[0005] As CRISPR/Cas systems have been adapted for genome editing in eukaryotic cells, the two technologies have the potential to permit the engineering of immune cells that have potent cytotoxicity versus the targeted cells, yet permit the reduction or elimination of cell markers that contribute to triggering unwanted recipient immune responses to transplants of such cells, especially in the case of allogeneic transplants of these cells. Accordingly, there exists a need for modified cells and methods to modify such cells into engineered CAR-T cells that exhibit these properties for use in immunotherapy treatment, for example allogeneic-based immunotherapy treatments.

SUMMARY

[0005a] In one aspect, the present disclosure provides a CasX:gNA system comprising a CasX protein and a first guide nucleic acid (gNA), wherein the gNA comprises a targeting sequence complementary to a target nucleic acid sequence of a gene encoding a first protein involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response.

[0005b] In another aspect, the present disclosure provides a polynucleotide comprising a sequence that encodes the CasX as disclosed herein.

[0005c] In another aspect, the present disclosure provides a polynucleotide comprising a sequence that encodes the gNA as disclosed herein.

[0005d] In another aspect, the present disclosure provides a vector comprising the polynucleotide as disclosed herein.

[0005e] In another aspect, the present disclosure provides a virus-like particle (VLP) comprising a. one or more components of a gag polyprotein selected from the group of matrix protein (MA), nucleocapsid protein (NC), capsid protein (CA), p1-p6 protein, and a protease cleavage site, and further comprising a targeting glycoprotein that provides for binding and fusion of the VLP to a target cell; b. the CasX protein and the gNA of any one of claims 1-11, c. wherein the CasX protein and the gNA are associated together in an RNP.

[0005f] In another aspect, the present disclosure provides an *ex vivo* method of modifying a target nucleic acid sequence of a gene in a population of cells, wherein the gene encodes a protein involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response, comprising introducing into each cell of the population: a. the CasX:gNA system of any one of claims 1-22; b. the polynucleotide of claim 23 or claim 24; c. the vector as in any one of claims 25-27; d. the VLP of claim 28; or e. combinations of two or more of (a) to (d), wherein the target nucleic acid sequence of the cells is modified by the CasX protein, wherein the modifying comprises introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the target nucleic acid sequence of the cells, resulting in a knock-down or knock-out of a gene encoding one or more proteins selected from the group consisting of B2M, TRAC, CIITA, TRBC1, TRBC2, HLA-A, HLA-B, TGFβRII, PD-1, CISH, LAG3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, CD244, and wild-type T-cell receptor.

[0005g] In another aspect, the present disclosure provides the use of a. the CasX:gNA system of any one of claims 1-22; b. the polynucleotide of claim 23 or claim 24; c. the vector as in any one of claims 25-27; d. the VLP of claim 28; or e. combinations of two or more of (a) to (d), in the manufacture of a medicament for modifying a target nucleic acid sequence of a gene in a population of cells in a subject for treating a cancer, wherein the gene encodes a protein involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response, comprising introducing into each cell of the population: wherein the target nucleic acid sequence of the cells is modified by the CasX protein, wherein the modifying comprises introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the target nucleic acid sequence of the cells, resulting in a knock-down or knock-out of a gene encoding one or more proteins selected from the group consisting of B2M, TRAC, CIITA, TRBC1, TRBC2, HLA-A, HLA-B, TGFβRII, PD-1, CISH, LAG3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, CD244, and wild-type T-cell receptor.

[0005h] In another aspect, the present disclosure provides a population of cells modified *ex vivo* by the method as disclosed herein.

[0005i] In another aspect, the present disclosure provides the use of the population of cells as disclosed herein in the manufacture of a medicament for providing anti-tumor immunity in a subject.

[0006] In some ~~aspects~~examples, the present disclosure provides compositions of CasX:guide nucleic acid systems (CasX:gNA system) and methods used to modify target nucleic acid sequences of cell genes encoding one or more proteins involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response. In the foregoing, the proteins are selected from the group consisting of beta-2-microglobulin (B2M), T cell receptor alpha chain constant region (TRAC, or TCRA), class II major histocompatibility complex transactivator (CIITA), T cell receptor beta constant 1 (TRBC1, or TCRB), T cell receptor beta constant 2 (TRBC2), programmed cell death 1 (PD-1), cytokine inducible SH2 (CISH), T cell immunoreceptor with Ig and ITIM domains (TIGIT), adenosine A2a receptor (ADORA2A), killer cell lectin like receptor C1 (NKG2A), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte activating 3 (LAG-3), T-cell immunoglobulin and mucin domain 3 (TIM-3), 2B4 (CD244), human leukocyte antigen A (HLA-A), human leukocyte antigen B (HLA-B), TGFβ Receptor 2 (TGFβRII), cluster of differentiation 247 (CD247), CD3d molecule (CD3D), CD3e molecule (CD3E), CD3g molecule (CD3G), CD52 molecule (CD52), human leukocyte antigen C (HLA-C), deoxycytidine kinase (dCK), or FKBP prolyl isomerase 1A (FKBP1A). The CasX:gNA systems can comprise a reference CasX protein, a CasX variant protein with improved properties relative to the reference CasX, a guide nucleic acid (gNA) that is a reference sequence or a gNA variant with improved properties relative to the reference sequence, as well as donor template nucleic acids that can be inserted into the break sites of the target nucleic acid sequences in cells introduced by the CasX nucleases to modify the target nucleic acid sequences. ~~Embodiment~~Examples of these components are described herein, below. In some ~~aspects~~examples, the present disclosure provides gene editing pairs of CasX and gNA as of any of the ~~embodiment~~examples described herein

complexed as a ribonuclear protein complex (RNP). In some ~~embodiment~~examples, the present disclosure provides methods to modify the genes of cells encoding the proteins involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response in which the gene are knocked-down or knocked out from expression of such proteins.

[0007] The cells modified by the CasX:gNA systems are useful for, among other things, immunotherapy applications; e.g. preparation and use of immune cells with reduced potential for graft-versus-host disease (GVHD), and that are also modified to express one or more chimeric antigen receptor (CAR) for use in the treatment of cancer or an autoimmune disease in a subject. Such cells also are also engineered to reduce host vs. graft complications. In other ~~embodiment~~examples, the CasX-gNA systems are used to knock-in nucleic acids into the cells that encode CAR and/or an engineered T cell receptor (TCR), the CAR and/or the TCR comprising binding domains specific for tumor cell antigens, including those listed herein, below. Such binding domains can be in the form of a linear antibody, a single domain antibody (sdAb) such as a VHH, or a single-chain variable fragment (scFv). The cells that can be used for the preparation of the modified cells include progenitor cells, hematopoietic stem cells, pluripotent stem cells, or immune cells selected from the group consisting of T cells, TREG cells, NK cells, B cells, macrophages, or dendritic cells.

[0008] In some ~~aspects~~examples, the present disclosure provides polynucleotides and vectors encoding or comprising the CasX proteins, gNAs, the gene editing pairs, or comprising the donor template nucleic acids described herein. In some ~~embodiment~~examples, the vectors are viral vectors such as an Adeno-Associated Viral (AAV) vector or a lentiviral vector. In other ~~embodiment~~examples, the vectors are non-viral particles such as virus-like particles (VLP) or nanoparticles.

[0009] In some ~~aspects~~examples, the disclosure provides methods of modifying a target nucleic acid sequence of in a population of cells, comprising introducing into each cell of the population: a) the CasX:gNA system of any of the ~~embodiment~~examples disclosed herein; or b) the nucleic acid of any of the ~~embodiment~~examples disclosed herein; or c) the vector of any of the ~~embodiment~~examples disclosed herein; d) the VLP of any of the ~~embodiment~~examples disclosed herein; or e) combinations of two or more of (a)-(d)), above, wherein the target nucleic acid sequence of the cells is modified by the CasX protein (e.g., a single- or double-stranded break, or an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the target nucleic acid sequence).

