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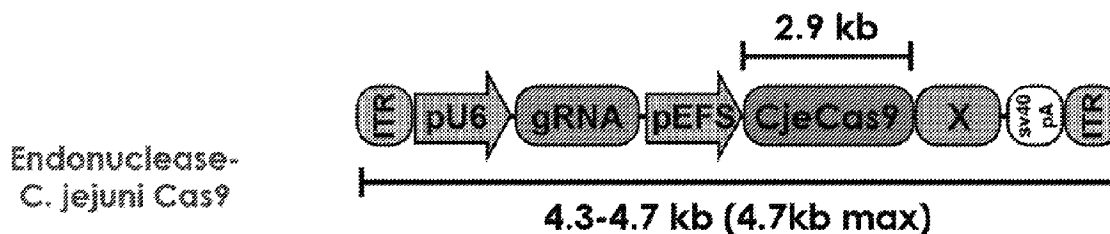
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(54) Titre : COMPOSITIONS ET PROCEDES DE MODULATION DE L'IMMUNITE ADAPTATIVE
(54) Title: COMPOSITIONS AND METHODS FOR THE MODULATION OF ADAPTIVE IMMUNITY

FIGURE 1A



(57) **Abrégé/Abstract:**

Disclosed are compositions and methods for simultaneously providing a gene therapy and preventing an adaptive immune response to a cell modified by the gene therapy by the immune system of a subject. In some embodiments, compositions of the disclosure modify a level of expression of an RNA molecule associated with a disease or disorder as well as inhibit expression or activity of a component of an adaptive immune response to mask the modified cell from a subject's immune system.

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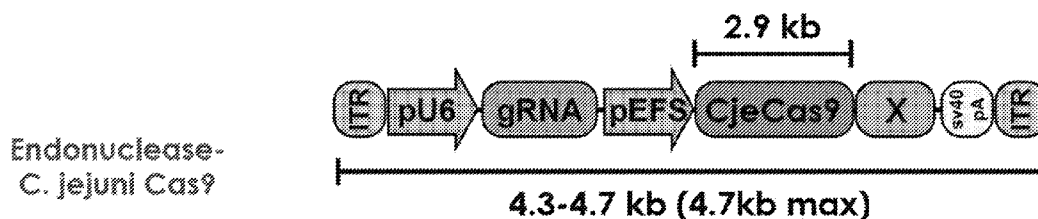
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(54) Title: COMPOSITIONS AND METHODS FOR THE MODULATION OF ADAPTIVE IMMUNITY

FIGURE 1A



(57) Abstract: Disclosed are compositions and methods for simultaneously providing a gene therapy and preventing an adaptive immune response to a cell modified by the gene therapy by the immune system of a subject. In some embodiments, compositions of the disclosure modify a level of expression of an RNA molecule associated with a disease or disorder as well as inhibit expression or activity of a component of an adaptive immune response to mask the modified cell from a subject's immune system.

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COMPOSITIONS AND METHODS FOR THE MODULATION OF ADAPTIVE IMMUNITY

FIELD OF THE DISCLOSURE

[01] The disclosure is directed to molecular biology, and more, specifically, to compositions and methods for modifying expression and activity of RNA molecules involved in an adaptive immune response.

RELATED APPLICATIONS

[02] This application claims priority to U.S. Patent Application No. 62/682,276, filed June 8, 2018, the contents of which are herein incorporated by reference in their entirety. The contents of International Application No. PCT/US2019/036021, filed June 7, 2019, U.S. Patent Application No. 16/434,689, filed June 7, 2019, and U.S. Patent Application No. 62/682,271, filed June 8, 2018, are herein incorporated by reference in their entirety.

INCORPORATION OF SEQUENCE LISTING

[03] The contents of the text file named “LOCN_003_001WO_SeqList_ST25”, which was created on June 6, 2019 and is 2.93 MB in size, are hereby incorporated by reference in their entirety.

BACKGROUND

[04] There has been a long-felt but unmet need in the art for simultaneously providing a gene therapy and suppressing the adaptive immune response that may arise when the gene therapy is delivered by, for example, a viral vector. The disclosure provides compositions and methods for specifically targeting RNA molecules in a sequence-specific manner that provides a gene therapy in vivo while masking the modified cells from the immune system of a subject, thereby preventing an adaptive immune response to the modified cell.

SUMMARY

[05] The disclosure provides a composition comprising a nucleic acid sequence comprising a guide RNA (gRNA) sequence that specifically binds a target RNA sequence, wherein the target RNA sequence encodes a protein component of an adaptive immune response, and wherein the

gRNA sequence comprises a spacer sequence comprising a portion of a nucleic acid sequence encoding the protein component, and wherein the protein component is selected from the group consisting of Beta-2-microglobulin (β 2M), Human Leukocyte Antigen A (HLA-A), Human Leukocyte Antigen B (HLA-B), Human Leukocyte Antigen C (HLA-C), Cluster of Differentiation 28 (CD28), Cluster of Differentiation 80 (CD80), Cluster of Differentiation 86 (CD86), Inducible T-cell Costimulator (ICOS), ICOS Ligand (ICOSLG), OX40L, Interleukin 12 (IL12), and CC Chemokine Receptor 7 (CCR7).

[06] The disclosure also provides a composition comprising (a) a first sequence comprising a guide RNA (gRNA) that specifically binds a target sequence within an RNA molecule, wherein the target sequence comprises a sequence encoding a component of an adaptive immune response and (b) a sequence encoding a fusion protein, the sequence comprising a sequence encoding a first RNA-binding polypeptide and a sequence encoding a second RNA-binding polypeptide, wherein neither the first RNA-binding polypeptide nor the second RNA-binding polypeptide comprises a significant DNA-nuclease activity, wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity.

[07] The disclosure provides a composition comprising: (a) a first sequence comprising a guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and (b) a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule and (c) a sequence encoding a fusion protein, the sequence comprising a sequence encoding a first RNA-binding polypeptide and a sequence encoding a second RNA-binding polypeptide, wherein neither the first RNA-binding polypeptide nor the second RNA-binding polypeptide comprises a significant DNA-nuclease activity, wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity.

[08] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a

second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the first target sequence or the second target sequence comprises at least one repeated sequence.

[09] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the first sequence comprising a first promoter capable of expressing the gRNA in a eukaryotic cell and/or the second sequence comprising a second promoter capable of expressing the gRNA in a eukaryotic cell. In some embodiments, the first promoter and the second promoter are identical. In some embodiments, the first promoter and the second promoter are not identical.

[010] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response, and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the first sequence and second sequence comprising a promoter capable of expressing the first gRNA and the second gRNA in a eukaryotic cell.

[011] In some embodiments of the compositions of the disclosure, including those wherein a gRNA sequence comprises a promoter capable of expressing the gRNA in a eukaryotic cell, the eukaryotic cell is an animal cell. In some embodiments, the animal cell is a mammalian cell. In some embodiments, the animal cell is a human cell.

[012] In some embodiments of the compositions of the disclosure, including those wherein a gRNA sequence comprises a promoter capable of expressing the gRNA in a eukaryotic cell, the promoter is a constitutively active promoter.

[013] In some embodiments of the compositions of the disclosure, including those wherein a gRNA sequence comprises a promoter capable of expressing the gRNA in a eukaryotic cell, the gRNA sequence comprises a sequence isolated or derived from a promoter capable of driving

expression of an RNA polymerase. In some embodiments, the promoter sequence is isolated or derived from a U6 promoter.

[014] In some embodiments of the compositions of the disclosure, including those wherein a gRNA sequence comprises a promoter capable of expressing the gRNA in a eukaryotic cell, the promoter comprises a sequence isolated or derived from a promoter capable of driving expression of a transfer RNA (tRNA). In some embodiments, the promoter sequence is isolated or derived from an alanine tRNA promoter, an arginine tRNA promoter, an asparagine tRNA promoter, an aspartic acid tRNA promoter, a cysteine tRNA promoter, a glutamine tRNA promoter, a glutamic acid tRNA promoter, a glycine tRNA promoter, a histidine tRNA promoter, an isoleucine tRNA promoter, a leucine tRNA promoter, a lysine tRNA promoter, a methionine tRNA promoter, a phenylalanine tRNA promoter, a proline tRNA promoter, a serine tRNA promoter, a threonine tRNA promoter, a tryptophan tRNA promoter, a tyrosine tRNA promoter, or a valine tRNA promoter. In some embodiments, the promoter sequence is isolated or derived from a valine tRNA promoter.

[015] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the sequence comprising the first gRNA further comprises a first spacer sequence that specifically binds to the first target RNA sequence. In some embodiments, the first spacer sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 87%, 90%, 95%, 97%, 99% or any percentage in between of complementarity to the first target RNA sequence. In some embodiments, the first spacer sequence has 100% complementarity to the target RNA sequence. In some embodiments, the first spacer sequence comprises or consists of 20 nucleotides. In some embodiments, the first spacer sequence comprises or consists of 21 nucleotides. In some embodiments, the first spacer sequence comprises or consists of 20 nucleotides of an amino acid sequence encoding a Beta-2-microglobulin (β 2M) protein. In some embodiments, the first spacer sequence comprises or consists of 20 nucleotides of an amino acid sequence of

MSRSVALAVL ALLSLSGLEA IQRTPKIQVY SRHPADIEVD LLKNGERIEK

VEHSDLSFSK DWSFYLLYYT EFTPTEKDEY ACRVNHVTLS QPKIVKWDRD

M (SEQ ID NO: 88).

[016] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the sequence comprising the first gRNA further comprises a first scaffold sequence that specifically binds to the first RNA binding protein. In some embodiments, the first scaffold sequence comprises a stem-loop structure. In some embodiments, the scaffold sequence comprises or consists of 90 nucleotides. In some embodiments, the scaffold sequence comprises or consists of 93 nucleotides. In some embodiments, the scaffold sequence comprises the sequence

GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUU
AUCACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 12) or
GUUUUAGAGCUAGAAAUAGCAAGUUAAAUAAGGCUAGUCCGUUAUCAACUUGA
AAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 13).

[017] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the sequence comprising the second gRNA further comprises a second spacer sequence that specifically binds to the second target RNA sequence. In some embodiments, the second spacer sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 87%, 90%, 95%, 97%, 99% or any percentage in between of complementarity to the first target RNA sequence. In some embodiments, the second spacer sequence has 100% complementarity to the target RNA sequence. In some embodiments, the second spacer sequence comprises or consists of 20 nucleotides. In some embodiments, the second spacer sequence comprises or consists of 21 nucleotides. In some embodiments, the second spacer sequence comprises or further comprises a sequence comprising at least 1, 2, 3, 4, 5, 6, or 7 repeats of the

sequence CUG (SEQ ID NO: 18), CCUG (SEQ ID NO: 19), CAG (SEQ ID NO: 80), GGGGCC (SEQ ID NO: 81) or any combination thereof.

[018] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the sequence comprising the second gRNA further comprises a second scaffold sequence that specifically binds to the first RNA binding protein. In some embodiments, the second scaffold sequence comprises a stem-loop structure. In some embodiments, the scaffold sequence comprises or consists of 85 nucleotides. In some embodiments, the scaffold sequence comprises the sequence

GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUU
AUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 12) or
GUUUUAGAGCUAGAAAUAGCAAGUUAAAUAAGGCUAGUCCGUUAUCAACUUGA
AAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 13).

[019] In some embodiments of the compositions of the disclosure, the gRNA does not bind or does not selectively bind to a second sequence within the RNA molecule.

[020] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the first gRNA does not bind or does not selectively bind to a second sequence within the first RNA molecule.

[021] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target

sequence within a second RNA molecule, the second gRNA does not bind or does not selectively bind to a second sequence within the second RNA molecule.

[022] In some embodiments of the compositions of the disclosure, an RNA genome or an RNA transcriptome comprises the RNA molecule.

[023] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, an RNA genome or an RNA transcriptome comprises the first RNA molecule or the second RNA molecule.

[024] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the first RNA binding protein comprises a CRISPR-Cas protein. In some embodiments, the CRISPR-Cas protein is a Type II CRISPR-Cas protein. In some embodiments, the first RNA binding protein comprises a Cas9 polypeptide or an RNA-binding portion thereof. In some embodiments, the CRISPR-Cas protein is a Type V CRISPR-Cas protein. In some embodiments, the first RNA binding protein comprises a Cpf1 polypeptide or an RNA-binding portion thereof. In some embodiments, the CRISPR-Cas protein is a Type VI CRISPR-Cas protein. In some embodiments, the first RNA binding protein comprises a Cas13 polypeptide or an RNA-binding portion thereof. In some embodiments, the CRISPR-Cas protein comprises a native RNA nuclease activity. In some embodiments, the native RNA nuclease activity is reduced or inhibited. In some embodiments, the native RNA nuclease activity is increased or induced. In some embodiments, the CRISPR-Cas protein comprises a native DNA nuclease activity and wherein the native DNA nuclease activity is inhibited. In some embodiments, the CRISPR-Cas protein comprises a mutation. In some embodiments, a nuclease domain of the CRISPR-Cas protein comprises the mutation. In some embodiments, the mutation occurs in a nucleic acid encoding the CRISPR-Cas protein. In some embodiments, the mutation

occurs in an amino acid encoding the CRISPR-Cas protein. In some embodiments, the mutation comprises a substitution, an insertion, a deletion, a frameshift, an inversion, or a transposition. In some embodiments, the mutation comprises a deletion of a nuclease domain, a binding site within the nuclease domain, an active site within the nuclease domain, or at least one essential amino acid residue within the nuclease domain.

