a core through hydrophobic packing in a domain of the CasX variant.~~

~~29. The CasX variant of any one of claims 23-28, wherein the modification is one or more of a deletion, an insertion, or a substitution of one or more amino acids of the region.~~

~~30. The CasX variant of any one of claims 23-28, wherein between 2 to 15 amino acid residues of the region of the CasX variant are substituted with charged amino acids.~~

~~31. The CasX variant of any one of claims 23-28, wherein between 2 to 15 amino acid residues of a region of the CasX variant are substituted with polar amino acids.~~

~~32. The CasX variant of any one of claims 23-28, wherein between 2 to 15 amino acid residues of a region of the CasX variant are substituted with amino acids that stack with DNA or RNA bases.~~

9~~33~~. The CasX variant of any one of claims 1-~~45~~, wherein the at least one modification compared to the reference CasX sequence of SEQ ID NO: 2 is selected from one or more of:

a. an amino acid substitution of L379R;

b. an amino acid substitution of A708K;

c. an amino acid substitution of T620P;

d. an amino acid substitution of E385P;

e. an amino acid substitution of Y857R;

f. an amino acid substitution of I658V;

g. an amino acid substitution of F399L;

h. an amino acid substitution of Q252K;

i. an amino acid substitution of L404K; and

j. an amino acid deletion of P793.

~~34~~10. The CasX variant of any one of claims 1-~~54~~, wherein the CasX variant has a sequence selected from the group consisting of the sequences of Tables 3, 8, 9, 10 and 12, 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 96%, or at least about 97%, or at least about 98%, or at least about 99%, sequence identity thereto.

~~35. The CasX variant of any one of claims 1 to 5, comprising a sequence selected from the group consisting of SEQ ID NOS: 258-327, 3508-3520, and 4412-4415.~~

~~36. The CasX variant of any one of claims 1-5, further comprising a substitution of an NTSB and/or a helical 1b domain from a different CasX.~~

~~37. The CasX variant of claim 36, wherein the substituted NTSB and/or the helical 1b domain is from the reference CasX of SEQ ID NO: 1.~~

11~~38~~. The CasX variant of any one of ~~claims 1 to 37~~the preceding claims, further comprising a heterologous protein or domain thereof fused to the CasX.

12. The CasX variant of claim 13, wherein the heterologous protein or domain thereof comprises one or more nuclear localization signals (NLS)~~.~~

~~39. The CasX variant of claim 38, wherein the one or more NLS~~that 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~~).~~), wherein the one or more NLS are positioned at or near the C-terminus of the CasX protein; at or near at the N-terminus of the CasX protein; or at or near the N-terminus and at or near the C-terminus of the CasX protein.

~~40. The CasX variant of claim 38, comprising a sequence of any one of SEQ ID NOS: 3540-3549.~~

~~41. The CasX variant of claim 38 or claim 39, wherein the one or more NLS are positioned at or near the C-terminus of the CasX protein.~~

~~42. The CasX variant of claim 38 or claim 39, wherein the one or more NLS are positioned at or near at the N-terminus of the CasX protein.~~

~~43. The CasX variant of claim 38 or claim 39, comprising at least two NLS, wherein the at least two NLS are positioned at or near the N-terminus and at or near the C-terminus of the CasX protein.~~

~~44~~13. The CasX variant of any one of claims 2-~~43~~12, wherein one or more of the improved characteristics of the CasX variant is at least about 1.1 to 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.

~~45. The CasX variant of claim 2-43, wherein one or more of the improved characteristics of the CasX variant 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.~~

~~46. The CasX variant of any one of claims 2-45, wherein the improved characteristic comprises editing efficiency, and the CasX variant comprises a 1.1 to 100-fold improvement in editing efficiency compared to the reference CasX protein of SEQ ID NO: 2.~~

1447. The CasX variant of any one of the preceding claims ~~claims 1 to 46~~, wherein the RNP comprising the CasX 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 of the protospacer 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 in a comparable assay system.