[0010] In some ~~aspects~~examples, the present disclosure provides populations of cells modified by the ex vivo methods of modification of the target nucleic acid by the CasX:gNA systems, vectors, or VLPs (or combinations thereof) of any of the ~~embodiment~~examples described herein, wherein the expression of MHC Class I molecules or T cell receptors or the proteins involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response have been reduced or eliminated in the modified cells. In some ~~embodiment~~examples, the present disclosure provides populations of cells modified by the ex vivo methods of modification of the target nucleic acid by the CasX:gNA systems, vectors, or VLPs (or combinations thereof) of any of the ~~embodiment~~examples described herein, wherein the modified cells express a detectable level of the CAR and/or TCR of any of the ~~embodiment~~examples described herein.

[0011] In some ~~aspects~~examples, the present disclosure provides methods of providing an anti-tumor immunity in a subject, the method comprising administering to the subject a therapeutically effective amount of the modified cells of any of the ~~embodiment~~examples described herein.

[0012] In some ~~aspects~~examples, the present disclosure provides methods of treating a subject having a disease associated with expression of a tumor antigen, the method comprising administering to the subject a therapeutically effective amount of the modified cells of any one of ~~embodiment~~examples described herein.

[0013] In another ~~aspect~~example, provided herein are compositions of immune cells modified by CasX and gNA gene editing pairs and, optionally, donor templates and/or polynucleotides encoding CAR and/or TCR for use as a medicament for the treatment of a subject having a disease associated with expression of a tumor antigen. In the foregoing, the CasX can be a CasX variant of any of the ~~embodiment~~examples described herein (e.g., the sequences of Table 4) and the gNA can be a gNA variant of any of the ~~embodiment~~examples described herein (e.g., the sequences of Table 2). In other ~~embodiment~~examples, the disclosure provides compositions cells modified by vectors comprising or encoding the gene editing pairs of CasX and gNA, donor templates and/or polynucleotides encoding CAR for use as a medicament for the treatment of a subject having a disease associated with expression of a tumor antigen.

[0014] In some ~~aspects~~examples, the present disclosure provides kits comprising the CasX:gNA systems, the vectors, or the VLP described herein, and further comprising an excipient and a container.

[0015] In another ~~aspect~~example, provided herein are CasX:gNA systems, compositions comprising CasX:gNA systems, vectors comprising or encoding CasX:gNA systems, VLP comprising

CasX:gNA systems, or populations of cells edited using the CasX:gNA systems, for use as a medicament for the treatment of a disease or disorder.

[0016] In another ~~aspect~~example, provided herein are CasX:gNA systems, composition comprising g CasX:gNA systems, or vectors comprising or encoding CasX:gNA systems, VLP comprising CasX:gNA systems, populations of cells edited using the CasX:gNA systems, for use in a method of treatment of a disease or disorder.

INCORPORATION BY REFERENCE

[0017] 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, which discloses CasX variants and gNA variants, are hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0019] FIG. 1 shows an SDS-PAGE gel of StX2 purification fractions visualized by colloidal Coomassie staining, as described in Example 1.

[0020] FIG. 2 shows the chromatogram from a size exclusion chromatography assay of the StX2, using of Superdex 200 16/600 pg Gel Filtration, as described in Example 1.

[0021] FIG. 3 shows an SDS-PAGE gel of StX2 purification fractions visualized by colloidal Coomassie staining, as described in Example 1.

[0022] FIG. 4 is a schematic showing the organization of the components in the pSTX34 plasmid used to assemble the CasX constructs, as described in Example 2.

[0023] FIG. 5 is a schematic showing the steps of generating the CasX 119 variant, as described in Example 2.

[0024] FIG. 6 shows an SDS-PAGE gel of purification samples, visualized on a Bio-Rad Stain-Free™ gel, as described in Example 2.

CLAIMS

What is claimed is:

1. A CasX:gNA system comprising a CasX protein and a first guide nucleic acid (gNA), wherein the gNA comprises a targeting sequence complementary to a target nucleic acid sequence of a gene encoding a first protein involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response.

~~**2.** The CasX:gNA system of claim 1, wherein the first protein is an immune cell surface marker or an immune checkpoint protein.~~

~~**3.** The CasX:gNA system of claim 1, wherein the first protein is an intracellular protein.~~

~~**4.**~~**2.** The CasX:gNA system of ~~any one of~~ claim~~s~~ 1~~-3~~, wherein the protein is selected from the group consisting of beta-2-microglobulin (B2M), T cell receptor alpha chain constant region (TRAC), class II major histocompatibility complex transactivator (CIITA), T cell receptor beta constant 1 (TRBC1), T cell receptor beta constant 2 (TRBC2), human leukocyte antigen A (HLA-A), human leukocyte antigen B (HLA-B), TGFβ Receptor 2 (TGFβRII), programmed cell death 1 (PD-1), cytokine inducible SH2 (CISH), lymphocyte activating 3 (LAG-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), adenosine A2a receptor (ADORA2A), killer cell lectin like receptor C1 (NKG2A), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin domain 3 (TIM-3), and 2B4 (CD244).

~~**5.**~~**3.** The CasX:gNA system of claim ~~4~~2, wherein the first protein is B2M and.

~~**6.** The CasX:gNA system of claim 5,~~ wherein the targeting sequence of the first gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 725-2100, 2281-7085, 547-551, 591-595 and 614-681- or a sequence having at least about 65%, at least about 75%, at least about 85%, or at least about 95% identity thereto.

~~**7.** The CasX:gNA system of claim 5, wherein the targeting sequence of the first gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 725-2100, 2281-7085, 547-551, 591-595, and 614-681.~~

~~**8.**~~**4.** The CasX:gNA system of claim ~~4~~2, wherein the first protein is TRAC, and~~.~~

~~**9.** The CasX:gNA system of claim 8,~~ wherein the targeting sequence of the first gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 7086-27454, 522-529 and 566-573, or a sequence having at least about 65%, at least about 75%, at least about 85%, or at least about 95% identity thereto.

~~10. The CasX:gNA system of claim 8, wherein the targeting sequence of the first gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 7086-27454, 522-529 and 566-573.~~

~~11.~~5. The CasX:gNA system of claim 42, wherein the first protein is CIITA, and.

~~12. The CasX:gNA system of claim 11,~~ wherein the targeting sequence of the first gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 27455-55572, or a sequence having at least about 65%, at least about 75%, at least about 85%, or at least about 95% identity thereto.

~~13. The CasX:gNA system of claim 11, wherein the targeting sequence of the first gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 27455-55572.~~

~~14.~~6. The CasX:gNA system of any one of claims 1-~~13~~5, further comprising a second gNA comprising a targeting sequence complementary to a target nucleic acid sequence of an immune cell gene encoding a second protein selected from the group consisting of beta-2-microglobulin (B2M), T cell receptor alpha chain constant region (TRAC), class II major histocompatibility complex transactivator (CIITA), T cell receptor beta constant 1 (TRBC1), T cell receptor beta constant 2 (TRBC2), human leukocyte antigen A (HLA-A), human leukocyte antigen B (HLA-B), TGFβRII, PD-1, CISH, LAG-3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, and CD244, wherein the second protein is different from the first protein.

~~15.~~7. The CasX:gNA system of claim 6~~14~~, wherein i) the first gNA targeting sequence is complementary to a B2M gene target nucleic acid sequence and the second gNA targeting sequence is complementary to a TRAC gene target nucleic acid sequence; ~~.~~

~~16. The CasX:gNA system of claim 14, wherein~~ ii) the first gNA targeting sequence is complementary to a B2M gene target nucleic acid sequence and the second gNA targeting sequence is complementary to a CIITA gene target nucleic acid sequence; or~~.~~

~~17. The CasX:gNA system of claim 14, wherein~~ iii) the first gNA targeting sequence is complementary to a TRAC gene target nucleic acid sequence and the second gNA targeting sequence is complementary to a CIITA gene target nucleic acid sequence.