[025] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the first RNA binding protein comprises a Pumilio and FBF (PUF) protein or an RNA binding portion thereof. In some embodiments, the first RNA binding protein comprises a Pumilio-based assembly (PUMBY) protein or an RNA binding portion thereof.

[026] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the first RNA binding protein does not require multimerization for RNA-binding activity. In some embodiments, the first RNA binding protein is not a monomer of a multimer complex. In some embodiments, a multimer protein complex does not comprise the first RNA binding protein.

[027] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the first RNA binding protein selectively binds to a target sequence within the RNA molecule. In some embodiments, the first RNA binding protein does not comprise an affinity for a second sequence within the RNA molecule. In some

embodiments, the first RNA binding protein does not comprise a high affinity for or selectively bind a second sequence within the RNA molecule. In some embodiments, an RNA genome or an RNA transcriptome comprises the RNA molecule.

[028] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the first RNA binding protein comprises between 2 and 1300 amino acids, inclusive of the endpoints.

[029] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the sequence encoding the first RNA binding protein further comprises a sequence encoding a nuclear localization signal (NLS). In some embodiments, the sequence encoding a nuclear localization signal (NLS) is positioned 3' to the sequence encoding the first RNA binding protein. In some embodiments, the first RNA binding protein comprises an NLS at a C-terminus of the protein.

[030] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the sequence encoding the first RNA binding protein further comprises a first sequence encoding a first NLS and a second sequence encoding a second NLS. In some embodiments, the sequence encoding the first NLS or the second NLS is positioned 3' to the sequence encoding the first RNA binding protein. In some embodiments, the first RNA binding protein comprises the first NLS or the second NLS at a C-terminus of the protein.

[031] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a nuclease domain. In some embodiments, the second RNA binding protein comprises or consists of an RNase. In some embodiments, the second RNA binding protein comprises or consists of an RNase1. In some embodiments, the RNase1 protein comprises or consists of SEQ ID NO: 20. In some embodiments, the second RNA binding protein comprises or consists of an RNase4. In some embodiments, the RNase4 protein comprises or consists of SEQ ID NO: 21. In some embodiments, the second RNA binding protein comprises or consists of an RNase6. In some embodiments, the RNase6 protein comprises or consists of SEQ ID NO: 22. In some embodiments, the second RNA binding protein comprises or consists of an RNase7. In some embodiments, the RNase7 protein comprises or consists of SEQ ID NO: 23. In some embodiments, the second RNA binding protein comprises or consists of an RNase8. In some embodiments, the RNase8 protein comprises or consists of SEQ ID NO: 24. In some embodiments, the second RNA binding protein comprises or consists of an RNase2. In some embodiments, the RNase2 comprises or consists of SEQ ID NO: 25. In some embodiments, the second RNA binding protein comprises or consists of an RNase6PL. In some embodiments, the RNase6PL protein comprises or consists of SEQ ID NO: 26. In some embodiments, the second RNA binding protein comprises or consists of an RNaseL. In some embodiments, the RNaseL protein comprises or consists of SEQ ID NO: 27. In some embodiments, the second RNA binding protein comprises or consists of an RNaseT2. In some embodiments, the RNaseT2 protein comprises or consists of SEQ ID NO: 28. In some embodiments, the second RNA binding protein comprises or consists of an RNase11. In some embodiments, the RNase11 protein comprises or consists of SEQ ID NO: 29. In some embodiments, the second RNA binding protein comprises or consists of an RNaseT2-like. In some embodiments, the RNaseT2-like protein comprises or consists of SEQ ID NO: 30.

[032] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that

specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a NOB1 polypeptide. In some embodiments, the NOB1 polypeptide comprises or consists of SEQ ID NO: 31.

[033] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of an endonuclease. In some embodiments, the second RNA binding protein comprises or consists of an endonuclease V (ENDOV). In some embodiments, the ENDOV comprises or consists of SEQ ID NO: 32. In some embodiments, the second RNA binding protein comprises or consists of an endonuclease G (ENDOG). In some embodiments, the ENDOG comprises or consists of SEQ ID NO: 33. In some embodiments, the second RNA binding protein comprises or consists of an endonuclease D1 (ENDOD1). In some embodiments, the ENDOD1 comprises or consists of SEQ ID NO: 34.

[034] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Human flap endonuclease-1 (hFEN1). In some embodiments, the hFEN1 comprises or consists of SEQ ID NO: 35.

[035] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a

second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a human Schlafen 14 (hSLFN14) polypeptide. In some embodiments, the hSLFN14 comprises or consists of SEQ ID NO: 36.

[036] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a human beta-lactamase-like protein 2 (hLACTB2) polypeptide. In some embodiments, the hLACTB2 comprises or consists of SEQ ID NO: 37.

[037] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of an apurinic/apyrimidinic (AP) endodeoxyribonuclease (APEX2) polypeptide. In some embodiments, the APEX2 comprises or consists of SEQ ID NO: 38. In some embodiments, the APEX2 comprises or consists of SEQ ID NO: 39.

[038] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of an angiogenin (ANG) polypeptide. In some embodiments, the ANG comprises or consists of SEQ ID NO: 40.

[039] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that

specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a heat responsive protein 12 (HRSP12) polypeptide. In some embodiments, the HRSP12 comprises or consists of SEQ ID NO: 41.

[040] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Zinc Finger CCCH-Type Containing 12A (ZC3H12A). In some embodiments, the ZC3H12A comprises or consists of SEQ ID NO: 42. In some embodiments, the ZC3H12A comprises or consists of SEQ ID NO: 43.

[041] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Reactive Intermediate Imine Deaminase A (RIDA) polypeptide. In some embodiments, the RIDA polypeptide comprises or consists of SEQ ID NO: 44.

[042] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Phospholipase D Family Member 6 (PDL6) polypeptide. In some embodiments, the PDL6 polypeptide comprises or consists of SEQ ID NO: 126.

[043] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Endonuclease III-like protein 1 (NTHL) polypeptide. In some embodiments, the NTHL polypeptide comprises or consists of SEQ ID NO: 123.

[044] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Mitochondrial ribonuclease P catalytic subunit (KIAA0391) polypeptide. In some embodiments, the KIAA0391 polypeptide comprises or consists of SEQ ID NO: 127.

[045] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of an apurinic or apyrimidinic site lyase (APEX1) polypeptide. In some embodiments, the APEX1 polypeptide comprises or consists of SEQ ID NO: 125.

[046] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists

of an argonaute 2 (AGO2) polypeptide. In some embodiments, encoding the AGO2 polypeptide comprises or consists of SEQ ID NO: 128.

[047] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a mitochondrial nuclease EXOG (EXOG) polypeptide. In some embodiments, the EXOG polypeptide comprises or consists of SEQ ID NO: 129.

[048] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Zinc Finger CCCH-Type Containing 12D (ZC3H12D) polypeptide. In some embodiments, the ZC3H12D polypeptide comprises or consists of SEQ ID NO: 130.

[049] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of an endoplasmic reticulum to nucleus signaling 2 (ERN2) polypeptide. In some embodiments, the ERN2 polypeptide comprises or consists of SEQ ID NO: 131.

[050] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target

sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a pelota mRNA surveillance and ribosome rescue factor (PELO) polypeptide. In some embodiments, the PELO polypeptide comprises or consists of SEQ ID NO: 132.

[051] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a YBEY metalloproteinase (YBEY) polypeptide. In some embodiments, the YBEY polypeptide comprises or consists of SEQ ID NO: 133.

[052] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule the second RNA binding protein comprises or consists of a cleavage and polyadenylation specific factor 4 like (CPSF4L) polypeptide. In some embodiments, the CPSF4L polypeptide comprises or consists of SEQ ID NO: 134.

[053] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of an hCG_2002731 polypeptide. In some embodiments, the hCG_2002731 polypeptide comprises or consists of SEQ ID NO: 135. In some embodiments, the sequence encoding the hCG_2002731 polypeptide comprises or consists of SEQ ID NO: 136.

[054] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target

sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of an Excision Repair Cross-Complementation Group 1 (ERCC1) polypeptide. In some embodiments, the ERCC1 polypeptide comprises or consists of SEQ ID NO: 137.

[055] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a ras-related C3 botulinum toxin substrate 1 isoform (RAC1) polypeptide. In some embodiments, the RAC1 polypeptide comprises or consists of SEQ ID NO: 138.

[056] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Ribonuclease A A1 (RAA1) polypeptide. In some embodiments, the RAA1 polypeptide comprises or consists of SEQ ID NO: 139.

[057] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Ras Related Protein (RAB1) polypeptide. In some embodiments, the RAB1 polypeptide comprises or consists of SEQ ID NO: 140.

[058] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that

specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a DNA Replication Helicase/Nuclease 2 (DNA2) polypeptide. In some embodiments, the DNA2 polypeptide comprises or consists of SEQ ID NO: 141.

[059] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a FLJ35220 polypeptide. In some embodiments, the FLJ35220 polypeptide comprises or consists of SEQ ID NO: 142.

[060] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a FLJ13173 polypeptide. In some embodiments, the FLJ13173 polypeptide comprises or consists of SEQ ID NO: 143.

[061] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule the second RNA binding protein comprises or consists of a DNA repair endonuclease XPF (ERCC4) polypeptide. In some embodiments, the ERCC4 polypeptide comprises or consists of SEQ ID NO: 124.

[062] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R)) polypeptide. In some embodiments, the Rnase1(K41R) polypeptide comprises or consists of SEQ ID NO: 116.

[063] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R, D121E)) polypeptide. In some embodiments, the Rnase1 (Rnase1(K41R, D121E)) polypeptide comprises or consists of SEQ ID NO: 117).

[064] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R, D121E, H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(K41R, D121E, H119N)) polypeptide comprises or consists of SEQ ID NO: 118.

[065] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists

of a mutated Rnase1 (Rnase1(H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(H119N)) polypeptide comprises or consists of SEQ ID NO: 119.

[066] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide comprises or consists of SEQ ID NO: 120.

[067] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E)) polypeptide comprises or consists of SEQ ID NO: 121.

[068] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D)) polypeptide comprises or consists of SEQ ID NO: 122.

[069] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that

specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Teneurin Transmembrane Protein 1 (TENM1) polypeptide. In some embodiments, the TENM1 polypeptide comprises or consists of SEQ ID NO: 144.

[070] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Teneurin Transmembrane Protein 1 (TENM2) polypeptide. In some embodiments, the TENM2 polypeptide comprises or consists of SEQ ID NO: 145.

[071] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a Ribonuclease Kappa (RNaseK) polypeptide. In some embodiments, the RNaseK protein comprises or consists of SEQ ID NO: 204.

[072] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a transcription activator-like effector nuclease (TALEN) polypeptide or a nuclease domain thereof. In some embodiments, the TALEN polypeptide comprises or consists of:

1 MRIGKSSGWL NESVSLEYEH VSPPTRPRDT RRRPRAAGDG GLAHLHRRLLA VGYAEDTPRT

61 EARSAPARRP LPVAPASAPP APSLVPEPPM PVSLPAVSSP RFSAGSSAAI TDPFPSLPPT
 121 PVLYAMAREL EALSDATWQP AVPLPAEPPT DARRGNTVFD EASASSPVIA SACPQAFASP
 181 PRAPRSARAR RARTGGDAWP APTFLSRPSS SRIGRDVFGK LVALGYSREQ IRKLKQESLS
 241 EIAKYHTTILT GQGFTHADIC RISRRRQSLR VVARNYPELA AALPELTRAH IVDIARQRS
 301 DLALQALLPV ATALTAAPLR LSASQIATVA QYGERPAIQA LYRLRRKLTR APLHLTPQOV
 361 VAIASNTGGK RALEAVCVQL PVLRAAPYRL STEQVVAIAS NKGKKQALEA VKAHLDDLGL
 421 APYVLDTEQV VAIASHNGGK QALEAVKADL LDLRGAPYAL STEQVVAIAS HNGGKQALEA
 481 VKADLLELRG APYALSTEQV VAIASHNGGK QALEAVKAHL LDLRGVPYAL STEQVVAIAS
 541 HNGGKQALEA VKAQLDLRG APYALSTAQV VAIASNGGK QALEGIGEQL LKLRTAPYGL
 601 STEQVVAIAS HDGGKQALEA VGAQLVALRA APYALSTEQV VAIASNKGK QALEAVKAQL
 661 LELRGAPYAL STAQVVAIAS HDGGNQALEA VGTQLVALRA APYALSTEQV VAIASHDGGK
 721 QALEAVGAQL VALRAAPYAL NTEQVVAIAS SHGGKQALEA VRALFPDLRA APYALSTAQL
 781 VAIASNPGGK QALEAVRALF RELRAAPYAL STEQVVAIAS NHGGKQALEA VRALFRGLRA
 841 APYGLSTAQV VAIASSNGGK QALEAVWALL PVLRTAPYDL NTAQIVAIAS HDGGKPALEA
 901 VWAKLPVLRG APYALSTAQV VAIACISGQQ ALEAIEAHMP TLRQASHSLS PERVAIACI
 961 GGRSAVEAVR QGLPVKAIRR IRREKAPVAG PPPASLGPTP QELVAVLHFF RAHQQPRQAF
 1021 VDALAAFQAT RPALLRLLSS VGVTEIEALG GTIPDATERW QRLGLRLGFR PATGAAAPSP
 1081 DSLQGFAQSL ERTLGSFGMA GQSACSPHRK RPAETAIAPR SIRRSPNNAG QPSEPWPDL
 1141 AWLQRRKRRTA RSHIRADSAA SVPANLHLGT RAQFTPDRLR AEPGPIMQAH TSPASVSFGS
 1201 HVAFEPGLPD PGTPTSADLA SFEAEFPVG PLDFHLDWLL QILET (SEQ ID NO: 205).