~~48. The CasX variant of claim 47, wherein the PAM sequence is TTC.~~

~~49. The CasX variant of claim 47, wherein the PAM sequence is ATC.~~

~~50. The CasX variant of claim 47, wherein the PAM sequence is CTC.~~

~~51. The CasX variant of claim 47, wherein the PAM sequence is GTC.~~

5215. The CasX variant of any one of the preceding claims, ~~47, wherein the improved editing efficiency and/or binding to the target DNA of the RNP comprising the CasX variant is at least about 1.1 to about 100-fold improved relative to the RNP comprising the reference CasX.~~

~~53. The CasX variant of any one of claims 1 to 52, wherein the CasX variant comprises between 400 and 2000 amino acids.~~

~~54. The CasX variant of any one of claims 1 to 53,~~ wherein the CasX variant protein comprises a nuclease domain having nickase activity~~.~~

~~55. The CasX variant of any one of claims 1-53~~, wherein the CasX variant protein comprises a nuclease domain having double-stranded cleavage activity~~.~~

~~56. The CasX variant of any one of claims 1-53,~~, or wherein the CasX protein is a catalytically inactive CasX (dCasX) protein, and wherein the dCasX and the gNA retain the ability to bind to the target DNA.

5716. The CasX variant of claim ~~56~~15, wherein the CasX protein is catalytically inactive and wherein the dCasX comprises a mutation at residues:

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

b. D659, and/or E756, and/or D922 corresponding to the CasX protein of SEQ ID NO: 2.

~~58. The CasX variant of claim 57, wherein the mutation is a substitution of alanine for the residue.~~

~~59~~17. The CasX variant of any one of the preceding claims ~~1 to 58~~, wherein the CasX variant comprises a first domain from a first CasX protein and second domain from a second CasX protein different from the first CasX protein.

~~60. The CasX variant of claim 59, wherein the first domain is selected from the group consisting of the NTSB, TSL, helical I, helical II, OBD, and RuvC domains.~~

~~61. The CasX variant of claim 59, wherein the second domain is selected from the group consisting of the NTSB, TSL, helical I, helical II, OBD, and RuvC domains.~~

~~62. The CasX variant of any one of claims 59 to 61, wherein the first and second domains are not the same domain.~~

18~~63~~. The CasX variant of any one of claim 17 ~~claims 59 to 62~~, wherein the first domain comprises a portion of the sequence selected from the group consisting of amino acids 1-56, 57-100, 101-191, 192-332, 333-509, 510-660, 661-824, 825-934, and 935-986 of SEQ ID NO: 1 and the second domain comprises a portion of the sequence selected from the group consisting of amino acids 1-58, 59-102, 103-192, 193-333, 334-501, 502-647, 648-812, 813-921, and 922-978 of SEQ ID NO: 2.

~~64~~19. The CasX variant of any one of claims 1-~~63~~18, wherein the CasX variant ~~is~~comprises a sequence selected from the group consisting of SEQ ID NOS: 328-336 and 4413-4415~~CasX variants SEQ ID NO: 328, SEQ ID NO: 3540, SEQ ID NO: 4413, SEQ ID NO: 4414, SEQ ID NO: 4415,SEQ ID NO: 329, SEQ ID NO: 3541,SEQ ID NO: 330, SEQ ID NO: 3542, SEQ ID NO: 331, SEQ ID NO: 3543, SEQ ID NO: 332, SEQ ID NO: 3544, SEQ ID NO: 333, SEQ ID NO: 3545, SEQ ID NO: 334, SEQ ID NO: 3546, SEQ ID NO: 335, SEQ ID NO: 3547, SEQ ID NO: 336 and SEQ ID NO: 3548~~.

~~65~~20. The CasX variant of any one of claims 1 ~~to 58~~-16, wherein the CasX variant comprises at least one chimeric domain comprising a first part from a first CasX protein and a second part from a second CasX protein different from the first CasX protein.

~~66. The CasX variant of claim 65, wherein the at least one chimeric domain is selected from the group consisting of the NTSB, TSL, helical I, helical II, OBD, and RuvC domains.~~

~~67. The CasX variant of claim 65 or claim 66, wherein the first CasX protein comprises a sequence of SEQ ID NO: 1 and the second CasX protein comprises a sequence of SEQ ID NO: 2.~~

~~68. The CasX variant of claim 66, wherein the at least one chimeric domain comprises a chimeric RuvC domain.~~

~~69. The CasX variant of claim 68, wherein the chimeric RuvC domain comprises amino acids 661 to 824 of SEQ ID NO: 1 and amino acids 922 to 978 of SEQ ID NO: 2.~~