~~8.~~

~~18.~~ The CasX:gNA system of ~~any one of~~ claim~~s 14-17~~7, ~~further~~ comprising a third gNA comprising a targeting sequence complementary to a target nucleic acid sequence of an immune cell gene encoding a third protein selected from the group consisting of ~~beta-2-microglobulin (B2M), T cell receptor alpha chain constant region (TRAC), class II major histocompatibility complex transactivator (CIITA),~~ T cell receptor beta constant 1 (TRBC1), T cell receptor beta constant 2 (TRBC2), human leukocyte antigen A (HLA-A), human

leukocyte antigen B (HLA-B), TGFβRII, PD-1, CISH, LAG-3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, ~~and~~ CD244, ~~wherein the third protein is different from the first and second proteins.~~

~~**19.** The CasX:gNA system of claim 18, wherein the first gNA targeting sequence is complementary to a target nucleic acid sequence of a gene encoding B2M, the second gNA targeting sequence is complementary to a target nucleic acid sequence of a gene encoding TRAC, and the third gNA targeting sequence is complementary to a target nucleic acid sequence of a gene encoding CIITA.~~

~~**20.** The CasX:gNA system of any one of claims 1-19, further comprising an additional gNA with a targeting sequence complementary to a target nucleic acid sequence of an immune cell gene encoding a protein selected from the group consisting of~~ cluster of differentiation 247 (CD247), CD3d molecule (CD3D), CD3e molecule (CD3E), CD3g molecule (CD3G), CD52 molecule (CD52), human leukocyte antigen C (HLA-C), deoxycytidine kinase (dCK), and FKBP prolyl isomerase 1A (FKBP1A).

~~**21.** The CasX:gNA system of any one of claims 1-20, wherein the first, second, third, and/or additional gNA is a guide RNA (gRNA).~~

~~**22.** The CasX:gNA system of any one of claims 1-20, wherein the gNA is a guide DNA (gDNA).~~

~~**23.** The CasX:gNA system of any one of claims 1-20, wherein the gNA is a chimera comprising DNA and RNA.~~

~~**24.** The CasX:gNA system of any one of claims 1-23, wherein the gNA is a single-molecule gNA (sgNA).~~

~~**25.** The CasX:gNA system of any one of claims 1-23, wherein the gNA is a dual-molecule gNA (dgNA).~~

~~**26.**__9.__ The CasX:gNA system of any one of claims 1-~~25~~8, wherein the targeting sequence of the gNA comprises 15, 16, 17, 18, 19, or 20 nucleotides.

~~**27.**__10.__ The CasX:gNA system of any one of claims 1-~~26~~9, wherein the gNA has a scaffold comprising a sequence selected from the group consisting of ~~reference gNA sequences of SEQ ID NOS: 4-16 or~~ gNA variant sequences of SEQ ID NOS:2101-2280, 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.

~~**28.** The CasX:gNA system of claim 27, wherein the gNA variant scaffold comprises a sequence having at least one modification relative to a reference gNA sequence selected from the group consisting of SEQ ID NOS:4-16.~~

~~**29.** The CasX:gNA system of claim 28, wherein the at least one modification of the reference gNA comprises at least one substitution, deletion, or substitution of a nucleotide of the gNA sequence.~~

~~**30.**__**11.**__ The CasX:gNA system of any one of ~~the preceding~~ claims __1-10__, wherein the gNA is chemically modified.

__**12.** The CasX:gNA system of any one of claims 1-11, wherein the CasX protein is a chimeric CasX protein comprising protein domains from two or more different CasX proteins.__

__**13.** The CasX:gNA system of claim 12, 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.__

__**14.** The CasX:gNA system of any one of claims 1-13, wherein the CasX protein comprises at least one chimeric domain.__

__**15.** The CasX:gNA system of any one of claims 1-11, wherein the CasX protein comprises a 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 engineered 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.__

~~**31.**__**16.**__ The CasX:gNA system of any one of ~~the preceding~~ claims __1-11__, wherein the CasX protein comprises ~~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: 49-143, 438, 440, 442, 444, 446, 448-460, 472, 474, 478, 480, 482, 484, 486, 488, 490, 612 or 613, 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.

17. The CasX:gNA system of any one of claims 1-11, wherein the CasX protein comprises a sequence of SEQ ID NO: 138, or a sequence having at least 90% identity thereto.

~~**32.** The CasX:gNA system of claim 31, 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.~~

~~**33.** The CasX:gNA system of claim 32, 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.~~

~~**34.** The CasX:gNA system of claim 33, 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.~~

~~**35.**~~**18.** The CasX:gNA system of any one of claims ~~31-34~~1-17, wherein the CasX protein further comprises one or more nuclear localization signals (NLS)~~.~~

~~**36.** The CasX:gNA system of claim 35,~~ wherein the one or more NLS are selected from the group of sequences consisting of PKKKRKV (SEQ ID NO: 158), KRPAATKKAGQAKKKK (SEQ ID NO: 159), PAAKRVKLD (SEQ ID NO: 160), RQRRNELKRSP (SEQ ID NO: 161), NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 162), RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 163), VSRKRPRP (SEQ ID NO: 164), PPKKARED (SEQ ID NO: 165), PQPKKKPL (SEQ ID NO: 166), SALIKKKKKMAP (SEQ ID NO: 167), DRLRR (SEQ ID NO: 168), PKQKKRK (SEQ ID NO: 169), RKLKKKIKKL (SEQ ID NO: 170), REKKKFLKRR (SEQ ID NO: 171), KRKGDEVDGVDEVAKKKSKK (SEQ ID NO: 172), RKCLQAGMNLEARKTKK (SEQ ID NO: 173), PRPRKIPR (SEQ ID NO: 174), PPRKKRTVV (SEQ ID NO: 175), NLSKKKKRKREK (SEQ ID NO: 176), RRPSRPFRKP (SEQ ID NO: 177), KRPRSPSS (SEQ ID NO: 178), KRGINDRNFWRGENERKTR (SEQ ID NO: 179), PRPPKMARYDN (SEQ ID NO: 180), KRSFSKAF (SEQ ID NO: 181), KLKIKRPVK (SEQ ID NO: 182), PKTRRRPRRSQRKRPPT (SEQ ID NO:184), RRKKRRPRRKKRR (SEQ ID NO: 187), PKKKSRKPKKKSRK (SEQ ID NO: 188), HKKKHPDASVNFSEFSK (SEQ ID NO: 189), QRPGPYDRPQRPGPYDRP (SEQ ID NO: 190), LSPSLSPLLSPSLSPL (SEQ ID NO: 191), RGKGGKGLGKGGAKRHRK (SEQ ID NO: 192), PKRGRGRPKRGRGR (SEQ ID NO: 193), MSRRRKANPTKLSENAKKLAKEVEN (SEQ ID NO: 185),

PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 183), and PKKKRKVPPPPKKKRKV (SEQ ID NO: 194).

~~37.~~19. The CasX:gNA system of claim ~~35 or claim 36~~18, wherein the one or more NLS are expressed at or near the C-terminus and/or the N-terminus of the CasX protein.

~~38.~~ ~~The CasX:gNA system of claim 35 or claim 36, wherein the one or more NLS are expressed at or near the N-terminus of the CasX protein.~~

~~39.~~ ~~The CasX:gNA system of claim 35 or claim 36, comprising one or more NLS located at or near the N-terminus and at or near the C-terminus of the CasX protein.~~

~~40.~~20. The CasX:gNA system of any one of claims ~~31-39~~1-19, wherein the CasX variant is capable of forming a ribonuclear protein complex (RNP) with the variant gNA, and wherein~~.~~

~~41.~~ ~~The CasX:gNA system of claim 40, wherein an~~ the RNP ~~of the CasX variant protein and the gNA variant~~ exhibits at least one or more improved characteristics as compared to an RNP of a reference CasX protein of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 and a gNA comprising a sequence of any one of SEQ ID NOS: 4-16.

~~42.~~21. The CasX:gNA system of claim ~~41~~20, wherein the improved characteristic is selected from one or more of 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 ability to form cleavage-competent RNP; and improved fusion characteristics.