In some embodiments, the TALEN polypeptide comprises or consists of:

1 mdpirsrtps parellpgpq pdrvqptadr ggappaggpl dglparrtms rtrlpsppap
 61 spafsagsfs dllrqfdpsl ldtslldsmp avgtphataa paecdevqsg lraaddpppt
 121 vrvavtaarp prakpaprrr aaqpsdaspa aqvdlrtlgy sqqqqekikp kvgstvaqhh
 181 ealvghgftah ahivalsrhp aalgtvavky qdmiaalpea thedivgvqk qwsgaralea
 241 lltvagelrg pplqltdgql vkiakrggvt aveavhasrn altgaplnlt paqvvaianas
 301 nggkqaletv qrllpvlcqa hgltpaqvva iashdggkqa letmqrlpvl lcqahglppd
 361 qvvaianasni gkqaletvqr llpvlcqhag ltpdqvvaia shgggkqale tvqrllpvlc
 421 qahgltpdqv vaiashdggk qaletvqrll pvlcqhaglt pdqvvaianas gggkqaletv
 481 qrllpvlcqa hgltpdqvva iasnggkqal etvqrllpvl cqahgltpdq vvaishdgg
 541 kqaletvqrl lpvlcqhthgl tpaqvvaia hdggkqalet vqqlpvlcqa ahgltpdqvv
 601 aiasniggkq alatvqrllp vlcqhagltq dqvvaianas gggkqaletv rllpvlcqh
 661 gltpdqvvai asngggkqal etvqrllpvl cqahgltpqv vvaianasni gkqaletvqr
 721 lpvlcqhagl tpaqvvaia hdggkqalet vqrllpvlcqa ahgltpdqvv aiasngggkq
 781 aletvqrllp vlcqhagltq eqvvaianasn ggkqaletvq rllpvlcqh gltpdqvvai
 841 asngggkqal etvqrllpvl cqahgltpaq vvaianasni gkqaletvqr llpvlcqhagl
 901 tlaqvvaia niggkqalet vqrllpvlcqa ahgltpdqvv aiasniggkq aletvqrllp
 961 vlcqhagltp dqvvaianas ggkqaletvq rllpvlcqh gltldqvva asngggkqale
 1021 tvqrllpvlc qdhgltpdqv vaianasnggk qaletvqrll pvlcqhagl pnvvaianas
 1081 ggkqaletiv aqlsrpdpal aaltndhlva laclggrpam davkkglpha pelirrnr
 1141 igertshrva dyaqvrvle ffqchshpay afdeamtqfg msrnlvqlf rrvgtvlea
 1201 rgtlppasq rwdrlqasq mkrakpspts aqtpdqaslh afadslerdl dapsmhegd
 1261 qtgassrkr rsdravtgps aqhsfevrp eqrdalhlpl swrvkrprtr igggldpdp
 1321 piaadlaass tvmweqdaap fagaaddfpa fneelawlm ellpqsgsvg gti (SEQ ID
 NO: 206).

[073] In some embodiments of the compositions of the disclosure, including those wherein the composition comprises a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target

sequence within a second RNA molecule, the second RNA binding protein comprises or consists of a zinc finger nuclease polypeptide or a nuclease domain thereof. In some embodiments, the second RNA binding protein comprises or consists of a ZNF638 polypeptide or a nuclease domain thereof. In some embodiments, the ZNF638 polypeptide polypeptide comprises or consists of:

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1  MSRPRFNPRG  DFPLQRPRAP  NPSGMRPPGP  FMRPGSMGLP  RFYPAGRARG  IPHRFAGHES
61  YQNMGPQRMN  VQVTQHRTPD  RLTKEKLDHF  EAQQKKGKPH  GSRWDDEPHI  SASVAVKQSS
121 VTQVTEQSPK  VQSRYTEKSA  SSILASFGLS  NEDLEELSR  PDEQLTPENM  PLILRDIRMR
181 KMGRRLPNLP  SQSRNKETLG  SEAVSSNVID  YGHASKYGYT  EDPLEVRIYD  PEIPTDEVEN
241 EFQSQQNISA  SVPNPNVICN  SMFPVEDVFR  QMDFPGESSN  NRSFFSVESG  TKMSGHLISG
301 GQSVLEPIKS  VNQSINQTVS  QTMSQSLIPP  SMNQPPFSSE  LISSVSQQER  IPHEPVINSS
361 NVHVGSRGSK  KNYQSQADIP  IRSPFGIVKA  SWLPKFSHAD  AQKMKRLPTP  SMMNDYYAAS
421 PRIFPHLCSL  CNVECSHLKD  WIQHONTSTH  IESCRQLRQQ  YPDWNPEILP  SRRNEGNRKE
481 NETPRRRSHS  PSPRRRRSS  SSHRFRRSRS  PMHYMYRPRS  RSPRICHRFI  SRYRSRSRSR
541 SPYRIRNPFR  GSPKCFRSVS  PERMSRRSVR  SDRKKALED  VVQRSRGHTE  FNKQKHLEAA
601 DKGHSPAQKP  KTSSGTPKPS  KPTSATKSDS  NLGGHSIRCK  SKNLEDDTLS  ECKQVSDKAV
661 SLQRKLRKEQ  SLHYGSVLLI  TELPEDGCTE  EDVRKLFQPF  GKVNDVLIVP  YRKEAYLEME
721 FKEAITAIMK  YIETTPITIK  GKSVKICVPG  KKAQNKEVK  KKTLESKKVS  ASTLKRDATA
781 SKAVEIVTST  SAAKTGQAKA  SVAKVNKSTG  KSASSVKS  TVAVKGNKAS  IKTAKSGGKK
841 SLEAKKTGNV  KNKDSNKPVT  IPENSEIKTS  IEVKATENCA  KEAISDAALE  ATENEPLNKE
901 TEEMCVMLVS  NLPNKGYSVE  EVYDLAKPFG  GLKDILILSS  HKKAYIEINR  KAAESMVKFY
961 TCFPVLMMDGN  QLSISMAPEN  MNIKDEEAI  ITLVKENDPE  ANIDTIYDRF  VHLDNLPEDG
1021 LQCVLCVGLQ  FGKVDHHVFI  SNRNKAILQL  DSPESAQSMY  SFLKQNPQNI  GDHMLTCSLS
1081 PKIDLPEVQI  EHDPELEKES  PGLKNSPIDE  SEVQTATDSP  SVKPNELEEE  STPSIQTTTL
1141 VQQEPCPEEE  AEKATCDSDF  AVETLELETQ  GEEVKEEIP  VASASVSIEP  FTENAEICAL
1201 NQQMFNSDLE  KKGAEIINPK  TALLPSDSVF  AEERNLKGIL  EESPSEAEDE  ISGITQTMVE
1261 AVAEVEKNET  VSEILPSTCI  VTLVPGIPTG  DEKTVDKKNI  SEKKGNMDEK  EEKEFNKTKET
1321 RMDLQIGTEK  AEKNEGRMDA  EKVEKMAAMK  EKPAENTLFK  AYPNKGVGQA  NKPDETSKTS
1381 ILAVSDVSSS  KPSIKAVIVS  SPKAKATVSK  TENQKSFPKS  VPRDQINAEK  KLSAKEFGLL
1441 KPTSARSGLA  ESSSKFKPTQ  SSLTRGSGSR  ISALQGKLSK  LDYRDITKQS  QETEARPSIM
1501 KRDDSNNKTL  AEQNTKNPKS  TTGRSSKSKE  EPLFPFNLDE  FVTVDEVIEE  VNPSQAKQNP
1561 LKGKRKETLK  NVPFSELNLK  KKKGKTSTPR  GVEGELSFTV  LDEIGEEEDA  AAHLAQALVT
1621 VDEVIDEEL  NMEEMVKNSN  SLFTLDELID  QDDCISHSEP  KDVTVLSVAE  EQDLLKQERL
1681 VTVDEIGEVE  ELPLNESADI  TFATLNTKGN  EGDTVRDSIG  FISSQVPEDP  STLVTVDEIQ
1741 DDSSDLHLVT  LDEVTEEDED  SLADFNNLKE  ELNFVTVDEV  GEEEDGDNDL  KVELAQSKND
1801 HPTDKKGNRK  KRAVDTKKTK  LESLSQVGPV  NENVMEEDLK  TMIERHLTAK  TPTKRVIRIG
1861 TLPSEKAVVT  EPAKGEEAFQ  MSEVDEESGL  KDSEPERKRK  KTEDSSSGKS  VASDVPEELD
1921 FLVPKAGFFC  PICSLFYSGE  KAMTNHCKST  RHKQNTKFKM  AKQRKEKEQN  EAEERSSR

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(SEQ ID NO: 207).

[074] In some embodiments of the compositions of the disclosure, the composition further comprises (a) a sequence comprising a gRNA that specifically binds within an RNA molecule and (b) a sequence encoding a nuclease. In some embodiments, the sequence encoding a nuclease comprises a sequence isolated or derived from a CRISPR/Cas protein. In some embodiments, the CRISPR/Cas protein is isolated or derived from any one of a type I, a type IA, a type IB, a type IC, a type ID, a type IE, a type IF, a type IU, a type III, a type IIIA, a type IIIB, a type IIIC, a type IIID, a type IV, a type IVA, a type IVB, a type II, a type IIA, a type IIB, a

type IIC, a type V, or a type VI CRISPR/Cas protein. In some embodiments, the sequence encoding a nuclease comprises a sequence isolated or derived from a TALEN or a nuclease domain thereof. In some embodiments, the sequence encoding a nuclease comprises a sequence isolated or derived from a zinc finger nuclease or a nuclease domain thereof. In some embodiments, the target sequence comprises a sequence encoding a component of an adaptive immune response.

[075] The disclosure provides a vector comprising a composition of the disclosure. In some embodiments, the vector is a viral vector. In some embodiments, the vector comprises a sequence isolated or derived from a lentivirus, an adenovirus, an adeno-associated virus (AAV) vector, or a retrovirus. In some embodiments, the vector is replication incompetent.

[076] The disclosure provides a vector comprising a composition of the disclosure. In some embodiments, the vector is a viral vector. In some embodiments, the vector comprises a sequence isolated or derived from an adeno-associated vector (AAV). In some embodiments, the adeno-associated virus (AAV) is an isolated AAV. In some embodiments, the adeno-associated virus (AAV) is a self-complementary adeno-associated virus (scAAV). In some embodiments, the adeno-associated virus (AAV) is a recombinant adeno-associated virus (rAAV). In some embodiments, the adeno-associated virus (AAV) comprises a sequence isolated or derived from an AAV of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or AAV12. In some embodiments, the adeno-associated virus (AAV) comprises a sequence isolated or derived from an AAV of serotype AAV9. In some embodiments, the adeno-associated virus (AAV) comprise a sequence isolated or derived from Anc80.

[077] The disclosure provides a vector comprising a composition of the disclosure. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retrovirus.

[078] The disclosure provides a vector comprising a composition of the disclosure. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a lentivirus.

[079] The disclosure provides a vector comprising a composition of the disclosure. In some embodiments, the vector is a non-viral vector. In some embodiments, the non-viral vector comprises a nanoparticle, a micelle, a liposome or lipoplex, a polymersome, a polyplex or a dendrimer.

[080] The disclosure provides a composition comprising a vector of the disclosure.

[081] The disclosure provides a cell comprising a vector of the disclosure.

[082] The disclosure provides a cell comprising a cell of the disclosure.

[083] In some embodiments of cells of the disclosure, the cell is a mammalian cell. In some embodiments, the cell is a human cell.

[084] In some embodiments of cells of the disclosure, the cell is an immune cell. In some embodiments, the immune cell is a T lymphocyte (T-cell). In some embodiments, the T-cell is an effector T-cell, a helper T-cell, a memory T-cell, a regulatory T-cell, a natural Killer T-cell, a mucosal-associated invariant T-cell, or a gamma delta T cell.

[085] In some embodiments of cells of the disclosure, the cell is an immune cell. In some embodiments, the immune cell is an antigen-presenting cell. In some embodiments, the antigen-presenting cell is a dendritic cell, a macrophage, or a B cell. In some embodiments, the antigen-presenting cell is a somatic cell.

[086] In some embodiments of cells of the disclosure, the cell is an immune cell. In some embodiments, the cell is a healthy cell. In some embodiments, the cell is not a healthy cell. In some embodiments, the cell is isolated or derived from a subject having a disease or disorder.

[087] The disclosure provides a composition comprising a cell of the disclosure.

[088] The disclosure provides a composition comprising a plurality of cells of the disclosure.