~~70. The CasX variant of claim 68, wherein the chimeric RuvC domain comprises amino acids 648 to 812 of SEQ ID NO: 2 and amino acids 935 to 986 of SEQ ID NO: 1.~~

~~71. The CasX variant of any one of claims 1 to 5, comprising a sequence selected from the group consisting of SEQ ID NOS: 247-337, 3301-3493, 3498-3501, 3505-3520, 3540-3549 and 4412-4415.~~

~~72. The CasX variant of any one of claims 1 to 5, comprising a sequence selected from the group consisting of SEQ ID NOS: 247-337, 3498-3501, 3505-3520, 3540-3549 and 4412-4415.~~

~~73. The CasX variant of any one of claims 1 to 5, comprising a sequence selected from the group consisting of SEQ ID NOS: 3498-3501, 3505-3520, and 3540-3549.~~

~~74. The CasX variant of any one claims of 1 to 73, comprising a heterologous protein or domain thereof fused to the CasX.~~

~~75. The CasX variant of claim 74, wherein the heterologous protein or domain thereof is a base editor.~~

~~76. The CasX variant of claim 75, wherein the base editor is an adenosine deaminase, a cytosine deaminase or a guanine oxidase.~~

~~77~~21. A variant of a reference guide nucleic acid scaffold (gNA variant) capable of binding a reference CasX protein or a CasX variant, wherein:

a. the gNA variant comprises at least one modification compared to the reference guide nucleic acid scaffold sequence; and

b. the gNA variant exhibits one or more improved characteristics compared to the reference guide nucleic acid scaffold comprising a sequence selected from the group consisting of SEQ ID NOS: 4-16.

~~78~~22. The gNA variant of claim ~~77~~21, wherein the one or more improved characteristics is selected from the group consisting of: improved stability; improved solubility; improved transcription of the gNA; improved resistance to nuclease activity; increased folding rate of the gNA; decreased side product formation during folding; increased productive folding; improved binding affinity to a CasX protein; improved binding affinity to a target DNA when complexed with the CasX protein; improved gene editing when complexed with the CasX protein; improved specificity of editing when complexed with the CasX protein; and 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 when complexed with the CasX protein.

~~79. The gNA variant of claim 77 or 78, wherein the reference guide scaffold comprises a sequence selected from the group consisting of the sequences of SEQ ID NOS: 4-16.~~

2380. The gNA variant of claim 21 or 22 ~~any one of claims 77 to 79~~, wherein the at least one modification comprises:

a. at least one nucleotide substitution in a region of the gNA variant;

b. at least one nucleotide deletion in a region of the gNA variant;

c. at least one nucleotide insertion in a region of the gNA variant;

d. a substitution of all or a portion of a region of the gNA variant;

e. a deletion of all or a portion of a region of the gNA variant; or

f. any combination of (a)-(e~~).~~);

~~81. The gNA variant of claim 80,~~ wherein the region of the gNA variant is selected from the group consisting of extended stem loop, scaffold stem loop, triplex, and pseudoknot.

8224. The gNA variant of claim 8123, wherein:

the scaffold stem ~~further~~ comprises a bubble~~.~~;

~~83. The gNA variant of claim 81 or claim 82, wherein~~ the scaffold further comprises a triplex loop region~~.~~; and/or

~~84. The gNA variant of any one of claims 81-83, wherein~~ the scaffold further comprises a 5' unstructured region.

8525. The gNA variant of ~~any one of claims 80 to 84~~claim 23 or 24, wherein the at least one modification comprises:

a. a substitution of 1 to 15 consecutive or non-consecutive nucleotides in the gNA variant in one or more regions;

b. a deletion of 1 to 10 consecutive or non-consecutive nucleotides in the gNA variant in one or more regions;

c. an insertion of 1 to 10 consecutive or non-consecutive nucleotides in the gNA variant in one or more regions;

d. a substitution of the scaffold stem loop or the extended stem loop with an RNA stem loop sequence from a heterologous RNA source with proximal 5' and 3' ends; or

e. any combination of (a)-(d).

8626. The gNA variant of any one of claims ~~77-85~~21-25, comprising an extended stem loop region comprising at least 10, at least 100, at least 500~~, at least 1000, or at least 10,000~~ nucleotides.