~~43.~~ ~~The CasX:gNA system of claim 41 or claim 42, 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 gNA of any one of SEQ ID NOS: 4-16.~~

~~44.~~ ~~The CasX:gNA system of claim 41 or claim 42, 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 the sequence of any one of SEQ ID NOS: 4-16.~~

~~45.~~22. The CasX:gNA system of ~~any one of~~ claim~~s 41-43~~21, wherein the improved characteristic comprises editing efficiency, and the RNP of the CasX variant protein and the gNA variant comprises a 1.1 to 100-fold improvement in editing efficiency compared to the RNP of the reference CasX protein of SEQ ID NO:2 and the gNA comprising the sequence of any one of SEQ ID NOS: 4-16.

~~46. The CasX:gNA system of any one of claims 40-45, 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 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 of SEQ ID NO:2 and the gNA comprising the sequence of any one of SEQ ID NOS: 4-16 in a comparable assay system.~~

~~47. The CasX:gNA system of claim 46, wherein the PAM sequence is TTC.~~

~~48. The CasX:gNA system of claim 46, wherein the PAM sequence is ATC.~~

~~49. The CasX:gNA system of claim 46, wherein the PAM sequence is CTC.~~

~~50. The CasX:gNA system of claim 46, wherein the PAM sequence is GTC.~~

~~51. The CasX:gNA system of any one of claims 46-50, wherein the increased binding affinity for the one or more PAM sequences is at least 1.5-fold to at least 10-fold greater compared to the binding affinity of any one of the reference CasX proteins of SEQ ID NOS: 1-3 for the PAM sequences.~~

~~52. The CasX:gNA system of any one of claims 40-51, 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 of SEQ ID NOS: 1-3 and the gNA comprising a sequence of any one of SEQ ID NOS: 4-16.~~

~~53. The CasX:gNA system of any one of claims 31-52, wherein the CasX variant protein comprises a RuvC DNA cleavage domain having nickase activity.~~

~~54. The CasX:gNA system of any one of claims 31-52, wherein the CasX variant protein comprises a RuvC DNA cleavage domain having double-stranded cleavage activity.~~

~~55. The CasX:gNA system of any one of claims 1-40, 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 SOD1 target nucleic acid.~~

~~**56.** The CasX:gNA system of claim 55, 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.~~

~~**57.** The CasX:gNA system of claim 56, wherein the mutation is a substitution of alanine for the residue.~~

~~**58.** The CasX:gNA system of any one of claims 1-54, further comprising a donor template nucleic acid.~~

~~**59.** The CasX:gNA system of claim 58, wherein the donor template comprises a polynucleotide comprising all or a portion of a gene encoding a protein selected from the group consisting of B2M, TRAC, CIITA, TRBC1, TRBC2, HLA-A, HLA-B, TGFβRII, PD-1, CISH, LAG-3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, and CD244, , wherein the polynucleotide comprises a deletion, insertion, or mutation of one or more nucleotides in comparison to a genomic polynucleotide sequence encoding the protein.~~

~~**60.**~~**23.** A polynucleotide comprising a sequence that encodes the CasX of any one of claims ~~31-57~~1-22.

~~**61.**~~**24.** A polynucleotide comprising a sequence that encodes the gNA of any one of claims 1-~~30~~11.

~~**62.** A polynucleotide comprising the donor template of claim 58 or claim 59.~~

~~**63.** A vector comprising one or more of the polynucleotides of claims 60-62.~~

~~**64.**~~**25.** A vector comprising the polynucleotide of ~~any one of~~ claim~~s 60-62~~23 or claim 24.

~~**65.** The vector of claim 63 or claim 64, wherein the vector further comprises a promoter.~~

~~**66.**~~**26.** The vector of ~~any one of claims 63-65~~claim 25, 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 virus-like particle (VLP), a herpes simplex virus (HSV) vector, a plasmid, a minicircle, a nanoplasmid, a DNA vector, and an RNA vector.

~~**67.** The vector of claim 66, wherein the vector is an AAV vector.~~

~~**68.**~~**27.** The vector of claim ~~67~~26, wherein the AAV vector is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-Rh74, or AAVRh10.

~~**69.** The vector of claim 66, wherein the vector is a retroviral vector.~~

~~**70.**~~**28.** A virus-like particle (VLP) comprising a. one or more components of a gag polyprotein selected from the group of matrix protein (MA), nucleocapsid protein (NC),

capsid protein (CA), p1-p6 protein, and a protease cleavage site, and further comprising a targeting glycoprotein that provides for binding and fusion of the VLP to a target cell; .

~~71. The VLP of claim 70 comprising~~ b. the CasX protein ~~of any one of claims 31-57,~~ and the gNA of any one of claims 1-~~30~~11, ~~and optionally comprising the polynucleotide of claim 62.~~

~~72.~~ ~~The VLP of claim 71,~~ c. wherein the CasX protein and the gNA are associated together in an RNP.

~~73.~~**29.** An *ex vivo* method of modifying a target nucleic acid sequence of a gene in a population of cells, wherein the gene encodes a protein involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response, comprising introducing into each cell of the population:

a. the CasX:gNA system of any one of claims 1-~~59~~22;

b. the polynucleotide of ~~any one of~~ claim~~s~~ 23 or claim 24~~60-62~~;

c. the vector as in any one of claims ~~63~~25-27;

d. the VLP of ~~any one of~~ claim~~s~~ ~~70-72~~28; or

e. combinations of two or more of (a) to (d),

wherein the target nucleic acid sequence of the cells is modified by the CasX protein, wherein the modifying comprises introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the target nucleic acid sequence of the cells, resulting in a knock-down or knock-out of a gene encoding one or more proteins selected from the group consisting of B2M, TRAC, CIITA, TRBC1, TRBC2, HLA-A, HLA-B, TGFβRII, PD-1, CISH, LAG3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, CD244, and wild-type T-cell receptor.

30. Use of

a. the CasX:gNA system of any one of claims 1-22;

b. the polynucleotide of claim 23 or claim 24;

c. the vector as in any one of claims 25-27;

d. the VLP of claim 28; or

e. combinations of two or more of (a) to (d),

in the manufacture of a medicament for modifying a target nucleic acid sequence of a gene in a population of cells in a subject for treating a cancer, wherein the gene encodes a protein involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response, comprising introducing into each cell of the population: wherein the target nucleic acid sequence of the cells is modified by the CasX protein, wherein the modifying comprises

introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the target nucleic acid sequence of the cells, resulting in a knock-down or knock-out of a gene encoding one or more proteins selected from the group consisting of B2M, TRAC, CIITA, TRBC1, TRBC2, HLA-A, HLA-B, TGFβRII, PD-1, CISH, LAG3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, CD244, and wild-type T-cell receptor.

31. The method of claim 29 or the use of claim 30, wherein the cells of the population have been modified such that expression of the one or more proteins is reduced by 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% in comparison to a cell that has not been modified.

32. The method or use of any one of claims 29 to 31, wherein the cells of the population have been modified such that 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% of the cells do not express a detectable level of the one or more proteins in comparison to a cell that has not been modified.

~~**74.** The method of claim 73, wherein the CasX:gNA system is introduced into the cells as an RNP.~~

~~**75.**~~**33.** The method or use of any one of claims ~~73 or claim 74~~29-32, wherein the cells are modified by introduction of a polynucleotide encoding: i) a chimeric antigen receptor (CAR) with binding affinity for a disease antigen, optionally a tumor cell antigen; and/or ii) ~~.~~

~~**76.** The method of claim 73 or claim 74, wherein the cells are modified by introduction of a polynucleotide encoding~~ an engineered T cell receptor (TCR) comprising a binding domain with binding affinity for a disease antigen, optionally a tumor cell antigen.