[089] The disclosure provides a method of masking a cell from an adaptive immune response comprising contacting a composition of the disclosure to the cell to produce a modified cell, wherein the composition modifies a level of expression of an RNA molecule of the modified cell and wherein the RNA molecule encodes a component of an adaptive immune response. In some embodiments, the cell is in vivo, in vitro, ex vivo or in situ. In some embodiments, the cell is in vitro or ex vivo. In some embodiments, a plurality of cells comprises the cell. In some embodiments, each cell of the plurality of cells contacts the composition, thereby producing a plurality of modified cells. In some embodiments, the method further comprises administering the modified cell to a subject. In some embodiments, the method further comprises administering the plurality of modified cells to a subject. In some embodiments, the cell is autologous. In some embodiments, the cell is allogeneic. In some embodiments, the plurality of modified cells is autologous. In some embodiments, the plurality of modified cells is allogeneic. In some embodiments, the component of an adaptive immune response comprises or consists of a component of a type I major histocompatibility complex (MHC I), a type II major

histocompatibility complex (MHC II), a T-cell receptor (TCR), a costimulatory molecule or a combination thereof. In some embodiments, the MHC I component comprises an $\alpha 1$ chain, an $\alpha 2$ chain, an $\alpha 3$ chain, or a $\beta 2M$ protein. In some embodiments, the component of an adaptive immune response comprises or consists of an MHC I $\beta 2M$ protein. In some embodiments, the MHC II component comprises an $\alpha 1$ chain, an $\alpha 2$ chain, a $\beta 1$ chain, or a $\beta 2$ chain. In some embodiments, the TCR component comprises an α -chain and a β -chain. In some embodiments, the costimulatory molecule comprises a Cluster of Differentiation 28 (CD28), a Cluster of Differentiation 80 (CD80), a Cluster of Differentiation 86 (CD86), an Inducible T-cell COStimulator (ICOS), or an ICOS Ligand (ICOSLG) protein. In some embodiments, a protein component of an adaptive immune response is, without limitation, Beta-2-microglobulin ($\beta 2M$), Human Leukocyte Antigen A (HLA-A), Human Leukocyte Antigen B (HLA-B), Human Leukocyte Antigen C (HLA-C), Cluster of Differentiation 28 (CD28), Cluster of Differentiation 80 (CD80), Cluster of Differentiation 86 (CD86), Inducible T-cell Costimulator (ICOS), ICOS Ligand (ICOSLG), OX40L, Interleukin 12 (IL12), or CC Chemokine Receptor 7 (CCR7).

[090] The disclosure provides a method of preventing or reducing an adaptive immune response in a subject comprising administering a therapeutically effective amount of a composition of the disclosure to the subject, wherein the composition contacts at least one cell in the subject producing a modified cell, wherein the composition modifies a level of expression of an RNA molecule of the modified cell and wherein the RNA molecule encodes a component of an adaptive immune response.

[091] The disclosure provides a method of treating a disease or disorder in a subject comprising administering a therapeutically effective amount of a composition of the disclosure to the subject, wherein the composition contacts at least one cell in the subject producing a modified cell, wherein the composition modifies a level of expression of an RNA molecule of the modified cell and wherein the composition prevents or reduces an adaptive immune response to the modified cell.

[092] In some embodiments of the methods of the disclosure, the component of an adaptive immune response comprises or consists of a component of a type I major histocompatibility complex (MHC I), a type II major histocompatibility complex (MHC II), a T-cell receptor (TCR), a costimulatory molecule or a combination thereof. In some embodiments, the MHC I component comprises an $\alpha 1$ chain, an $\alpha 2$ chain, an $\alpha 3$ chain, or a $\beta 2M$ protein. In some

embodiments, the component of an adaptive immune response comprises or consists of an MHC I β 2M protein. In some embodiments, the MHC II component comprises an α 1 chain, an α 2 chain, a β 1 chain, or a β 2 chain. In some embodiments, the TCR component comprises an α -chain and a β -chain. In some embodiments, the costimulatory molecule comprises a Cluster of Differentiation 28 (CD28), a Cluster of Differentiation 80 (CD80), a Cluster of Differentiation 86 (CD86), an Inducible T-cell COStimulator (ICOS), or an ICOS Ligand (ICOSLG) protein.

[093] In some embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is a genetic disease or disorder. In some embodiments, the disease or disorder is a single gene genetic disease or disorder. In some embodiments, the disease or disorder results from microsatellite instability. In some embodiments, the microsatellite instability occurs in a DNA sequence at least 1, 2, 3, 4, 5 or 6 repeated motifs. In some embodiments, an RNA molecule comprises a transcript of the DNA sequence and wherein the composition binds to a target sequence of the RNA molecule comprising at least 1, 2, 3, 4, 5, or 6 repeated motifs.

[094] In some embodiments of the methods of the disclosure, the composition is administered systemically. In some embodiments, the composition is administered intravenously. In some embodiments, the composition is administered by an injection or an infusion.

[095] In some embodiments of the methods of the disclosure, the composition is administered locally. In some embodiments, the composition is administered by an intraosseous, intraocular, intracerebral, or intraspinal route. In some embodiments, the composition is administered by an injection or an infusion.

[096] In some embodiments of the methods of the disclosure, a therapeutically effective amount of the composition is a single dose.

[097] In some embodiments of the methods of the disclosure, the composition is non-genome integrating.

BRIEF DESCRIPTION OF THE DRAWINGS

[098] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0099] Figure 1A is a schematic diagram depicting an exemplary RNA Endonuclease-C. jejuni Cas9 fusion protein.

[0100] Figure 1B is a graph depicting changes in expression levels of Zika NS5 in the presence of both E43 and E67 CjeCas9-endonuclease fusions with sgRNAs containing the various NS5-targeting spacer sequences as indicated in Table 8. Zika NS5 expression is displayed as fold change relative to the endonuclease loaded with an sgRNA containing a control (Lambda) spacer sequence.

[0101] Figure 2A is a fluorescence microscopy image of cells transfected with CjeCas9-endonuclease fusions loaded with an sgRNA containing a Zika NS5-targeting spacer sequence.

[0102] Figure 2B is a graph depicting changes of expression of Zika NS5 in the presence of CjeCas9-endonuclease fusions loaded with the appropriate Zika NS5-targeting sgRNA as compared to CjeCas9-endonuclease fusions loaded with a non-Zika NS5 targeting sgRNA.

[0103] Figure 3 is a list of exemplary endonucleases for use in the compositions of the disclosure.

[0104] Figure 4 is a schematic diagram depicting a construct encoding an exemplary RNA Endonuclease-C. jejuni Cas9 fusion protein and two gRNA molecules for modulating immune response in the context of a gene therapy. The present invention describes a means to address human disease using a CRISPR-based gene therapy or other non-self protein encoded in AAV while simultaneously altering host gene expression to prevent adaptive immune response to the non-self protein. In one embodiment, the AAV particle (left) carries a pair of guide RNAs and a CRISPR-associated (Cas) protein. The guides target a gene associated with adaptive immune response and a gene (or gene product) to promote therapeutic benefit, respectively. Upon delivery to target tissue, the immune response-targeted guide reduces expression of genes associated with antigen presentation (beta-2-microglobulin, B2M) or co-stimulation of T cells (ICOSLG, CD80, CD86, OX40L, IL12, CCR7). Antigen presentation inhibition prevents formation of T helper (Th) cells specific to the therapeutic transgenes such as Cas proteins while co-stimulation inhibition prevents the activation of Th cells that are specific to the transgene.

DETAILED DESCRIPTION

[0105] The disclosure provides compositions and methods for the simultaneous treatment of disease by targeting RNA molecules of a modified cell while masking the modified cell from an

adaptive immune response. By inhibiting or reducing expression of a component of an adaptive immune response in the modified cell, the modified cell is invisible to a host immune system. For example, compositions of the disclosure may simultaneously target an RNA molecule associated with a genetic disease or disorder and an RNA molecule that encodes the $\beta 2M$ subunit of the MHC I. By selectively targeting an RNA molecule that encodes the $\beta 2M$ subunit of the MHC I, the composition prevents the modified cell from displaying one or more antigen peptides derived from an RNA targeting construct, vector, or combination thereof on the surface of the modified cell. Consequently, a subject's immune system does not identify the modified cell as containing foreign sequences and does not attempt to mount an immune response directed at the modified cell. This method increases the therapeutic efficacy of the treatment of the genetic disease or disorder while avoiding a common side effect of gene therapy.

RNA-Targeting Fusion Protein Compositions

[0106] The disclosure provides a composition comprising (a) a sequence comprising a guide RNA (gRNA) that specifically binds a target sequence within an RNA molecule and (b) a sequence encoding a fusion protein, the sequence comprising a sequence encoding a first RNA-binding polypeptide and a sequence encoding a second RNA-binding polypeptide, wherein neither the first RNA-binding polypeptide nor the second RNA-binding polypeptide comprises a significant DNA-nuclease activity, wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity.

[0107] In some embodiments of the compositions of the disclosure, the target sequence comprises at least one repeated sequence.

[0108] In some embodiments of the compositions of the disclosure, the gRNA sequence comprises a promoter capable of expressing the gRNA in a eukaryotic cell.

[0109] In some embodiments of the compositions of the disclosure, the eukaryotic cell is an animal cell. In some embodiments, the animal cell is a mammalian cell. In some embodiments, the animal cell is a human cell.

[0110] In some embodiments of the compositions of the disclosure, the promoter is a constitutively active promoter. In some embodiments, the promoter sequence is isolated or

derived from a promoter capable of driving expression of an RNA polymerase. In some embodiments, the promoter sequence is isolated or derived from a U6 promoter. In some embodiments, the promoter sequence is isolated or derived from a promoter capable of driving expression of a transfer RNA (tRNA). In some embodiments, the promoter sequence is isolated or derived from an alanine tRNA promoter, an arginine tRNA promoter, an asparagine tRNA promoter, an aspartic acid tRNA promoter, a cysteine tRNA promoter, a glutamine tRNA promoter, a glutamic acid tRNA promoter, a glycine tRNA promoter, a histidine tRNA promoter, an isoleucine tRNA promoter, a leucine tRNA promoter, a lysine tRNA promoter, a methionine tRNA promoter, a phenylalanine tRNA promoter, a proline tRNA promoter, a serine tRNA promoter, a threonine tRNA promoter, a tryptophan tRNA promoter, a tyrosine tRNA promoter, or a valine tRNA promoter. In some embodiments, the promoter sequence is isolated or derived from a valine tRNA promoter.

[0111] In some embodiments of the compositions of the disclosure, the sequence comprising the gRNA further comprises a spacer sequence that specifically binds to the target RNA sequence. In some embodiments, the spacer sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 87%, 90%, 95%, 97%, 99% or any percentage in between of complementarity to the target RNA sequence. In some embodiments, the spacer sequence has 100% complementarity to the target RNA sequence. In some embodiments, the spacer sequence comprises or consists of 20 nucleotides. In some embodiments, the spacer sequence comprises or consists of 21 nucleotides. In some embodiments, the spacer sequence comprises or consists of the sequence UGGAGCGAGCAUCCCCCAA (SEQ ID NO: 1), GUUUGGGGGAUGCUCGCUCCA (SEQ ID NO: 2), CCCUCACUGCUGGGGAGUCC (SEQ ID NO: 3), GGACUCCCCAGCAGUGAGGG (SEQ ID NO: 4), GCAACUGGAUCAAUUUGCUG (SEQ ID NO: 5), GCAGCAAUUGAUCCAGUUGC (SEQ ID NO: 6), GCAUUCUUAUCUGGUCAGUGC (SEQ ID NO: 7), GCACUGACCAGAUAAAGAAUG (SEQ ID NO: 8), GAGCAGCAGCAGCAGCAGCAG (SEQ ID NO: 9), GCAGGCAGGCAGGCAGGCAGG (SEQ ID NO: 10), GCCCGGGCCCCGGCCCCGGC (SEQ ID NO: 11), or GCTGCTGCTGCTGCTGCTGC (SEQ ID NO: 84), GGGGCCGGGGCCGGGGCCGG (SEQ ID NO: 74), GGGCCGGGGCCGGGGCCGGG (SEQ ID NO: 75), GGCCGGGGCCGGGGCCGGG (SEQ ID NO: 76),

GCCGGGGCCGGGGCCGGGGC (SEQ ID NO: 77), CCGGGGCGGGGGCCGGGGCC (SEQ ID NO: 78), or CGGGGCGGGGGCCGGGGCCG (SEQ ID NO: 79).

[0112] In some embodiments of the compositions of the disclosure, the sequence comprising the gRNA further comprises a spacer sequence that specifically binds to the target RNA sequence. In some embodiments, the spacer sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 87%, 90%, 95%, 97%, 99% or any percentage in between of complementarity to the target RNA sequence. In some embodiments, the spacer sequence has 100% complementarity to the target RNA sequence. In some embodiments, the spacer sequence comprises or consists of 20 nucleotides. In some embodiments, the spacer sequence comprises or consists of 21 nucleotides. In some embodiments, the spacer sequence comprises or consists of the sequence GUGAUAAGUGGAAUGCCAUG (SEQ ID NO: 14), CUGGUGAACUCCGAUAGUG (SEQ ID NO: 15), or GAGATATAGCCTGGTGGTTC (SEQ ID NO: 16).