~~87. The gNA variant of claim 85, wherein the heterologous RNA stem loop sequence increases the stability of the gNA.~~

~~88. The gNA variant of claim 87, wherein the heterologous RNA stem loop is capable of binding a protein, an RNA structure, a DNA sequence, or a small molecule.~~

2789. The gNA variant of claim 26 87 or claim 88, wherein the heterologous RNA stem loop sequence is selected from MS2, Qβ, U1 hairpin II, Uvsx, or PP7 stem loops.

9028. The gNA variant of any one of claims 85-8925-27, wherein the at least one modification compared to the reference guide scaffold of SEQ ID NO: 5 is selected from one or more of:

a. a C18G substitution in the triplex loop;

b. a G55 insertion in the stem bubble;

c. a U1 deletion;

d. a modification of the 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 that results in a loop-distal base that is fully base-paired.

29. The gNA variant of claim 28, comprising the sequence of any one of SEQ ID NOS: 2236, 2237, 2238, 2241, 2244, 2248, 2249, or 2259-2280,

91. The gNA variant of any one of claims 77-90, wherein the gNA variant comprises two or more modifications in one region.

92. The gNA variant of any one of claims 77-91, wherein the gNA variant comprises modifications in two or more regions.

93. The gNA variant of any one of claims 77-92, wherein the gNA variant further comprises a targeting sequence, wherein the targeting sequence is complementary to the target DNA sequence.

3094. The gNA variant of claim 2993, wherein the targeting sequence has 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 nucleotides.

95. The gNA variant of any one of claims 93 or claim 94, wherein the targeting sequence has 20 nucleotides.

3196. The gNA variant of claim 29 or 30 any one of claims 93-95, wherein the gNA is a single-guide gNA comprising the scaffold sequence linked to the targeting sequence.

9732. The gNA variant of any one of claims 77 to 9621-31, wherein the one or more of the improved characteristics of the CasX variant is at least about 1.1 to about 100-fold or more improved relative to the reference gNA of SEQ ID NO: 4 or SEQ ID NO: 5.

98. The gNA variant of any one of claims 77 to 96, wherein one or more of the improved characteristics of the gNA variant is at least about 1.1, at least about 2, at least about 10, or at least about 100-fold or more improved relative to the reference gNA of SEQ ID NO: 4 or SEQ ID NO: 5.

~~99. The gNA variant of any one of claims 77-98, comprising a scaffold region having at least 60% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 5 exclusive of the extended stem region.~~

~~100. The gNA variant of any one of claims 77-98, comprising a scaffold stem loop having at least 60% sequence identity to SEQ ID NO: 14.~~

~~101. The gNA variant of claim 100, comprising a scaffold stem loop sequence of CCAGCGACUAUGUCGUAGUGG (SEQ ID NO: 245).~~

~~102. The gNA variant of any one of claims 77-101, wherein the scaffold of the gNA variant sequence has at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, or at least 70%, at least 80%, at least 85%, 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% identity to the sequence of SEQ ID NO: 4 or SEQ ID NO: 5.~~

33~~103~~. The gNA variant of any one of claims ~~77-101~~21-32, wherein the scaffold of the gNA variant sequence comprises a sequence selected from the group of sequences of SEQ ID NOS: 2101-2280, or having 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% identity thereto.

~~104~~34. The gNA variant of claim ~~103~~33, wherein the scaffold of the gNA variant sequence consists of a sequence selected from the group of sequences of SEQ ID NOS: 2101-2280.

~~105. The gNA variant of any one of claims 77-104, further comprising one or more ribozymes.~~

~~106. The gNA variant of claim 105, wherein the one or more ribozymes are independently fused to a terminus of the gNA variant.~~

~~107. The gNA variant of claim 105 or claim 106, wherein at least one of the one or more ribozymes are an hepatitis delta virus (HDV) ribozyme, hammerhead ribozyme, pistol ribozyme, hatchet ribozyme, or tobacco ringspot virus (TRSV) ribozyme.~~

35~~108~~. The gNA variant of any one of claims 21-34 ~~77-107~~, further comprising a protein binding motif~~.~~

~~109. The gNA variant of any one of claims 77-108, further comprising~~ ; and/or a thermostable stem loop.