~~**77.**~~**34.** The method or use of claim ~~74 or claim 75~~33, wherein the tumor cell antigen is selected from the group consisting of Cluster of Differentiation 19 (CD19), cluster of differention 3 (CD3), CD3d molecule (CD3D), CD3g molecule (CD3G), CD3e molecule (CD3E), CD247 molecule (CD247, or CD3Z), CD8a molecule (CD8), CD7 molecule (CD7), membrane metalloendopeptidase (CD10), membrane spanning 4-domains A1 (CD20), CD22 molecule (CD22), TNF receptor superfamily member 8 (CD30), C-type lectin domain family 12 member A (CLL1), CD33 molecule (CD33), CD34 molecule (CD34), CD38 molecule (CD38), integrin subunit alpha 2b (CD41), CD44 molecule (Indian blood group) (CD44), CD47 molecule (CD47), integrin alpha 6 (CD49f), neural cell adhesion molecule 1 (CD56), CD70 molecule (CD70), CD74 molecule (CD74), CD99 molecule (Xg blood group) (CD99), interleukin 3 receptor subunit alpha (CD123), prominin 1 (CD133), syndecan 1 (CD138), carbonix anhydrase IX (CAIX), CC chemokine receptor 4 (CCR4), ADAM metallopeptidase

domain 12 (ADAM12), adhesion G protein-coupled receptor E2 (ADGRE2), alkaline phosphatase placental-like 2 (ALPPL2), alpha 4 Integrin, angiopoietin-2 (ANG2), B-cell maturation antigen (BCMA), CD44V6, carcinoembryonic antigen (CEA), CEAC, CEA cell adhesion molecule 5 (CEACAM5), Claudin 6 (CLDN6), claudin 18 (CLDN18), C-type lectin domain family 12 member A (CLEC12A), mesenchymal-epithelial transition factor (cMET), cytotoxic T-lymphocyte-associated protein 4 (CTLA4), epidermal growth factor receptor 1 (EGF1R), epidermal growth factor receptor variant III (EGFRvIII), epithelial glycoprotein 2 (EGP-2), epithelial cell adhesion molecule (EGP-40 or EpCAM), EPH receptor A2 (EphA2), ectonucleotide pyrophosphatase/phosphodiesterase 3 (ENPP3), erb-b2 receptor tyrosine kinase 2 (ERBB2), erb-b2 receptor tyrosine kinase 3 (ERBB3), erb-b2 receptor tyrosine kinase 4 (ERBB4), folate binding protein (FBP), fetal nicotinic acetylcholine receptor (AChR), folate receptor alpha (FRalpha or FOLR1), G protein-coupled receptor 143 (GPR143), glutamate metabotropic receptor 8 (GRM8), glypican-3 (GPC3), ganglioside GD2, ganglioside GD3, human epidermal growth factor receptor 1 (HER1), human epidermal growth factor receptor 2 (HER2), human epidermal growth factor receptor 3 (HER3), Integrin B7, intercellular cell-adhesion molecule-1 (ICAM-l), human telomerase reverse transcriptase (hTERT), Interleukin-13 receptor α2 (IL-l3R-a2), K-light chain, Kinase insert domain receptor (KDR), Lewis-Y (LeY), chondromodulin-1 (LECT1), Ll cell adhesion molecule (L1CAM), Lysophosphatidic acid receptor 3 (LPAR3), melanoma-associated antigen 1 (MAGE-A1), mesothelin (MSLN), mucin 1 (MUC1), mucin 16, cell surface associated (MUC16), melanoma-associated antigen 3 (MAGE-A3), tumor protein p53 (p53), Melanoma Antigen Recognized by T cells 1 (MART1), glycoprotein 100 (GPl00), Proteinase3 (PR1), ephrin-A receptor 2 (EphA2), Natural killer group 2D ligand (NKG2D ligand), New York esophageal squamous cell carcinoma 1 (NY-ESO-1), oncofetal antigen (h5T4), prostate-specific membrane antigen (PSMA), programmed death ligand 1 (PDL-1), receptor tyrosine kinase-like orphan receptor 1 (ROR1), trophoblast glycoprotein (TPBG), tumor-associated glycoprotein 72 (TAG-72), tumor-associated calcium signal transducer 2 (TROP-2), tyrosinase (TYR), survivin, vascular endothelial growth factor receptor 2 (VEGF- R2), Wilms tumor-1 (WT-1), leukocyte immunoglobulin-like receptor B2 (LILRB2), Preferentially Expressed Antigen In Melanoma (PRAME), T cell receptor beta constant 1 (TRBC1), TRBC2, and (T-cell immunoglobulin mucin-3) TIM-3.

35. The method or use of claim 33 or claim 34, wherein the CAR and/or the TCR comprises an antigen binding domain, wherein the antigen binding domain is an scFv with

binding affinity to the tumor cell antigen, and wherein the CAR comprises at least one intracellular signaling domain, an extracellular hinge domain, and a transmembrane domain.

~~**78.** The method of any one of claims 75-77, wherein the CAR and/or the TCR comprises an antigen binding domain selected from the group consisting of a linear antibody, single domain antibody (sdAb), and single-chain variable fragment (scFv).~~

~~**79.** The method of claim 78, wherein the antigen binding domain is an scFv with binding affinity to the tumor cell antigen.~~

~~**80.**~~**36.** The method or use of claim ~~79~~35, wherein the antigen binding domain is an scFv comprising variable heavy (VH) and variable light (VL) and/or heavy chain and light chain CDRs selected from the group consisting of the sequences set forth in Table 5.

~~**81.** The method of claim 80, wherein the VH, VL, and/or the CDRs of the scFv have one or more amino acid modifications wherein the scFv retains binding affinity to the tumor antigen, and wherein the modification is selected from the group consisting of a substitution, deletion, and insertion.~~

~~**82.** The method of any one of claims 75-81, wherein the CAR further comprises at least one intracellular signaling domain.~~

~~**83.**~~**37.** The method or use of claim 35 or claim 36~~82~~, wherein the at least one intracellular signaling domain comprises at least one intracellular signaling domain isolated or derived from CD247 molecule (CD3-zeta), CD27 molecule (CD27), CD28 molecule (CD28), TNF receptor superfamily member 9 (4-1BB), inducible T cell costimulator (ICOS), or TNF receptor superfamily member 4 (OX40).

38. The method or use of any one of claims -35-37, wherein the hinge domain is an immunoglobulin like domain.

~~**84.** The method of claim 83, wherein the at least one intracellular signaling domain comprises:~~

~~a. a CD3-zeta intracellular signaling domain;~~

~~b. a CD3-zeta intracellular signaling domain and a 4-1BB or CD28 intracellular signaling domain;~~

~~c. a CD-zeta intracellular signaling domain, a 4-1BB intracellular signaling domain, and a CD28 intracellular signaling domain; or~~

~~d. a CD-zeta intracellular signaling domain, a CD28 intracellular signaling domain, a 4-1BB intracellular signaling domain, and a CD27 or OX40 intracellular signaling domain.~~

~~**85.** The method of any one of claims 75-84, wherein the CAR further comprises an extracellular hinge domain.~~

~~**86.** The method of claim 85, wherein the hinge domain is an immunoglobulin like domain.~~

~~**87.**~~**39.** The method or use of claim ~~86~~38, wherein the hinge domain is isolated or derived from IgG1, IgG2, ~~or~~ IgG4, a CD8a molecule (CD8) or CD28.

~~**88.** The method of claim 86, wherein the hinge domain is isolated or derived from CD8a molecule (CD8) or CD28.~~

~~**89.** The method of any one of claims 75-88, wherein the CAR further comprises a transmembrane domain.~~

~~**90.**~~**40.** The method or use of any one of claims ~~89~~35-39, wherein the transmembrane domain is isolated or derived from the group consisting of CD3-zeta, CD4, CD8, and CD28.

~~**91.**~~**41.** The method or use of any one of claims ~~76-81~~35-40, wherein the TCR comprises one or more subunits selected from the group consisting of TCR alpha, TCR beta, CD3-delta, CD3-epsilon, CD-gamma or CD3-zeta.

~~**92.**~~**42.** The method or use of any one of claims-35-41~~91~~, wherein the TCR ~~further~~ comprises one or more intracellular signaling domains selected from the group consisting of CD247 molecule (CD3-zeta), CD27 molecule (CD27), CD28 molecule (CD28), TNF receptor superfamily member 9 (4-1BB), inducible T cell costimulator (ICOS), or TNF receptor superfamily member 4 (OX40).