[0113] In some embodiments of the compositions of the disclosure, the sequence comprising the gRNA further comprises a spacer sequence that specifically binds to the target RNA sequence. In some embodiments, the spacer sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 87%, 90%, 95%, 97%, 99% or any percentage in between of complementarity to the target RNA sequence. In some embodiments, the spacer sequence has 100% complementarity to the target RNA sequence. In some embodiments, the spacer sequence comprises or consists of 20 nucleotides. In some embodiments, the spacer sequence comprises or consists of 21 nucleotides. In some embodiments, the spacer sequence comprises or consists of a sequence comprising at least 1, 2, 3, 4, 5, 6, or 7 repeats of the sequence CUG (SEQ ID NO: 18), CCUG (SEQ ID NO: 19), CAG (SEQ ID NO: 80), GGGGCC (SEQ ID NO: 81) or any combination thereof.

[0114] In some embodiments of the compositions of the disclosure, the sequence comprising the gRNA further comprises a scaffold sequence that specifically binds to the first RNA binding protein. In some embodiments, the scaffold sequence comprises a stem-loop structure. In some embodiments, the scaffold sequence comprises or consists of 90 nucleotides. In some embodiments, the scaffold sequence comprises or consists of 93 nucleotides. In some embodiments, the scaffold sequence comprises or consists of the sequence GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUU AUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 83). In some embodiments, the scaffold sequence comprises or consists of the sequence

GGACAGCAUAGCAAGUUA AAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG
CACCGAGUCGGUGCUUUUUU (SEQ ID NO: 17). In some embodiments, the scaffold

sequence comprises or consists of the sequence

GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUA AAAUAAGGCUAGUCCGUU
AUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUUU (SEQ ID NO: 82) or

GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAGGCUAGUCCGUUAUCAACUUGA
AAAAGUGGCACCGAGUCGGUGCUUUUUUUU (SEQ ID NO: 13).

[0115] In some embodiments of the compositions of the disclosure, the gRNA does not bind or does not selectively bind to a second sequence within the RNA molecule.

[0116] In some embodiments of the compositions of the disclosure, an RNA genome or an RNA transcriptome comprises the RNA molecule.

[0117] In some embodiments of the compositions of the disclosure, the first RNA binding protein comprises a CRISPR-Cas protein. In some embodiments, the CRISPR-Cas protein is a Type II CRISPR-Cas protein. In some embodiments, the first RNA binding protein comprises a Cas9 polypeptide or an RNA-binding portion thereof. In some embodiments, the CRISPR-Cas protein comprises a native RNA nuclease activity. In some embodiments, the native RNA nuclease activity is reduced or inhibited. In some embodiments, the native RNA nuclease activity is increased or induced. In some embodiments, the CRISPR-Cas protein comprises a native DNA nuclease activity and the native DNA nuclease activity is inhibited. In some embodiments, the CRISPR-Cas protein comprises a mutation. In some embodiments, a nuclease domain of the CRISPR-Cas protein comprises the mutation. In some embodiments, the mutation occurs in a nucleic acid encoding the CRISPR-Cas protein. In some embodiments, the mutation occurs in an amino acid encoding the CRISPR-Cas protein. In some embodiments, the mutation comprises a substitution, an insertion, a deletion, a frameshift, an inversion, or a transposition. In some embodiments, the mutation comprises a deletion of a nuclease domain, a binding site within the nuclease domain, an active site within the nuclease domain, or at least one essential amino acid residue within the nuclease domain.

[0118] In some embodiments of the compositions of the disclosure, the first RNA binding protein comprises a CRISPR-Cas protein. In some embodiments, the CRISPR-Cas protein is a Type V CRISPR-Cas protein. In some embodiments, the first RNA binding protein comprises a Cpf1 polypeptide or an RNA-binding portion thereof. In some embodiments, the CRISPR-Cas

protein comprises a native RNA nuclease activity. In some embodiments, the native RNA nuclease activity is reduced or inhibited. In some embodiments, the native RNA nuclease activity is increased or induced. In some embodiments, the CRISPR-Cas protein comprises a native DNA nuclease activity and the native DNA nuclease activity is inhibited. In some embodiments, the CRISPR-Cas protein comprises a mutation. In some embodiments, a nuclease domain of the CRISPR-Cas protein comprises the mutation. In some embodiments, the mutation occurs in a nucleic acid encoding the CRISPR-Cas protein. In some embodiments, the mutation occurs in an amino acid encoding the CRISPR-Cas protein. In some embodiments, the mutation comprises a substitution, an insertion, a deletion, a frameshift, an inversion, or a transposition. In some embodiments, the mutation comprises a deletion of a nuclease domain, a binding site within the nuclease domain, an active site within the nuclease domain, or at least one essential amino acid residue within the nuclease domain.

[0119] In some embodiments of the compositions of the disclosure, the first RNA binding protein comprises a CRISPR-Cas protein. In some embodiments, the CRISPR-Cas protein is a Type VI CRISPR-Cas protein. In some embodiments, the first RNA binding protein comprises a Cas13 polypeptide or an RNA-binding portion thereof. In some embodiments, the first RNA binding protein comprises a Cas13d polypeptide or an RNA-binding portion thereof. In some embodiments, the CRISPR-Cas protein comprises a native RNA nuclease activity. In some embodiments, the native RNA nuclease activity is reduced or inhibited. In some embodiments, the native RNA nuclease activity is increased or induced. In some embodiments, the CRISPR-Cas protein comprises a native DNA nuclease activity and the native DNA nuclease activity is inhibited. In some embodiments, the CRISPR-Cas protein comprises a mutation. In some embodiments, a nuclease domain of the CRISPR-Cas protein comprises the mutation. In some embodiments, the mutation occurs in a nucleic acid encoding the CRISPR-Cas protein. In some embodiments, the mutation occurs in an amino acid encoding the CRISPR-Cas protein. In some embodiments, the mutation comprises a substitution, an insertion, a deletion, a frameshift, an inversion, or a transposition. In some embodiments, the mutation comprises a deletion of a nuclease domain, a binding site within the nuclease domain, an active site within the nuclease domain, or at least one essential amino acid residue within the nuclease domain.

[0120] In some embodiments of the compositions of the disclosure, the first RNA binding protein comprises a Pumilio and FBF (PUF) protein. In some embodiments, the first RNA

binding protein comprises a Pumilio-based assembly (PUMBY) protein. In some embodiments, a PUF1 protein of the disclosure comprises or consists of the amino acid sequence of

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MDKSKQMNIN NLSNIPEVID PGITIPYEE EYENNGESNS QLQQQPQKLK SYRSRAGKFS 60
NTLSNLLPSI SAKLHHSKKN SHGKNGAEFS SSNNSSQSTV ASKTPRASFS RSKMMESSID 120
GVTMDRPGSL TPPQDMEKLV HFPDSSNNFL IPAPRGSSDS FNLPHQISRT RNNTMSSQIT 180
SISSIAPKPR TSSGIWSSNA SANDPMQQHL LQQQLQPTTSN NTTNSNTLND YSTKTAYFDN 240
MVSTSGSQMA DNKMNNTNLA IPNSVWSNTR QRSQSNASSI YTDAPLYEQP ARASISSHYT 300
IPTQESPLIA DEIDPQSINW VTMDPTVPSI NQISNLLPTN TISISNVFPL QHQQPQLNNA 360
INLTSTSLAT LCSKYGEVIS ARTLRNLNMA LVEFSSVESA VKALDSLQK EVSMIGAPSK 420
ISFAKILPMH QQQPQFLINS QGLPLGLENN NLQPQPLLQE QLFNGAVTFQ QQGNVSIPVF 480
NQSQSQSQHQ NHSSGSAGFS NVLHGYNNNN SMHGNNNNSA NEKEQCFFPL PPPNVNEKED 540
LLREIIELEF ANSDEYQINS LIKKSLSNHHK TSDTQNEFGPL PEPLSGREFD PPKLRELKRS 600
IDSNAFSDLE IEQLAIAMLD ELPESLSDYL GNTIVQKLF HSSDIKDIM LRKTSKYLTS 660
MGVHKNGTWA CQKMITMAHT PRQIMQVTQG VKDYCTPLIN DQFGNYVIQC VLKFGFPWNQ 720
FIFESIIANF WVIVQNRGA RAVRACLEAH DIVTPEQSIV LSAMIVTYAE YLSTNSNGAL 780
LVTWFLDTSV LPNRHSILAP RLTKRIVELC GHRLASLTIL KVLNRYGDDN ARKIILDSLF 840
GNVNAHDSSP PKELTKLLCE TNYGPTFVHK VLAMPLLEDD LRAHIKQVR KVLTDSTQIQ 900
PSRRLLEEVG LASPSTHNK TKQQQQQHHN SSISHMFATP DTSGQHMRGL SVSSVKSGGS 960
KHTTMNTTTT NGSSASTLSP GQPLNANSNS SMGYFSYPGV FPVSGFSGNA SNGYAMNDD 1020
LSSQFDMLEF NNGTRLSLPQ LSLTNHNNNTT MELVNNVGS3 QPHTNNNNNN NNTNYNDDNT 1080
VFETLTLSA N 1091
(SEQ ID NO: 208).
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In some embodiments, a PUF3 protein of the disclosure comprises or consists of the amino acid sequence of

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1 MEMNMDMD MELASIVSSL SALSHSNNNG GQAAAAGIVN GGAAGSQQIG GFRRSSFTTA
61 NEVDSEILL HGSSESSPIF KKTALSVGTA PPFSTNSKKF FGNGGNYQQY RSTDTASLSS
121 ASYNNYHTH TAANLGKNNK VNHLGQYSA SIAGPVYNG NDNNSNGGEG FFEKFGKSLI
181 DGTRELESQ RPDVANTQSQ FISKSVSNAS LDTQNTFEQN VESDKNFNKL NRNTTNSGSL
241 YHSSNSGSS ASLESENAHY PKRNIWVAN TPVFRPSNNP AAVGATNVAL PNQDGPANN
301 NFPPYMGFF PNQFHQGPY QNFPNYLIGS PSNFISQMIS VQIPANEDTE DSNKKKKKA
361 NRSSSVSSP SPENNSFFPF AYPNFMFEMP FPPLSAPQQQ QQQQQQQQEE DQQQQQQQEN
421 PYIYYPTNP IPVKMPKDEK TFKKRNKNH PANNSNNANK QANPYLENSI PTKNTSKKNA
481 SSKSNESTAN NHKSHSHSH HSQSLQQQQQ TYHRSPLEEQ LRNSSSDKNS NSNMSLKDIF
541 GHSLEFCKDQ HGSRFIQREL ATSPASEKEV IFNEIRDDAI ELSNDVFGNY VIQKFFEFGS
601 KIQKNTLVDQ FKGNMQLSL QMYACRVIQ ALEYIDSNQR IELVLELSDS VLQMIKDQNG
661 NHVIQKAJET IPIEKLPIFIL SSLTGHYHL STHSYGCRVI QRLLFSGSSE DQESILNELK
721 DFIPYLIQDQ YGNYVIQYVL QQDQFTNKEM VDIKQEIET VANNVVEYSK HKFASNVVEK
781 SILYGSKNQK DLIISKILPR DKNHALNLED DSPMILMIK QFANYVIQKL VNVSEGEK
841 LIVIAIRAYL DKLKNSNSLG NRHLASVEKL AALVENAEV (SEQ ID NO: 209).
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In some embodiments, a PUF4 protein of the disclosure comprises or consists of the amino acid sequence of

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1 MSTKGLKEEI DDVPSVDFV SETVNSALEQ LQLDDPEENA TSNAFANKVS QDSQFANGPP
61 SQMFPHQMM GGMGFMPYSQ MMQVPHNCP FFPFDFNDP TAPLSSSPLN AGGPPMLFKN
121 DSLPFQMLSS GAAVATQGGQ NLNPLINDNS MKVLPASAD PLWTHSNVPG SASVAIETT
181 ATLQESLPSK GRESNNKASS FRRQTFHALS PTDLINAANN VTLSKDFQSD MQNFSAKAKP
241 SVGANN TAKT RTQISFDNT PSSTSFIPPT NSVSEKLSDF KIETSKEDLI NKTAPAKKES
301 PPTYGAAPY GGPLLQPNPI MPGHPHNISS PIYGIRSPFP NSYEMGAQFQ PFSPILNPTS
361 HSLNANSPIL LTQSPIHLAP VLNPSNSVA FSDMKNDGGK PTTDNDKAGP NVRMDLINPN
421 LGPSMQPFHI LPPQONTPPP PWLYSTPPPF NAMVPPHLLA QNHMPLMNSA NNKHHGRNNN
481 SMSSHNDNDN IGNSNYNNKD TGRSNVGKMK NMKNSYHGY NNNNNNNNNN NNNNNSNATN
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541 SNSAEKQRKI EESSRFADAV LDQYIGSIHS LCKDQHGC RF LQKQLDILGS KAADAIFEET
 601 KDYTVELMTD SFGNYLIQKL LEEVTTEQRI VLTKEISSPHF VEISLNPHGT RALQKLIIECI
 661 KTDEEAQIVV DSLRPYTVQL SKDLNGNHVI QKCLQRLKPE NFQFIFDAIS DSCIDIATHR
 721 HGCCVLQRCL DHGTTEQCDN LCDKLLALVD KLTLDPFNGY VVQYIITKEA EKNKYDYTHK
 781 IVHLLKPRAI ELSIHKFGSN VIEKILKTAI VSEPMILEIL NNGGETGIQS LLNDSYGNVY
 841 LQTALDISHK QNDYLYKRLS EIVAPLLVGP IRNTPHGKRI IGMHLHDS (SEQ ID NO:

210).