~~110. The gNA variant of any one of claims 77-109, wherein the gNA is chemically modified.~~

~~111. The gNA variant of any one of claims 77 to 110, wherein the gNA comprises a first region from a first gNA and a second region from a second gNA different from the first gNA.~~

~~112. The gNA variant of claim 111, wherein the first region is selected from the group consisting of a triplex region, a scaffold stem loop, and an extended stem loop.~~

~~113. The gNA variant of claim 111 or claim 112, wherein the second region is selected from the group consisting of a triplex region, a scaffold stem loop, and an extended stem loop.~~

~~114. The gNA variant of any one of claims 111 to 113, wherein the first and second regions are not the same region.~~

~~115. The gNA variant of any one of claims 111 to 113, wherein the first gNA comprises a sequence of SEQ ID NO: 4 and the second gNA comprises a sequence of SEQ ID NO: 5.~~

~~116. The gNA variant of any one of claims 77 to 115, comprising at least one chimeric region comprising a first part from a first gNA and a second part from a second gNA.~~

~~117. The gNA variant of claim 116, wherein the at least one chimeric region is selected from the group consisting of a triplex region, a scaffold stem loop, and an extended stem loop.~~

~~118. The gNA variant of claim 77, comprising the sequence of any one of any one of SEQ ID NOS: 2101-2280.~~

~~119. The gNA variant of claim 77, comprising the sequence of any one of SEQ ID NOS: 2236, 2237, 2238, 2241, 2244, 2248, 2249, or 2259-2280.~~

~~120~~36. A gene editing pair comprising a CasX protein variant of any one of claims 1-20 and a ~~first~~ gNA~~.~~ comprising:

~~121. The gene editing pair of claim 120, wherein the CasX and the gNA are capable of associating together in a ribonuclear protein complex (RNP).~~

~~122. The gene editing pair of claim 120, wherein the CasX and the gNA are associated together in a ribonuclear protein complex (RNP).~~

~~123. The gene editing pair of any one of claims 120-122, wherein the first gNA comprises:~~

a. the~~a~~ gNA variant of any one of claims ~~93-119; or~~21-35; and

b. ~~a reference guide nucleic acid of SEQ ID NOS: 4 or 5 and~~ a targeting sequence, wherein the targeting sequence is complementary to the target DNA.

~~124. The gene editing pair of any one of claims 120-123, wherein the CasX comprises:~~

~~a. a CasX variant of any one of claims 1-76; or~~

~~b. a reference CasX protein of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.~~

~~125. The gene editing pair of any one of claims 120 to 124, wherein the first gNA comprises:~~

~~a. a gNA variant of any one of claims 93-119; and~~

~~b. a CasX variant of any one of claims 1-76.~~

~~126. The gene editing pair of claim 125, wherein the gene editing pair of the CasX variant and the gNA variant has one or more improved characteristics compared to a gene editing~~

pair comprising a reference CasX protein of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 and a reference guide nucleic acid of SEQ ID NOS: 4 or 5.

127. The gene editing pair of claim 126, wherein the one or more improved characteristics comprises improved CasX:gNA (RNP) complex stability, improved binding affinity between the CasX 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.

128. The gene editing pair of claim 126 or claim 127, wherein the at least one or more of the improved characteristics is at least about 1.1 to about 100-fold or more improved relative to a gene editing pair of the reference CasX protein and the reference guide nucleic acid.

129. The gene editing pair of claim 126 or 127, wherein one or more of the improved characteristics of the CasX variant is at least about 1.1, at least about 2, at least about 10, or at least about 100-fold or more improved relative to a gene editing pair of the reference CasX protein and the reference guide nucleic acid.

130. The gene editing pair of claim 126 or claim 127, wherein the improved characteristic comprises a 4 to 9 fold increase in editing activity compared to a reference editing pair of SEQ ID NO: 2 and SEQ ID NO: 5.

131. The gene editing pair of claim 130, comprising a CasX selected from any one of SEQ ID NO: 270, SEQ ID NO: 292, SEQ ID NO: 311, SEQ ID NO: 333, SEQ ID NO: 336, SEQ ID NOS: 3498-3501, SEQ ID NOS: 3505-3520, and SEQ ID NOS: 3540-3549, and a gNA selected from any one of SEQ ID NOS: 2104, 2106, or 2238.