~~.~~

~~**93.**~~**43.** The method or use of any one of claims-35-42~~90 or claim 91~~, wherein the antigen binding domain of the TCR is operably linked to one or more TCR subunits selected from the group consisting of ~~of~~ TCR alpha, TCR beta, CD3-delta, CD3-epsilon, CD-gamma or CD3-zeta.

~~**94.** The method of claim 93, wherein the antigen binding domain of the TCR is an scFv comprising variable heavy (VH) and variable light (VL) and/or heavy chain and light chain CDRs selected from the group consisting of the sequences set forth in Table 5.~~

~~**95.** The method of claim 94, wherein the VH, VL, and/or the CDRs of the scFv have one or more amino acid modifications wherein the scFv retains binding affinity to the tumor antigen, and wherein the modification is selected from the group consisting of a substitution, deletion, and insertion.~~

~~96.~~44. The method or use of any one of claims ~~73-95~~29-43, wherein the cells are selected from the group consisting of rodent cells, mouse cells, rat cells, ~~and~~ non-human primate, and human cells.

~~97. The method of any one of claims 73-95, wherein the cells are human cells.~~

~~98.~~45. The method or use of any one of claims ~~73-97~~29-44, wherein the cells are selected from the group consisting of progenitor cells, hematopoietic stem cells, ~~and~~ pluripotent stem cells, and induced pluripotent stem cells.

~~99. The method of claim 98, wherein the cells are induced pluripotent stem cells.~~

~~100.~~46. The method or use of any one of claims ~~73-97~~29-44, wherein the cells are immune cells~~.~~

~~101. The method of claim 100, wherein the immune cells are~~ selected from the group consisting of T cells, tumor infiltrating lymphocytes, NK cells, B cells, monocytes, macrophages, or dendritic cells.

~~102.~~47. The method or use of claim ~~101~~46, wherein the T cells are selected from the group consisting of CD4+ T cells, CD8+ T cells, cytotoxic T cells, terminal effector T cells, memory T cells, naïve T cells, regulatory T cells, natural killer T cells, gamma-delta T cells, cytokine-induced killer (CIK) T cells, and tumor infiltrating lymphocytes, or a combination thereof.

~~103. The method of any one of claims 73-102, wherein the modifying comprises introducing one or more single-stranded breaks in the target nucleic acid sequence of the cells of the population.~~

~~104. The method of any one of claims 73-102, wherein the modifying comprises introducing one or more double-stranded breaks in the target nucleic acid sequence of the cells of the population.~~

~~105. The method of any one of claims 73-104, wherein the modifying comprises introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the target nucleic acid sequence of the cells of the population, resulting in a knock-down or knock-out of a gene in the cells of the population encoding one or more proteins selected from the group consisting of B2M, TRAC, CIITA, TRBC1, TRBC2, HLA-A, HLA-B, TGFβRII, PD-1, CISH, LAG3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, and CD244.~~

~~106. The method of any one of claims 73-104, wherein the method comprises insertion of the donor template of claim 58 or claim 59 into the break site(s) of the target nucleic acid sequence of the cells of the population.~~

~~**107.** The method of claim 106, wherein the insertion of the donor template is mediated by homology-directed repair (HDR) or homology-independent targeted integration (HITI).~~

~~**108.** The method of claim 106 or claim 107, wherein insertion of the donor template results in a knock-down or knock-out of the gene in the cells of the population encoding one or more proteins selected from the group consisting of B2M, TRAC, CIITA, TRBC1, TRBC2, HLA-A, HLA-B, TGFβRII, PD-1, CISH, LAG-3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, and CD244.~~

~~**109.** The method of any one of claims 105-108, wherein the cells of the population have been modified such that expression of the one or more proteins is reduced by 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% in comparison to a cell that has not been modified.~~

~~**110.** The method of any one of claims 105-109, wherein the cells of the population have been modified such that 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% of the cells do not express a detectable level of the one or more proteins in comparison to a cell that has not been modified.~~

~~**111.** The method of any one of claims 105-110, wherein the one or more proteins are selected from the group consisting of B2M, TRAC, and CIITA.~~

~~**112.** The method of claim 111, wherein the cells of the population have been modified such that 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% of the cells do not express a detectable level of at least two of the proteins selected from the group consisting of B2M, TRAC, and CIITA.~~

~~**113.** The method of any one of claims 105-112, wherein the cells have been modified such that 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% of the population of cells do not express a detectable level of MHC Class I molecules.~~

~~**114.** The method of any one of claims 105-113, wherein the cells have been modified such that 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% of the population of cells do not express a detectable level of wild-type T cell receptor.~~

~~**115.**~~**48.** The method or use of any one of claims ~~105-114~~33-47, wherein the population of cells expresses a detectable level of the CAR and/or the TCR.

~~**116.** The method of any one of claims 105-115, wherein the population of cells expresses a detectable level of the TCR.~~

~~**117.** The method of any one of claims 73-115, wherein the method is conducted ex vivo on the population of cells.~~

~~**118.** The method of any one of claims 73-115, wherein the method is conducted in vivo in a subject.~~

~~**119.**~~**49.** The method or use of any one of claims ~~118~~30-48, wherein the subject is selected from the group consisting of a rodent, a mouse, a rat, ~~and~~ a non-human primate, and a human.

~~**120.** The method of claim 118, wherein the subject is human.~~

~~**121.**~~**50.** A population of cells modified *ex vivo* by the method of any one of claims ~~73-117~~29-49.

~~**122.** The population of cells of claim 121, 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 population of cells do not express a detectable level of MHC Class I molecules.~~

~~**123.** The population of cells of claim 121 or claim 122, 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 population of cells do not express a detectable level of wild-type T cell receptor.~~

~~**124.** The population of cells of any one of claims 121-123, wherein the cell has 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 population of cells express a detectable level of the chimeric antigen receptor (CAR).~~

~~**125.** The population of cells of claim 121-124, wherein the cell has 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 population of cells express a detectable level of an immune stimulatory cytokine selected from the group consisting of interleukin 7 (IL-7), IL-12, IL-15, and IL-18.~~

~~**126.** The population of cells of any one of claims 121-125, wherein the cell has 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 population of cells express a detectable level of the TCR.~~

~~**127.**~~**51.** The population of cells of ~~any one of~~ claim~~s~~ 50~~124-126~~, wherein upon binding of the CAR to the tumor antigen of a cell bearing said tumor antigen, the population of cells are capable of a response selected from: i) becoming activated; ii) inducing proliferation of the population of cells; iii) cytokine secretion by the population of cells;iv) inducing cytotoxicity of the cell bearing said tumor antigen, or v) a combination of any one of (i)-(iv).

~~128.~~**52.** Use of the population of cells of claim 50 or 51 in the manufacture of a medicament for ~~A method of~~ providing anti-tumor immunity in a subject~~, the method comprising administering to the subject a therapeutically effective amount of the population of cells of any one of claims 121-127~~.

~~**129.** A method of treating a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the population of cells of any one of claims 121-127.~~

~~**130.**~~**53.** The ~~method~~ use of claim ~~129~~52, wherein the subject has a cancer ~~or an autoimmune disease~~.

~~**131.**~~**54.** The ~~method~~ use of claim ~~130~~52 or 53, wherein the cancer selected from the group consisting of colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers, chronic lymphocytic leukemia (CLL), acute leukemias, acute lymphoid leukemia (ALL), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, pre-leukemia, combinations of said cancers, and metastatic lesions of said cancers.

~~132.~~55. The ~~method~~ use of claim ~~130 or 131~~54, wherein the cancer expresses a tumor cell antigen.

~~133.~~56. The ~~method~~ use of claim ~~132~~55, wherein the CAR and/or TCR has a specific binding affinity to the tumor cell antigen.

~~134.~~57. The ~~method~~ use of claim ~~133~~56, wherein upon binding of the CAR and/or TCR to the tumor antigen , the population of cells are capable of: i) becoming activated; ii) inducing proliferation of the population of cells; iii) cytokine secretion by the population of cells; iv) inducing cytotoxicity of the cell bearing said tumor antigen, or v) a combination of any one of (i)-(iv). ~~.~~

~~135.~~58. The ~~method~~ use of any one of claims ~~128-134~~52-57, wherein the population of cells is to be administered to the subject by a route of administration selected from intraparenchymal, intravenous, intra-arterial, intracerebroventricular, intracisternal, intrathecal, intracranial, lumbar, intraperitoneal, subcutaneous, intraocular, periocular, subretinal, intravitreal, intrapulmonary, intranasal, and combinations thereof.