In some embodiments, a PUF5 protein of the disclosure comprises or consists of the amino acid sequence of

1 MSDSTGRINS KASDSSSISD HQTADLSIFN GSFDGGAFSS SNIPLFNFMG TGNQRFQYSP
 61 HPFAKSSDPC RLAALTPSTP KGPLNLTPAD FGLADFSVGN ESFADFTANN TSFVGNVQSN
 121 VRSTRLLPAW AVDNNGNIRD DLTLDQDVVN GSLIDFAMDR TGVKFLERHF PEDHDNEMHF
 181 VLFDKLTEQG AVFTSLCRSA AGNFIIQKFV EHATLDEQER LVRKMCNDGL IEMCLDKFAC
 241 RVVQMSIQKF DVSIAKMLVE KISSLDLPL CTDQCAIHVL QKVVKLLPIS AWSFFVKFLC
 301 RDDNIMTVCQ DRYGCRVLVQ TIDKLSNPK LHCFTNRLQL LHGLMTSVAR NCFRLSSNEF
 361 ANYVVQYVIK SSGVMEMYRD TIEKCLLRN ILSMSQDKYA SHVVEGAFLF APPLLLSEMM
 421 DEIFDGYVKD QETNRDALDI LLFHQYGNV VQQMISICIS ALLGKEERKM VASEMRLYAK
 481 WFDRIKNRVN RHSGRLERFS SGKKIIESLQ KLNVPMTMTN EPMYPWAMPT PLMDISAHFM
 541 NKLNFQKNSV FDE (SEQ ID NO: 211).

In some embodiments, a PUF6 protein of the disclosure comprises or consists of the amino acid sequence of

1 MTPNRRSTDS YNMLGASFDF DPDFSLLSNK THKNKNPKPP VKLLPYRHGS NTTSSDLNDY
 61 IFNSGSGSSD DETPPPAPI FISLEEVLLN GLLIDFAIDP SGVKFLEANY PLDSEDQIRK
 121 AVFEKLTEST TLFVGLCHSR NGNFIVQKLV ELATPAEQRE LLRQMIDGGL LVMCKDKFAC
 181 RVVQLALQKF DSHNVFQLIQ ELSTFDLAAM CTDQISIHVI QRVVKQLPVD MWTFVVFHFLS
 241 SGDSLMAVCQ DRYGCRVLVQ VIDRLAENPK LPCFKFRIQL LHSMLTCIVR NCFRLSSNEF
 301 ANYVIQYVIK SSGIMEMYRD TIIDKCLLRN LLSMSQDKYA SHVIEGAFLF APPALLHEMM
 361 EEIFSGYVKD VELNRDALDI LLFHQYGNV VQQMISICTA ALIGKEERQL PPAILLLYSG
 421 WYEKMKQKRVL QHASRLERFS SGKKIIDSVM RHGVPTAAAI NAQAAPSLME LTAQFDAMFP
 481 SFLAR (SEQ ID NO: 212).

In some embodiments, a PUF7 protein of the disclosure comprises or consists of the amino acid sequence of

1 MTPNRRSTDS YNMLGASFDF DPDFSLLSNK THKNKNPKPP VKLLPYRHGS NTTSSDSDSY
 61 IFNSGSGSSD AETPAFVAPI FISLEDVLLN GLLIDFAIDP SGVKFLEANY PLDSEDQIRK
 121 AVFEKFTEST TLFVGLCHSR NGNFIVQKLV ELATPAEQRE LLRQMIDGGL LAMCKDKFAC
 181 RVVQLALQKF DSHNVFQLIQ ELSTFDLAAM CTDQISIHVI QRVVKQLPVD MWTFVVFHFLS
 241 SGDSLMAVCQ DRYGCRVLVQ VIDRLAENPK LPCFKFRIQL LHSMLTCIVR NCFRLSSNEF
 301 ANYVIQYVIK SSGIMEMYRD TIIDKCLLRN LLSMSQDKYA SHVIEGAFLF APPALLHEMM
 361 EEIFSGYVKD VESNRDALDI LLFHQYGNV VQQMISICTA ALIGKEEREL PPAILLLYSG
 421 WYEKMKQKRVL QHASRLERFS SGKKIIDSVM RHGVPTAAAV NAQAAPSLME LTAQFDAMFP
 481 SFLAR (SEQ ID NO: 213).

In some embodiments, a PUF8 protein of the disclosure comprises or consists of the amino acid sequence of

1 MSRPISIGNT CTFDPSASPI ESLGRSIGAQ KIVDSVCGSP IRSYGRHIST NPKNERLPDT
 61 PEFQFATYMH QGGKVIQNT LHMFGTPPSC YCAQENIPIS SNVGHVLSTI NNNYMNHQYN
 121 GSNMFSNQMT QMLQAQAYND LQMHQAHSQS IRVPVQPSAT GIFSNPYREP TTTDDLTRY
 181 RANPAMKKNL KLSDIRGALL KFAKDQVGSF FIQQELASSK DRFEKDSIFD EVVSNADLV
 241 DDIFGNYVVQ KFFEYGEERH WARLVDAIID RVPEYAFQMY ACRVLQKALE KINEPLQIKI
 301 LSQIRHVIHR CMKDQNGNHV VQKAIEKVSP QYVQFIVDTL LESSNTIYEM SVDPYGCRV
 361 QRCLEHCSPS QTKPVIQIH KRFDEIANNQ YGNVYVQHV EHGSEEDRMV IVTRVSNNLF
 421 EFATHKYSSN VIEKCLEQGA VYHKSMIVGA ACHHQEGSV IVVQMMKDQY ANYVVQKMF

481 QVTSEQRREL ILTVRPHIPV LRQFPHGKHI LAKLEKYFOK PAVMSYPYQD MQGSH (SEQ ID NO: 214). In some embodiments, a PUF9 protein of the disclosure comprises or consists of the amino acid sequence of

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1  MADPNWAYAP PTNYYADHSI AKPIMISGGH PSQDQGHSPK SESFGQSVTT AFNGMVDNLV
61  GSPSSSVQQR NYFTTTPFPI SRSPNDRNDD KIMGNGSYGV PIPFQDGVF QGTPDFQMTF
121 FLQQGGHLIG GSPNGPVQVS GNWYSGGAGI FSTMQQADPS NGMPGMAAEF VNNENGMPGP
181 NGMHQQAMIS GSPPFPYQNM MNLTTSTFGAM GLGPQQIQQR DPQMFQQPIL HEPIQGMAQN
241 GFGQQVFFTQ MQNQQHPQGG AQQQLQQLAQ QHQQQQNSQQ FFGQGPNGMG NGGVMNDWSQ
301 RSFGMPQQQA QQNGLPENFS QNPERRRGPE DPNGQTPKTL QDIKNNVIEF AKDQHGSRFI
361 QQKLERASLR DKAAIFTFVL ENAEELMTDV FGNYVIQKFF EFGNNEQRNQ LVGTIRGNVM
421 KLALQMYGCR VIQKALEYVE EKYQHEILGE MEGQVLKCVK DQNGNHVIQK VIERVEPERL
481 QFIIDAFKN NSDNVYTLSV HPYGCVRVQR VLEYCNEEQK QPVLDAQIH LKQLVLDQYG
541 NYVIQHVIEH GSPSDKEQIV QDVISDDLK FAQHKFASNV IEKCLTFGGH AERNLIIDKV
601 CGDPNDPSPF LLQMMKDPFA NYVQKMLDV ADPQHRKKIT LTIKPHIATL RKNYFGKHIL
661 LKLEKYFAKQ APANSSNSSS NDQIYEHSPF DIPLGADFSN HPF (SEQ ID NO:
215).

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[0121] In some embodiments of the compositions of the disclosure, the first RNA binding protein does not require multimerization for RNA-binding activity. In some embodiments, the first RNA binding protein is not a monomer of a multimer complex. In some embodiments, a multimer protein complex does not comprise the first RNA binding protein.

[0122] In some embodiments of the compositions of the disclosure, the first RNA binding protein selectively binds to a target sequence within the RNA molecule. In some embodiments, the first RNA binding protein does not comprise an affinity for a second sequence within the RNA molecule. In some embodiments, the first RNA binding protein does not comprise a high affinity for or selectively bind a second sequence within the RNA molecule.

[0123] In some embodiments of the compositions of the disclosure, an RNA genome or an RNA transcriptome comprises the RNA molecule.

[0124] In some embodiments of the compositions of the disclosure, the first RNA binding protein comprises between 2 and 1300 amino acids, inclusive of the endpoints.

[0125] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein further comprises a nuclear localization signal (NLS). In some embodiments, the sequence encoding a nuclear localization signal (NLS) is positioned 3' to the sequence encoding the first RNA binding protein. In some embodiments, the first RNA binding protein comprises an NLS at a C-terminus of the protein.

[0126] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein further comprises a first sequence encoding a first NLS and a second

sequence encoding a second NLS. In some embodiments, the sequence encoding the first NLS or the second NLS is positioned 3' to the sequence encoding the first RNA binding protein. In some embodiments, the first RNA binding protein comprises the first NLS or the second NLS at a C-terminus of the protein.

[0127] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a nuclease domain. In some embodiments, the second RNA binding protein binds RNA in a manner in which it associates with RNA. In some embodiments, the second RNA binding protein associates with RNA in a manner in which it cleaves RNA.

[0128] In some embodiments of the compositions of the disclosure, the sequence encoding the second RNA binding protein comprises or consists of an RNase. In some embodiments, the second RNA binding protein comprises or consists of an RNase1 polypeptide. In some embodiments, the RNase1 polypeptide comprises or consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGLCKPVNTFVHEPLVDVQNV
CFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIIVACEGSPYV
PVHFDASVEDST (SEQ ID NO: 20). In some embodiments, the second RNA binding protein comprises or consists of an RNase4 polypeptide. In some embodiments, the RNase4 polypeptide comprises or consists of:

QDGM YQRFLRQH VHP EETGGSDRYCDLMMQRRKMTLYHCKRFNTFIHEDIWNIRSICS
TTNIQCKNGKMNCHEGVVKVTDCRDTGSSRAPNCRYRAIASTRRVVIACEGNPQVPVH
FDG (SEQ ID NO: 21). In some embodiments, the second RNA binding protein comprises or consists of an RNase6 polypeptide. In some embodiments, the RNase6 polypeptide comprises or consists of:

WPKRLTKAHWF EIQHIQPSPLQCN RAMSGINNYTQHCKHQNTFLHDSFQNVA AVCDLL
SIVCKNRRHNCHQSSKPVNMTDCRLTSGKYPQCRYSA AAYKFFIVACDPPQKSDPPYK
LVPVHLD SIL (SEQ ID NO: 22). In some embodiments, the second RNA binding protein comprises or consists of an RNase7 polypeptide. In some embodiments, the RNase7 polypeptide comprises or consists of:

APARAGFCPL LLLLLLLGLWVAEIPVSAKPKGMTSSQWFKIQHMQPSPQACNSAMKNINK
HTKRCKDLNTFLHEPFSSVAATCQTPKIACKNGDKNCHQSHGPVSLTMCKLTSGKYPNC
RYKEKRQNKSYVVACKPPQKKDSQQFHLVPVHLDRVL (SEQ ID NO: 23). In some embodiments, the second RNA binding protein comprises or consists of an RNase8 polypeptide.