132. A composition comprising the gene editing pair of any one of claims 120-131, further comprising:

a. a second gene editing pair comprising the CasX variant of any one of claims 1-76, or the reference CasX protein of any one of SEQ ID NOS: 1-3; and

b. a second gNA variant of any one of claims 77-119 or a second reference guide nucleic acid, wherein the second gNA variant or the second reference guide nucleic acid has a targeting sequence complementary to a different or overlapping portion of the target DNA compared to the targeting sequence of the first gNA.

133. The gene editing pair of any one of claims 120-132, wherein the RNP of the CasX variant and the gNA variant has a higher percentage of cleavage-competent RNP compared to an RNP of a reference CasX protein and a reference guide nucleic acid.

~~134. The gene editing pair of any one of claims 120-133, wherein the RNP is capable of binding and cleaving a target DNA.~~

~~135. The gene editing pair of any one of claims 120-132, wherein the RNP is capable of binding a target DNA but is not capable of cleaving the target DNA.~~

~~136. The gene editing pair of any one of claims 120-132, wherein the RNP is capable of binding a target DNA and generating one or more single-stranded nicks in the target DNA.~~

~~137~~37. A method of editing a target DNA, comprising contacting the target DNA with ~~a~~the gene editing pair of ~~any one of claims 120-136~~claim 36, wherein the contacting results in editing or modification of the target DNA.

~~138~~38. The method of claim ~~137~~37, comprising contacting the target DNA with a plurality of gNAs comprising targeting sequences complementary to different or overlapping regions of the target DNA.

~~139~~39. The method of claim ~~137~~37 or ~~claim 138~~38, wherein:

the contacting by the gene editing pair comprises binding the target DNA and results in introducing a mutation, an insertion, or a deletion in the target DNA~~.~~;

~~140. The method of claim 137 or claim 138, wherein~~ the contacting introduces one or more single-stranded breaks in the target DNA and wherein the editing comprises introducing a mutation, an insertion, or a deletion in the target DNA~~.~~; or

~~141. The method of claim 137 or claim 138, wherein~~ the contacting comprises introducing one or more double-stranded breaks in the target DNA and wherein the editing comprises introducing a mutation, an insertion, or a deletion in the target DNA.

~~142~~40. The method of claim ~~140 or claim 141~~39, further comprising contacting the target DNA with a nucleotide sequence of a donor template nucleic acid wherein the donor template comprises a nucleotide sequence having homology to the target DNA, ~~.~~

~~143. The method of claim 142,~~ wherein the donor template comprises homologous arms on the 5' and 3' ends of the donor template.

~~144. The method of claim 142 or claim 143, wherein the donor template is inserted in the target DNA at the break site by homology-directed repair.~~

~~145. The method of claim 142 or claim 143, wherein the donor template is inserted in the target DNA at the break site by non-homologous end joining (NHEJ) or micro-homology end joining (MMEJ).~~

~~146~~41. The method of any one of claims ~~137-144~~37-40, wherein editing occurs in vitro outside of a cell~~.~~

~~147. The method of any one of claims 137-144, wherein~~, editing occurs in vitro inside of a cell~~.~~

~~148. The method of any one of claims 137-144, wherein editing occurs~~ , or in vivo inside of a cell.

~~14942~~. The method of ~~claims 147 or~~ claim ~~14841~~, wherein the cell is a eukaryotic cell.

~~150. The method of claim 149, 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.~~

~~151. The method of claim 149, wherein the eukaryotic cell is a human cell.~~

~~152. The method of claim 151, 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.~~

43~~153~~. The method of claim ~~151 or 152~~41 or 42, wherein the cell is in a subject.