~~136.~~59. The ~~method~~ use of any one of claims ~~128-135~~52-58, wherein the administration of the therapeutically effective amount of the population of cells results in an improvement in a clinical parameter or endpoint associated with the disease in the subject selected from one or more of tumor shrinkage as a complete, partial or incomplete response; time-to-progression, time to treatment failure, biomarker response; progression-free survival; disease free-survival; time to recurrence; time to metastasis; time of overall survival; improvement of quality of life; and improvement of symptoms.

~~137. The method of any one of claims 128-136, wherein the method further comprises administering a chemotherapeutic agent.~~

~~138. A method of preparing cells for immunotherapy in a subject, comprising modifying immune cells by reducing or eliminating expression of one or more proteins involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response.~~

~~139. The method of claim 138, comprising contacting a target nucleic acid sequence of the immune cell with a CasX:gNA system comprising a CasX protein and one or more gNA, wherein each gNA comprises a targeting sequence complementary to a target nucleic acid sequence of one or more genes encoding the one or more proteins involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response.~~

~~140. The method of claim 138 or claim 139, wherein the one or more proteins are selected from the group consisting of B2M, TTRAC, CIITA, TRBC1, TRBC2, HLA-A, HLA-B, TGFβRII, PD-1, CISH, LAG-3, TIGIT, ADORA2A, NKG2A, CTLA-4, TIM-3, and CD244.~~

141. The method of claim 140, wherein the one or more proteins are selected from the group consisting of B2M, TRAC, and CIITA.

142. The method of claim 140 or claim 141, further comprising a gNA comprising a targeting sequence complementary to a nucleic acid sequence of a gene encoding a protein selected from the group consisting of CD247, CD3D, CD3E, CD3G, CD52, human leukocyte antigen C (HLA-C), deoxycytidine kinase (dCK), and FKBP1A.

143. The method of any one of claims 138-142, wherein the cells have been modified such that expression of the one or more proteins is reduced by 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% in comparison to a cell that has not been modified.

144. The method of any one of claims 138-143, wherein the cells have been modified such that the cells do not express a detectable level of the one or more proteins.

145. The method of any one of claims 138-144, 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 MHC Class I molecules.

146. The method of claim 138-145, 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 wild-type T cell receptor.

147. The method of any one of claims 138-146, further comprising introducing into the immune cell a polynucleic acid encoding a chimeric antigen receptor (CAR) with specific binding affinity for a tumor cell antigen.

148. The method of any one of claims 138-147, further comprising introducing into the immune cell a polynucleic acid encoding an engineered T cell receptor (TCR) comprising a binding domain with binding affinity for a disease antigen, optionally a tumor cell antigen.

149. The method of claim 147, wherein the tumor cell antigen is selected from the group consisting ofCD19, CD3, CD3D, CD3G, CD3E, CD247, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD47, CD49f, CD56, CD70, CD74, CD99, CD123, CD133, CD138, CAIX, CCR4, ADAM12, ADGRE2, ALPPL2, ANG2, BCMA, CD44V6, CEA, CEAC, CEACAM5, CLDN6, CLDN18, CLEC12A, cMET, CTLA-4, EGF1R, EGFR-vIII, EGP-2, EGP-40, EphA2, ENPP3, EpCAM, ERBB2, ERBB3, ERBB4, FBP, AChR, FRalpha, GPR143, GRM8, gGPC3, ganglioside GD2, ganglioside GD3, HER1, HER2, HER3, Integrin B7, ICAM-1, hTERT, IL-l3R-a2, K-light chain, KDR, Lewis-Y, LECT1, L1CAM, LPAR3, MAGE-A1, MSLN, MUC1, MUC16, MAGE-A3, p53, MART1, GPl00, PR1, EphA2, NKG2D ligand, NY-ESO-1, h5T4, PSMA, PDL-1, ROR1,

TPBG, TAG-72, TROP-2, TYR, survivin, VEGF-R2, WT-1, LILRB2, PRAME, TRBC1, TRBC2, and TIM-3. .

150. The method of claim 147 or claim 148, wherein the CAR comprises an antigen binding domain selected from the group consisting of linear antibody, single domain antibody (sdAb), and single-chain variable fragment (scFv).

151. The method of claim 150, wherein the antigen binding domain is a scFv comprising variable heavy (VH) and variable light (VL) and/or heavy chain and light chain CDRs selected from the group consisting of the sequences set forth in Table 5.

152. The method of claim 151, wherein the VH, VL, and/or the CDRs of the scFv have one or more amino acid modifications wherein the scFv retains binding affinity to the tumor antigen, and wherein the modification is selected from the group consisting of a substitution, deletion, and insertion.

153. The method of any one of claims 147-152, wherein the CAR further comprises at least one intracellular signaling domain.

154. The method of claim 153, wherein the at least one intracellular signaling domain comprises at least one intracellular signaling domain isolated or derived from CD247 molecule (CD3-zeta), CD27 molecule (CD27), CD28 molecule (CD28), TNF receptor superfamily member 9 (4-1BB), inducible T-cell costimulator (ICOS), or TNF receptor superfamily member 4 (OX40).

155. The method of claim 154, wherein the at least one intracellular signaling domain comprises:

a. a CD3-zeta intracellular signaling domain;

b. a CD3-zeta intracellular signaling domain and a 4-1BB or CD28 intracellular signaling domain;

c. a CD-zeta intracellular signaling domain, a 4-1BB intracellular signaling domain, and a CD28 intracellular signaling domain;

d. a CD-zeta intracellular signaling domain, a CD28 intracellular signaling domain, a 4-1BB intracellular signaling domain, and a CD27 or OX40 intracellular signaling domain.

156. The method of any one of claims 147-155, wherein the CAR further comprises an extracellular hinge domain.

157. The method of claim 156, wherein the hinge domain is an immunoglobulin like domain.

158. The method of claim 157, wherein the hinge domain is isolated or derived from IgG1, IgG2, or IgG4.

159. The method of claim 157, wherein the hinge domain is isolated or derived from CD8a molecule (CD8) or CD28.

160. The method of any one of claims 147-159, wherein the CAR further comprises a transmembrane domain.

161. The method of claim 160, wherein the transmembrane domain is isolated or derived from from the group consisting of CD3-zeta, CD4, CD8, and CD28.

162. The method of any one of claims 148-161, wherein the TCR comprises one or more subunits selected from the group consisting of TCR-alpha, TCR-beta, CD3-delta, CD3-epsilon, CD-gamma or CD3-zeta.

163. The method of claim 162, wherein the TCR further comprises an intracellular domain comprising a stimulatory domain from an intracellular signaling domain.

164. The method of claim 162 or claim 163, wherein the antigen binding domain of the TCR is operably linked to the TCR-alpha or the TCR-beta subunits.

165. The method of claim 164, wherein the antigen binding domain of the TCR is an scFv comprising variable heavy (VH) and variable light (VL) and/or heavy chain and light chain CDRs selected from the group consisting of the sequences set forth in Table 5.

166. The method of claim 165, wherein the VH, VL, and/or the CDRs of the scFv have one or more amino acid modifications wherein the scFv retains binding affinity to the tumor antigen, and wherein the modification is selected from the group consisting of a substitution, deletion, and insertion.

167. The method of any one of claims 147-166, further comprising introducing into the immune cell a polynucleotide encoding an immune stimulatory cytokine selected from the group consisting of IL-7, IL-12, IL-15, and IL-18.

168. The method of any one of claims 138-167, further comprising expanding a population of said cells by in vitro culture in an appropriate medium under appropriate growth conditions.