In some embodiments, the RNase8 polypeptide comprises or consists of:

TSSQWFKTQHVQPSPQACNSAMSIINKYTERCKDLNTFLHEPFSSVAITCQTPNIACKNSC
KNCHQSHGPMSTMGELTSGKYPNCRYKEKHLNTPYIVACDPPQQGDPGYPLVPVHLD
KVV (SEQ ID NO: 24). In some embodiments, the second RNA binding protein comprises or
consists of an RNase2 polypeptide. In some embodiments, the RNase2 polypeptide comprises
or consists of:

KPPQFTWAQWFETQHINMTSQQCTNAMQVINNYQRRCKNQNTFLLTTFANVVNVCGN
PNMTCPSNKTRKNCHHSGSQVPLIHCNLTTPSPQNISNCRYAQTPANMFYIVACDNRDQ
RRDPPQYPVVPVHLDRII (SEQ ID NO: 25). In some embodiments, the second RNA binding
protein comprises or consists of an RNase6PL polypeptide. In some embodiments, the
RNase6PL polypeptide comprises or consists of:

DKRLRDNHEWKKLIMVQHPETVCEKIQNDCRDPPDYWTIHGLWPKSEGCNRSWPF
NLEEIKKNWMEITDSSLPSPSMGPAPPRWMRSTPRRSTLAEAWNSTGSWTSTGGCALPP
AALPSGDLCCRPSLTAGSRGVGVDLTALHQLLHVHYSATGIIPEECSEPTKPFQIILHHDH
TEWVQSIGMPIWGTISSSESAIGKNEESQPACAVLSHDS (SEQ ID NO: 26). In some
embodiments, the second RNA binding protein comprises or consists of an RNaseL
polypeptide. In some embodiments, the RNaseL polypeptide comprises or consists of:

AAVEDNHLLIKAVQNEDVDLVQQLLEGGANVNFQEEEGGWTPLHNAVQMSREDIVEL
LLRHGADPVLRRKNGATPFILAAIAGSVKdLLKLFLSKGADVNECDFYGFATFMEAAVY
GKVKALKFLYKRGANVNLRRKTKEDQERLRKGGATALMDAAEKGHVEVLKILLDEM
GADVNAACDNMGRNALIHALLSSDDSDVEAITHLLLDHGADVNVRRGERGKTPLILAVEK
KHLGLVQRLLEQEHIEINDTSDGKTALLLAVELKLKKIAELLCKRGASTDCGDLVMTA
RRNYDHSLVKVLLSHGAKEDFHPPAEDWKPQSSHWGAALKDLHRIYRPMIGKLKFFID
EKYKIADTSEGGIYLGfYEKQEVAVKTFCEGSPRAQREVSCSQSSRENSHLVTFYGSESH
RGHLFVCVTLCEQTLEACLDVHRGEDVENEDEFARNVLSSIFKAVQELHLSCGYTHQD
LQPQNILIDSKKAAHLADFDKSIKWAGDPQEVKRDLEDLGRVLVYVVKKGSISFEDLKA
QSNEEVVQLSPDEETKDLIHRLFHPGEHVRDCLSDLLGHPFFWTWESRYRTLNRVGNES
DIKTRKSESEILRLLQPGPSEHSKSFDKWTTKINECVMKKMKNKFYEKRGNFYQNTVGDL
LKFIRNLGEHIDEKHKMKMLKIGDPSLYFQKTFPDLVIYVYTKLQNTYRKHFHPQTHSP
NKPQCDGAGGASGLASPGC (SEQ ID NO: 27). In some embodiments, the second RNA
binding protein comprises or consists of an RNaseT2 polypeptide. In some embodiments, the

RNAseT2 polypeptide comprises or consists of:

VQHWPETVCEKIQNDCRDPPDYWTIHGLWPDKSEGCNRSWPFNLEEIKDLLPEMRAYW
PDVIHSFPNRSRFRWKHEWEKHGTCAAQVDALNSQKKYFGRSLELYRELDLNSVLLKLG
KPSINYYQVADFKDALARVYGVIPKIQCLPPSQDEEVQTIGQIELCLTKQDQQLQNCTEP
GEQPSPKQEVWLANGAAESRGLRVCEGDPVFYPPPKKTKH (SEQ ID NO: 28). In some

embodiments, the second RNA binding protein comprises or consists of an RNAse11 polypeptide. In some embodiments the RNAse11 polypeptide comprises or consists of:

EASESTMKIIKEEFTDEEMQYDMAKSGQEKQTIEILMNPILLVKNTSLMSKDDMSSTLL
TFRSLHYNDPKGNSSGNDKECCNDMTVWRKVSEANGSCKWSNNFIRSSTEVMRRVHR
APSCKFVQNPGISCCESLELENTVCQFTTGKQFPRCQYHSVTSLEKILTVLTGHSLMSWL
VCGSKL (SEQ ID NO: 29). In some embodiments, the second RNA binding protein comprises

or consists of an RNAseT2-like polypeptide. In some embodiments, the RNAseT2-like polypeptide comprises or consists of:

XLGGADKRLRDNHEWKKLIMVQHWPETVCEKIQNDCRDPPDYWTIHGLWPDKSEGCN
RSWPFNLEEIKDLLPEMRAYWPDVIHSFPNRSRFRWKHEWEKHGTCAAQVDALNSQKKY
FGRSLELYRELDLNSVLLKLGKPSINYYQTTEEDLNLDVEPTTEDTAAEVTHVLLHSAL
FGEIGPRRW (SEQ ID NO: 30).

[0129] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a mutated RNAse. In some embodiments, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R)) polypeptide. In some embodiments, the Rnase1(K41R) polypeptide comprises or consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCRPVNTFVHEPLVDVQNV
CFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIIVACEGSPYV
PVHFDASVEDST (SEQ ID NO: 116). In some embodiments, the second RNA binding protein

comprises or consists of a mutated Rnase1 (Rnase1(K41R, D121E)) polypeptide. In some embodiments, the Rnase1 (Rnase1(K41R, D121E)) comprises or consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCRPVNTFVHEPLVDVQNV
CFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIIVACEGSPYV
PVHFEASVEDST (SEQ ID NO: 117). In some embodiments, the second RNA binding protein

comprises or consists of a mutated Rnase1 (Rnase1(K41R, D121E, H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(K41R, D121E, H119N)) polypeptide comprises or

consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCRPVNTFVHEPLVDVQNV
CFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACEGSPYV
PVNFEASVEDST (SEQ ID NO: 118). In some embodiments, the second RNA binding protein

comprises or consists of a mutated Rnase1. In some embodiments, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(H119N)) polypeptide comprises or consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCKPVNTFVHEPLVDVQNV
CFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACEGSPYV
PVNFDASVEDST (SEQ ID NO: 119). In some embodiments, the second RNA binding protein

comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide comprises or consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGDCKPVNTFVHEPLVDVQNV
CFQEKVTCKDGQGNCYKSNSSMHITDCRLTADSDYPNCAYRTSPKERHIIVACEGSPYV
PVNFDASVEDST (SEQ ID NO: 120). In some embodiments, the second RNA binding protein

comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E)) polypeptide comprises or consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGDCRPVNTFVHEPLVDVQNV
CFQEKVTCKDGQGNCYKSNSSMHITDCRLTADSDYPNCAYRTSPKERHIIVACEGSPYV
PVNFEASVEDST (SEQ ID NO: 121). In some embodiments, the second RNA binding protein

comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide. In some embodiments, the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D)) polypeptide comprises or consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGDCKPVNTFVHEPLVDVQNV
CFQEKVTCKDGQGNCYKSNSSMHITDCRLTADSDYPNCAYRTSPKERHIIVACEGSPYV
PVHFDASVEDST (SEQ ID NO: 122).

In some embodiments, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1 (R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E)) polypeptide comprises or consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGDCRPVNTFVHEPLVDVQNV
CFQEKVTCKDGQGNKYKSNSSMHITDCRLTADSDYPNCAYRTSPKERHIIVACEGSPYV
PVNFEASVEDST (SEQ ID NO: 225).

[0130] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a NOB1 polypeptide. In some embodiments, the NOB1 polypeptide comprises or consists of:

APVEHVADAGAFRLRHAALQDIGKNIYTIREVVTEIRDKATTRRLAVLPYELRFKEPLPE
YVRLVTEFSKKTGDYPSLSATDIQVLALTYQLEAEFVGVSHLKQEPQKVKSSSIQHPET
PLHISGFHLPYKPKPPQETEKGHSACEPENLEFSSFMFWRNPLPNIDHELQELLIDRGEDV
PSEEEEEENGFEEDRKDDSDDDGGGWITPSNIKQIQQEQCDVPEDVRVGCLTTDFAM
QNVLLQMGLHVLAVNGMLIREARSYL RCHGCFKTTSDMSRVFCSHCGNKTLKKVSVT
V (SEQ ID NO: 31).

[0131] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an endonuclease. In some embodiments, the second RNA binding protein comprises or consists of an endonuclease V (ENDOV). In some embodiments, the ENDOV polypeptide comprises or consists of:

AFSGLQRVGGVDVSFVKGDSVRACASLVLSFPELEVVEESRMVSLTAPYVSGFLAFR
EVPFLELVQQLREKEPGLMPQVLLVDGNGVLHHRGFGVACHLGVLTDLPCVGVAKKL
LQVDGLENNALHKEKIRLLQTRGDSFPLLGDSTVLGMALRSHDRSTRPLYISVGHMS
LEAAVRLTCCCCRFRIPEPVRQADICSREHIRKS (SEQ ID NO: 32). In some embodiments, the second RNA binding protein comprises or consists of an endonuclease G (ENDOG)

polypeptide. In some embodiments, the ENDOG polypeptide comprises or consists of:

AELPPVPGGPRGPGELAKYGLPGLAQLKSRESYVLCYDPRTRGALWVVEQLRPERLRG
DGDRRECDFREDDSVHAYHRATNADYRGSGFDRGHLAAAANHRWSQKAMDDTFYLS
NVAPQVPHLNQNAWNNLEKYSRSLTRSYQNVYVCTGPLFLPRTEADGKSYVKYQVIGK
NHVAVPTHFFKVLILEAAGGQIELRTYVMPNAPVDEAIPLERFLVPIESIERASGLLFVPNI
LARAGSLKAITAGSK (SEQ ID NO: 33). In some embodiments, the second RNA binding

protein comprises or consists of an endonuclease D1 (ENDOD1) polypeptide. In some embodiments, the ENDOD1 polypeptide comprises or consists of:

RLVGEEEAGFGECDKFFYAGTPPAGLAADSHVKICQRAEGAERFATLYSTRDRIPVYSA
FRAPRPAPGGAEQRWLVEPQIDDPNSNLEEAINAEAITSVNSLGSKQALNTDYLDSDYQ

RGQLYPFSLSSDVQVATFTLTNSAPMTQSFQERWYVNLHSLMDRALTPQCGSGEDLYIL
TGTVPSPDYRVKDKVAVPEFVWLAACCAVPGGGWAMGFVKHTRDSDIIEDVMVKDLQ
KLLPFNPQLFQNNCGETEQDTEKMKKILEVVNQIQDEERMVQSQKSSSPLSSTRSKRSTL
LPPEASEGSSSFLGKLMGFIATPFIKLFQLIYYLVVAILKNIVYFLWCVTQKQVINGIESCLY
RLGSATISYFMAIGEELVSIPWKVLKVVAKVIRALLRILCCLLKAICRVLSIPVRVLVDVA
TFPVYTMGAIPVCKDIALGLGGTVSLLFDTAFGTLGGLFQVVFSVCKRIGYKVTFDMSG
EL (SEQ ID NO: 34). In some embodiments, the second RNA binding protein comprises or
consists of a Human flap endonuclease-1 (hFEN1) polypeptide. In some embodiments, the
hFEN1 polypeptide comprises or consists of:

MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSIYQFLIAVRQGGDVLQNEEGET
TSHLMGMFYRTIRMMENGIKPVYVFDGKPPQLKSGELAKRSERRAEAEKQLQQAQAAG
AEQEVEKFTKRLVKVTKQHNDECKHLLSLMGIPYLDAPSEAEASCAALVKAGKVYAAA
TEDMDCLTFGSPVLMRHLTASEAKKLPIQEFHLSRILQELGLNQEQFV DLCILLGSDYCE
SIRGIGPKRAVDLIQKHK SIEEIVRR LDPNKYPVPENWLHKEAHQLFLEPEVLD PESVELK
WSEPNEEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGR LDDFFKVTGSLSSAKRKE
PEPKGSTKKKAKTGAAGKFKRGK (SEQ ID NO: 35). In some embodiments, the second

RNA binding protein comprises or consists of a DNA repair endonuclease XPF (ERCC4)
polypeptide. In some embodiments, the ERCC4 polypeptide comprises or consists of:

MESGQPARRIAMAPLLEYERQLVLELLD TDGLVVCARGLGADRLLYHFLQLHCHPACL
VLVLNTQPAEEYFINQLKIEGVEHLPRRVTNEITSNSRYEVYTQGGVIFATSRILVVD FL
TDRIPSDLITGILVYRAHRIESCQEAFILRLFRQKNKRGFIKFTDN AVAFDTGFCHVERV
MRNLFVRKLYLWPRFHVA VNSFLEQHKPEVVEIHVSMTPTMLAIQTAILDILNACLKEL
KCHNPSLEVEDLSLENAIGKPFDKTIRHYLDPLWHQLGAKTKSLVQDLKILRTLLQYLSQ
YDCVTFLNLLESLRATEKAFGQNSGWLFLDSSTSMFINARARVYHLPDAKMSKKEKISE
KMEIKEGEGILWG (SEQ ID NO: 124).

[0132] In some embodiments of the compositions of the disclosure, the second RNA binding
protein comprises or consists of an Endonuclease III-like protein 1 (NTHL) polypeptide. In some
embodiments, the NTHL polypeptide comprises or consists of:

CSPQESGMTALSARMLTRSRSLGPGAGPRGCREEPGLRRREAAAEARKSHSPVKRPRK
AQRLRVAYEGSDSEKGEAEPLKVPVWEPQDWQQQLVNIRAMRNKKDAPVDHLGTEH
CYDSSAPPKVRRYQVLLSLMLSSQTKDQV TAGAMQRLRLRAGLTVDSILQTDDATLGKLI

YPVGFWRSKVKYIKQTSAILQQHYGGDIPASVAELVALPGVGPKMAHLAMAVAWGTV
SGIAVDTHVHRIANRLRWTKKATKSPEETRAALEEWLPRELWHEINGLLVGFGQQTCLP
VHPRCHACLNQALCPAAQGL (SEQ ID NO: 123).