~~154. The method of claim 153, wherein editing occurs in the subject having a mutation in an allele of a gene wherein the mutation causes a disease or disorder in the subject.~~

~~155. The method of claim 154, wherein the editing changes the mutation to a wild type allele of the gene.~~

~~156. The method of claim 154, wherein the editing knocks down or knocks out an allele of a gene causing a disease or disorder in the subject.~~

~~157. The method of claim 151, wherein editing occurs in vitro inside of the cell prior to introducing the cell into a subject.~~

~~158. The method of claim 157, wherein the cell is autologous or allogeneic.~~

~~159. The method of any one of claims 147-151, wherein greater editing of a target sequence in the target DNA is achieved in a cellular assay system comprising an RNP comprising the CasX variant when any one of the PAM sequences TTC, ATC, GTC, or CTC is located 1 nucleotide 5' to the non-target strand of the protospacer having identity with the~~

~~targeting sequence of the gNA in a cellular assay system, compared to the editing efficiency of an RNP comprising a reference CasX protein in a comparable assay system.~~

~~44~~160. The method of~~-~~ claim 42 or 43~~any one of claims 149-159~~, wherein the method comprises contacting the eukaryotic cell with a vector encoding or comprising the CasX protein and the gNA, and optionally further comprising the donor template.

~~16~~145. The method of claim ~~160~~44, wherein the vector is an Adeno-Associated Viral (AAV) vector~~.~~

~~162. The method of claim 161, wherein the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-Rh74, or AAVRh10.~~

~~163. The method of claim 160, wherein the vector is,~~ a lentiviral vector~~.~~

~~164. The method of claim 160, wherein the vector is,~~ a non-viral particle~~.~~

~~165. The method of claim 160, wherein the vector is~~ or a virus-like particle (VLP).

~~166. The method of any one of claims 160-164, wherein the vector is administered to a subject in need using a therapeutically effective dose.~~

~~167. The method of claim 164, wherein the subject is selected from the group consisting of mouse, rat, pig, and non-human primate.~~

~~168. The method of claim 166, wherein the subject is a human.~~

~~169. The method of any one of claims 166-168, wherein the vector is administered at a dose of at least about 1 x 109 vector genomes (vg), at least about 1 x 1010 vg, at least about 1 x 1011 vg, at least about 1 x 1012 vg, at least about 1 x 1013 vg, at least about 1 x 1014 vg, at least about 1 x 1015 vg, or at least about 1 x 1016 vg.~~

~~170. The method of any one of claims 166-169, wherein the vector is administered by a route of administration selected from the group consisting of intraparenchymal, intravenous, intra-arterial, intracerebroventricular, intracisternal, intrathecal, intracranial, and intraperitoneal routes.~~

~~171. The method of claim 147, wherein the cell is a prokaryotic cell.~~

~~172. A cell comprising a target DNA edited by the gene editing pair or composition of any one of claims 120 to 136.~~

~~173. A cell edited by the method of any one of claims 137-165.~~

~~174. The cell of claim 172 or 173, wherein the cell is a prokaryotic cell.~~

~~175. The cell of claim 172 or 173, wherein the cell is a eukaryotic cell.~~

~~176. The cell of claim 175, 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.~~

~~177. The cell of claim 175, wherein the eukaryotic cell is a human cell.~~

~~178~~46. A polynucleotide encoding the CasX variant of any one of claims 1-~~76.~~20, and/or

~~179. A polynucleotide encoding~~ the gNA variant of any one of claims ~~77-119~~21-35.

~~180~~47. A vector comprising the polynucleotide of claim 46.

~~comprising the polynucleotide of claim 178 or claim 179.~~

~~181~~48. A vector comprising encoding the CasX variant of any one of claims 1-~~76~~20 and/or the gNA variant of any one of claims ~~77-119~~21-35.

~~182. The vector of claim 180, wherein the vector is an Adeno-Associated Viral (AAV) vector.~~

~~183. The vector of claim 182, wherein the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-Rh74, or AAVRh10.~~

~~184. The vector of claim 180, wherein the vector is a lentiviral vector.~~

~~185. The vector of claim 180, wherein the vector is a virus-like particle (VLP).~~

~~186. The vector of claim 180, wherein the vector is a non-viral particle.~~

~~187. A cell comprising the polynucleotide of claim 178, or the vector of any one of claims 180-186.~~

~~188. A composition, comprising the CasX variant of any one of claims 1-76.~~

~~189. The composition of claim 188, further comprising:~~

~~a. a gNA variant of any one of claims 77 to 119, or~~

~~b. the reference guide scaffold of SEQ ID NOS: 4 or 5 and a targeting sequence.~~

~~190~~49. The composition of claim ~~188 or claim 189~~48, wherein the CasX protein and the gNA are associated together in a ribonuclear protein complex (RNP).