169. The method of any one of claims 138-168, wherein the cells are autologous to the subject to receive the cells.

170. The method of any one of claims 138-168, wherein the cells are allogeneic to the subject to receive the cells.

171. The method of any one of claims 138-170, wherein the subject has cancer or an autoimmune disease.

172. The method of claim 171, wherein the cancer selected from the group consisting of colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of

~~the lung, cancer of the small intestine, cancer of the esophagus, melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers, chronic lymphocytic leukemia (CLL), acute leukemias, acute lymphoid leukemia (ALL), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), B-cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, pre-leukemia, combinations of said cancers, and metastatic lesions of said cancers.~~

~~173.~~ ~~The method of claim 171 or claim 172, wherein the cancer expresses a tumor cell antigen.~~

~~174.~~ ~~The method of claim 173, wherein the CAR has a specific binding affinity to the tumor cell antigen.~~

~~175.~~ ~~The method of claim 174, wherein upon binding of the CAR to the tumor antigen, the cells are capable of: i) becoming activated; ii) inducing proliferation of the cells; iii) inducing cytokine secretion by the cells; iv) inducing cytotoxicity of the cell bearing said tumor antigen, or v) a combination of any one of (i)-(iv).~~

~~176.~~ ~~The method of any one of claims 138-175, wherein the cells are administered to the subject by a route of administration selected from intraparenchymal, intravenous, intra-arterial, intracerebroventricular, intracisternal, intrathecal, intracranial, lumbar,~~

intraperitoneal, subcutaneous, intraocular, periocular, subretinal, intravitreal, intrapulmonary, intranasal, and combinations thereof.

177. The method of any one of claims 138-176, wherein the administration of a therapeutically effective amount of the cells results in an improvement in a clinical parameter or endpoint associated with the disease in the subject selected from one or more of tumor shrinkage as a complete, partial or incomplete response; time-to-progression, time to treatment failure, biomarker response; progression-free survival; disease-free survival; time to recurrence; time to metastasis; time of overall survival; improvement of quality of life; and improvement of symptoms.

178. The method of any one of claims 138-177, wherein the method further comprises administering a chemotherapeutic agent.

179. A kit, comprising

a. the CasX system of any one of claims 1-59;

b. the vector of any one of claims 63-69 or

c. the VLP of any one of claims 70-72;

and further comprising an excipient and a container.

180. The kit of claim 179, 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.

181. The CasX:gNA system of any one of claims 1-54, the polynucleotide of any one of claims 60-62, the vector of any one of claims 63-69, the VLP of any one of claims 70-72, or the population of cells of any one of claims 121-127 for use as a medicament for the treatment of a disease or disorder.

182. The CasX:gNA system of any one of claims 1-54, the polynucleotide of any one of claims 60-62, the vector of any one of claims 63-69, the VLP of any one of claims 70-72, or the population of cells of any one of claims 121-127 for use in a method of treatment of a disease or disorder in a subject in need thereof.

183. The CasX:gNA system, polynucleotide, vector, VLP or population of cells of claim 181 or 182, wherein the disease or disorder is cancer or an autoimmune disease.

184. A guide nucleic acid (gNA) comprising a targeting sequence complementary to a target nucleic acid sequence in the target strand of a gene encoding a protein involved in antigen processing, antigen presentation, antigen recognition, and/or antigen response, wherein the gNA is capable of forming a complex with a CRISPR protein that is specific to a protospacer adjacent motif (PAM) sequence comprising a TC motif in the complementary

non-target strand, and wherein the PAM sequence is located 1 nucleotide 5' of the sequence in the non-target strand that is complementary to the target nucleic acid sequence in the target strand.

185. The gNA of claim 184, wherein the CRISPR protein is specific for a TC PAM sequence.

186. The gNA of claim 184, wherein the CRISPR protein is specific for a TTC PAM sequence.

187. The gNA of claim 184, wherein the CRISPR protein is specific for an ATC PAM sequence.

188. The gNA of claim 184, wherein the CRISPR protein is specific for a CTC PAM sequence.

189. The gNA of claim 184, wherein the CRISPR protein is specific for a GTC PAM sequence.

190. The gNA any one of claims 184-189, wherein the targeting sequence is located at the 3' end of the gNA.

191. The gNA of any one of claims 184-190, wherein the CRISPR protein is a Type V CRISPR protein.

192. The gNA sequence of claim 184-191, wherein the protein is an immune cell surface marker.

193. The gNA sequence of claim 184-191, wherein the protein is an immune checkpoint protein.

194. The gNA sequence of claim 184-191, wherein the protein is an intracellular protein.

195. The gNA sequence of claim 184-191, wherein the protein is selected from the group consisting of beta-2-microglobulin (B2M), T cell receptor alpha chain constant region (TRAC), class II major histocompatibility complex transactivator (CIITA), T cell receptor beta constant 1 (TRBC1), T cell receptor beta constant 2 (TRBC2), human leukocyte antigen A (HLA-A), human leukocyte antigen B (HLA-B), TGFβ Receptor 2 (TGFβRII), programmed cell death 1 (PD-1), cytokine inducible SH2 (CISH), lymphocyte activating 3 (LAG-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), adenosine A2a receptor (ADORA2A), killer cell lectin like receptor C1 (NKG2A), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin-domain 3 (TIM-3), and 2B4 (CD244).

196. The gNA of claim 195, wherein the protein is B2M.

197. The gNA of claim 196, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 725-2100, 2281-7085, 547-551, 591-595, and 614-681 or a sequence having at least about 65%, at least about 75%, at least about 85%, or at least about 95% identity thereto.

198. The gNA of claim 196, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 725-2100, 2281-7085, 547-551, 591-595 and 614-681.

199. The gNA of claim 195, wherein the protein is TRAC.

200. The gNA of claim 199, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 7086-27454, 522-529 and 566-573, or a sequence having at least about 65%, at least about 75%, at least about 85%, or at least about 95% identity thereto.

201. The gNA of claim 199, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 7086-27454, 522-529 and 566-573.

202. The gNA of claim 195, wherein the protein is CIITA.

203. The gNA of claim 202, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 27455-55572, or a sequence having at least about 65%, at least about 75%, at least about 85%, or at least about 95% identity thereto.

204. The gNA of claim 202, wherein the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOs: 27455-55572.

205. The gNA of any one of claims 184-204, wherein the gNA is a guide RNA (gRNA).

206. The gNA of any one of claims 184-204, wherein the gNA is a guide DNA (gDNA).

207. The gNA of any one of claims 184-204, wherein the gNA is a chimera comprising DNA and RNA.

208. The gNA of any one of claims 184-204, wherein the gNA is a single-molecule gNA (sgNA).

209. The gNA of any one of claims 184-208, wherein the gNA is a dual-molecule gNA (dgNA).

210. The gNA of any one of claims 184-209, wherein the targeting sequence of the gNA comprises 15, 16, 17, 18, 19, or 20 nucleotides.

211. The gNA of any one of claims 184-210, wherein the gNA has a scaffold comprising a sequence selected from the group consisting of reference gNA sequences of SEQ ID NOS: 4-

16 or gNA variant sequences of SEQ ID NOS:2101-2280, 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.

212. The gNA of claim 211, wherein the gNA variant scaffold comprises a sequence having at least one modification relative to a reference gNA sequence selected from the group consisting of SEQ ID NOS:4-16.

213. The gNA of claim 212, wherein the at least one modification of the reference gNA comprises at least one substitution, deletion, or substitution of a nucleotide of the gNA sequence.

214. The gNA of any one of claims 184-213, wherein the gNA is chemically modified.

215. The gNA of any one of claims 184-214, wherein the gNA can from a ribonuclear protein complex (RNP) with a Class II Type V CRISPR-Cas protein.

216. The gNA of claim 215, wherein the Class II Type V CRISPR-Cas protein is selected from a protein comprising any one of the SEQ ID NOS:1-3, a protein comprising a sequence of SEQ ID NOs: 49-143, 438, 440, 442, 444, 446, 448-460, 472, 474, 478, 480, 482, 484, 486, 488, 490, 612 or 613, 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.

217. A Class II Type V CRISPR protein, wherein an RNP comprising the CRISPR protein and a gNA at a concentration of 20 pM or less is capable of cleaving a double stranded DNA target with an efficiency of at least 80%.