~~191~~50. The composition ~~of any one of claims 188-190~~claim 48 or 49, further comprising a donor template nucleic acid wherein the donor template comprises a nucleotide sequence having homology to a target DNA.

~~192. The composition of any one of claims 188-191, further comprising 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.~~

~~193. A composition, comprising a gNA variant of any one of claims 77 to 119.~~

~~194. The composition of claim 193, further comprising the CasX variant of any one of claims 1-76, or the CasX protein of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.~~

~~195. The composition of claim 194, wherein the CasX protein and the gNA are associated together in a ribonuclear protein complex (RNP).~~

~~196. The composition of any one of claims 193-195, further comprising a donor template nucleic acid wherein the donor template comprises a nucleotide sequence having homology to a target DNA.~~

~~197. The composition of any one of claims 193-196, further comprising 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.~~

~~198. A composition, comprising the gene editing pair of any one of claims 120 to 136.~~

~~199. The composition of claim 198, further comprising a donor template nucleic acid wherein the donor template comprises a nucleotide sequence having homology to a target DNA.~~

~~200. The composition of claim 198 or claim 199, further comprising 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.~~

~~201.~~ 51. A kit, comprising the CasX variant of any one of claims 1-~~76 and a container~~20, the gNA of any one of claims 21-35 or the gene editing pair of claim 36.

~~202. The kit of claim 201, further comprising:~~

~~a. a gNA variant of any one of claims 93 to 119, or~~

~~b. the reference guide RNA of SEQ ID NOS: 4 or 5 and a targeting sequence.~~

~~203. The kit of claim 201 or claim 202, further comprising a donor template nucleic acid wherein the donor template comprises a nucleotide sequence having homology to a target sequence of a target DNA.~~

~~204. The kit of any one of claims 201-203, further comprising 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.~~

~~205. A kit, comprising a gNA variant of any one of claims 77 to 119.~~

~~206. The kit of claim 205, further comprising the CasX variant of any one of claims 1-76, or the CasX protein of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.~~

~~207. The kit of claim 205 or claim 206, further comprising a donor template nucleic acid wherein the donor template comprises a nucleotide sequence having homology to a target sequence of a target DNA.~~

~~208. The kit of any one of claims 205-207, further comprising 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.~~

~~209. A kit, comprising the gene editing pair or composition of any one of claims 120 to 136.~~

~~210. The kit of claim 209, further comprising a donor template nucleic acid wherein the donor template comprises a nucleotide sequence having homology to a target DNA.~~

~~211. The kit of claim 209 or claim 210, further comprising 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.~~

~~212. A CasX variant comprising any one of the sequences listed in Table 3.~~

~~213. A gNA variant comprising any one of the sequences listed in Table 2.~~

~~214. The gNA variant of claim 213, further comprising a targeting sequence of at least 10 to 30 nucleotides complementary to a target DNA.~~

~~215. The gNA variant of claim 214, wherein the targeting sequence has 20 nucleotides.~~

~~216. The gNA variant of claim 214, wherein the targeting sequence has 19 nucleotides.~~

~~217. The gNA variant of claim 214, wherein the targeting sequence has 18 nucleotides.~~

~~218. The gNA variant of claim 214, wherein the targeting sequence has 17 nucleotides.~~

~~219. A CasX variant comprising substitutions L379R and A708K and a deletion of P793 of SEQ ID NO: 2.~~

~~220. A gNA variant comprising a sequence of ACUGGCGCUUUUAUCUGAUUACUUUGAGAGCCAUCACCAGCGACUAUGUCGUAGUGGGUAAAGCUCCCUCUUCGGAGGGAGCAUCAAAG (SEQ ID NO: 2238).~~

~~221. A gene editing pair, or composition, comprising the gene editing pair or composition of any one of claims 120 to 136, or a vector of any one of claims 180 to 186, for use as a medicament.~~

~~222. A gene editing pair, or composition comprising the gene editing pair, or composition, of any one of claims 120 to 136, or a vector of any one of claims 180 to 186, for use in a method of treatment, wherein the method comprises editing or modifying a target DNA; optionally wherein the editing occurs in a subject having a mutation in an allele of a gene wherein the mutation causes a disease or disorder in the subject, preferably wherein the editing changes the mutation to a wild type allele of the gene or knocks down or knocks out an allele of a gene causing a disease or disorder in the subject.~~