[0133] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a human Schlafen 14 (hSLFN14) polypeptide. In some embodiments, the hSLFN14 polypeptide comprises or consists of:

ESTHVEFKRFTTKKVIPRIKEMLPHYVSAFANTQGGYVLIGVDDKSKEVVGCKWEKVN
DLLKKEIENCIEKLPTFHFCEKPKVNFTTKILNVYQKDVLDGYVCVIQVEPFCCVFAE
APDSWIMKDNSVTRLTAEQWVVMMLDTQSAPPSLVTDYNSCLISSASSARKSPGYPIKV
HKFKEALQ (SEQ ID NO: 36).

[0134] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a human beta-lactamase-like protein 2 (hLACTB2) polypeptide. In some embodiments, the hLACTB2 polypeptide comprises or consists of:

TLQGTNTYLVGTGPRRILDTGEPAlPEYISCLKQALTEFNIAIQEIVVTHWHRDHSGGIG
DICKSINNDTTYCIKKLPRNPQREEIIGNGEQYVYLKDGDKVIKTEGATLRVLYTPGHTD
DHMALLLEEENAI FSGDCILGEGTTVFEDLYDYMNSL KELLKIKADIIPGHGPVIHNAE
AKIQQYISHRNIREQQILTLFRENFEKSFTVMELVKIYKNTPENLHEMAKHNNLLHLKKL
EKEGKIFSNTDPDKKWK A HL (SEQ ID NO: 37).

[0135] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an apurinic/apyrimidinic (AP) endodeoxyribonuclease (APEX) polypeptide. In some embodiments, the second RNA binding protein comprises or consists of an apurinic/apyrimidinic (AP) endodeoxyribonuclease (APEX2) polypeptide. In some embodiments, the APEX2 polypeptide comprises or consists of:

MLRVVSWNINGIRRPLQGVANQEPSNCAAVAVGRILDELDA DIVCLQETKVTRDALTEP
LAIVEGYNSYFSFSRNRSGYSGVATFCKDNATPVAAEEGLSGLFATQNGDVGCYGNMD
EFTQEELRALDSEGRALLTQHKIRTWEGKEKTLTLINVYCPHADPGRPERLVFKMRFYR
LLQIRAEALLAAGSHVILGDLNTAHRPIDHWDVNLECFEEDPGRKWMDSLLSNLGCQ
SASHVGPFIDSYRCFQPKQEGAFTCWSAVTGARHLNYGSRLDYVLGDRTLVIDTFQASF
LLPEVMGSDHCPVGAVLSVSSVPAKQCPLCTRFLPEFAGTQLKILRFLVPLEQSPVLEQ
STLQHNNQTRVQTCQNK AQVRSTRPQPSQVGSSRGQKNLKS YFQPSPCPQASPDIELPS
LPLMSALMTPKTPEEKAVAKVVKGQAKTSEAKDEKELRTSFWKSVLAGPLRTPLCGGH

REPCVMRTVKKPGPNLGRRFYMCARPRGPPTDPSSRCNFFLWSRPS (SEQ ID NO: 38). In some embodiments, the APEX2 polypeptide comprises or consists of:

MLRVVSWNINGIRRPLQGVANQEPSNCAAVAVGRILDELDADIVCLQETKVTRDALTEP
LAIVEGYNSYFSFSRNRSGYSGVATFCKDNATPVAAEEGLSGLFATQNGDVGCYGNMD
EFTQEELRALDSEGRALLTQHKIRTWEGKEKTLTLINVYCPHADPGRPERLVFKMRFYR
LLQIRAEALLAAGSHVILGDLNTHRPIDHWDVNLECFEEDPGRKWMDSLLSNLGCQ
SASHVGPFIDSYRCFQPKQEGAFTCWSAVTGARHLNYGSRLDYVLGDRTLVIDTFQASF
LLPEVMGSDHCPVGAVLSVSSVPAKQCPPLCTRFLPEFAGTQLKILRFLVPLEQSP (SEQ

ID NO: 39). In some embodiments, the second RNA binding protein comprises or consists of an apurinic or apyrimidinic site lyase (APEX1) polypeptide. In some embodiments, the APEX1 polypeptide comprises or consists of:

PKRGKKGAVAEDGDELRTPEAKKSKTAAKKNDKEAAGEGPALYEDPPDQKTSPSGKP
ATLKICSWNV DGLRAWIKKKGLDWVKEEAPDILCLQETKCSENKLPAELQELPGLSHQ
YWSAPSDKEGYSGVGLLSRQCPLKVSYGIGDEEHDQEGRVIVAEFDSFVLVTAYVPNAG
RGLVRLEYRQRWDEAFRKFLKGLASRKPLVLCGDLNVAHEEIDLRNPKGNKKKNAGFTP
QERQGF GELLQAVPLADSFRLYPNTPYAYTFWTYMMNARSKNVGWRLDYFLLS
(SEQ ID NO: 125).

[0136] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an angiogenin (ANG) polypeptide. In some embodiments, the ANG polypeptide comprises or consists of:

QDNSRYTHFLTQHYDAKPQGRDDRYCESIMRRRGLTSPCKDINTFIHGNKRSIKAICENK
NGNPHRENLRISKSSFQVTTCKLHGGSPWPPCQYRATAGFRNVVVACENGLPVHLDQSI
FRRP (SEQ ID NO: 40).

[0137] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a heat responsive protein 12 (HRSP12) polypeptide. In some embodiments, the HRSP12 polypeptide comprises or consists of:

SSLIRR VISTAKAPGAIGPYSQAVLVDR TIYISGQIGMDPSSGQLVSGGVAEEAKQALKN
MGEILKAAGCDFTNVVKTTVLLADINDFNTVNEIYKQYFKSNFPARAAYQVAALPKGS
RIEIEAVAIQGPLTTASL (SEQ ID NO: 41).

[0138] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a Zinc Finger CCCH-Type Containing 12A (ZC3H12A)

polypeptide. In some embodiments, the ZC3H12A polypeptide comprises or consists of:

GGGTPKAPNLEPPLPEEEKEGSDLRPVVIDGSNVAMSHGNKEVFSCRGILLAVNWFLER
GHTDITVFVPSWRKEQPRPDVPITDQHILRELEKKKILVFTPSRRVGGKRVVCYDDRFIV
KLAYESDGIVVSNPTYRDLQGERQEWKRFIEERLLMYSFVNDKFMPPDDPLGRHGPSLD
NFLRKKPLTLE (SEQ ID NO: 42). In some embodiments, the ZC3H12A polypeptide
comprises or consists of:

SGPCGEKPVLEASPTMSLWEFEDSHSRQGTTPRGQELAAEEASALELQMKVDFFRKLGY
SSTEIHSVLQKLG VQADTNTVLGELVKHGTATERERQTSPDPCPQLPLVPRGGGTPKAP
NLEPPLPEEEKEGSDLRPVVIDGSNVAMSHGNKEVFSCRGILLAVNWFLERGHTDITVFV
PSWRKEQPRPDVPITDQHILRELEKKKILVFTPSRRVGGKRVVCYDDRFIVKLAYESDGI
VVSNPTYRDLQGERQEWKRFIEERLLMYSFVNDKFMPPDDPLGRHGPSLDNFLRKKPL
TLEHRKQPCPYGRKCTYGIKCRFFHPERPSCPQRSVADELARANALLSPPRAPSKDKNGRR
PSPSSQSSSLTTESEQCSLDGKKLGAQASPGSRQEGLTQTYAPSGRSLAPSGGSGSSFGPT
DWLPQTLDSLPHYVSQDCLDSGIGSLESQMSELWGVRGGGPGEPPRAPPYTGYSPYGSE
LPATAAFSAFGRAMGAGHFSVPADYPPAPPAPPPREYWSEPYPLPPPTSVLQEPPVQSPG
AGRSPWGRAGSLAKEQASVYTKLCGVFPFHLVEAVMGRFPQLLDPQQLAAEILSYKSQ
HPSE (SEQ ID NO: 43).

[0139] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a Reactive Intermediate Imine Deaminase A (RIDA) polypeptide. In some embodiments, the RIDA polypeptide comprises or consists of:

SSLIRRVISTAKAPGAIGPYSQAVLVDRITIIYISGQIGMDPSSGQLVSGGVAEEAKQALKN
MGEILKAAGCDFTNVVKTTVLLADINDFNTVNEIYKQYFKSNFPARAAYQVAALPKGS
RIEIEAVAIQGPLTTASL (SEQ ID NO: 44).

[0140] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a Phospholipase D Family Member 6 (PDL6) polypeptide. In some embodiments, the PDL6 polypeptide comprises or consists of:

EALFFPSQVTCTEALLRAPGAELAEPEGCPGLPHGESALSRLLRALLAARASLDLCLF
AFSSPQLGRAVQLLHQRGVRVRVVTDCDYMALNGSQIGLLRKAGIQVRHDQDPGYMH
HKFAIVDKRVLITGSLNWTQAIQNNRENVLITEDDEYVRLFLEEFERIWEQFNPTKYTF
FPPKKSHGSCAPPVSRAGGRLLSWHRTCSTSESQT (SEQ ID NO: 126).

[0141] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a mitochondrial ribonuclease P catalytic subunit (KIAA0391) polypeptide. In some embodiments, the KIAA0391 polypeptide comprises or consists of:

KARYKTLEPRGYSLIRGLIHSDRWREALLLLEDIKKVITPSKKNYNDICIQGALLHQDVN
TAWNLYQELLGHDIVPMLETLKAFFDFGKDIKDDNYSNKLLDILSYLRNNQLYPGESFA
HSIKTWFEVPGKQWKQFTTVRKSGQCSGCGKTIESIQLSPEEYECLKGKIMRDVIDGG
DQYRKTTTPQELKRFENFIKSRPPFDVVIDGLNVAKMFPKVRESQLLLNVVSQLAKRNLR
LLVLGRKHMLRRSSQWSRDEMEEVQKQASCFFADDISEDDPFLLYATLHSGNHCRFITR
DLMRDHKACLPDAKTQRLFFKWQQGHQLAIVNRFPGSKLTFQRILSYDTVVQTTGDSW
HIPYDEDLVERCSCEVPTKWLC LHQKT (SEQ ID NO: 127).

[0142] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an argonaute 2 (AGO2) polypeptide.

In some embodiments of the compositions of the disclosure, the AGO2 polypeptide comprises or consists of:

SVEPMFRHLKNTYAGLQLVVVILPGKTPVYAEVKRVGDTVLG MATQCVQMKNVQRTT
PQTL SNLCLKINVKLGGVNNILLPQGRPPVFQQPVI FLGADVTHPPAGDGKKPSIAAVVG
SMDAHPNRYCATVRVQQHRQEIIQDLAAMVRELLIQFYKSTRFKPTRIIFYRDGVSEGQF
QQVLHHELLAIREACIKLEKDYQPGITFIVVQKRHHTRLFCTDKNERVGKSGNIPAGTTV
DTKITHPTFEFD FYLC SHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTYVRCTRS
VSIPAPAYYAHLVAFRARYHLVDKEHDSAEGSHTSGQSNGRDHQALAKAVQVHQDTL
RTMYFA (SEQ ID NO: 128).

[0143] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a mitochondrial nuclease EXOG (EXOG) polypeptide. In some embodiments, the EXOG polypeptide comprises or consists of:

QGAEGALTGKQPDGSAEKAVLEQFGFPLTGTEARCYTNHALSYDQAKRVPRWVLEHIS
KSKIMGDADRKHCKFKPDPNIPPTFS AFNEDYVGSGWSRGHMAPAGNNKFSSKAMAET
FYLSNIVPQDFDNNSGYWNRIEMYCRELTERFEDVWVVS GPLTLPQTRGDGKKIVSYQV
IGEDNVAVPSHLYKVILARRSSVSTEPLALGAFVVPNEAIGFQPQLTEFQVSLQDLEKLSG
LVFFPHLDRTSDIRNICSVDTC KLLDFQEFTLYLSTRKIEGARSVLRLEKIMENLKN AEIEP
DDYFMSRYEKKLEELKAKEQSGTQIRKPS (SEQ ID NO: 129).

[0144] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a Zinc Finger CCCH-Type Containing 12D (ZC3H12D) polypeptide. In some embodiments, the ZC3H12D polypeptide comprises or consists of: EHPSKMEFFQKLGYDREDVLRVLGKLGEGALVNDVLQELIRTGSRPGALEHPAAPRLVP RGSCGVPDSAQRGPGTAEEDFRTLASSLRPIVIDGSNVAMSHGNETFSCRGIKLAVD WFRDRGHTYIKVFVPSWRKDPPrADTPIREQHVLAEERQAVLVYTPSRKVH GKRLVC YDDRYIVKVAYEQDGVIVSNDNYRDLQSENPEWKWFIEQRLLMFSFVNDRFMPPDDPL GRHGPSLSNFLSRKPKPPEPSWQHCPYGGKCTYGIKCKFYHPERPHHAQLAVADELRAK TGARPGAGAEERPPRAPGGSAGARAAPREPFHSLPPARGSPDLAALRGFSRLAFSD DLGPLGPPLPVPACSLTPRLGGPDWVSAGGRVPGPLSLSPESQFSPGDLPPPPGLQLQPR GEHRPRDLHGDLLSPRRPPDDPWARPPRSDFPGRSVWAEPAWGDGATGGLSVYATED DEGDAARARIALYSVFPRDQVDRVMAAFPELSDLARLILLVQRCQSAGAPLGKP (SEQ ID NO: 130).

[0145] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an endoplasmic reticulum to nucleus signaling 2 (ERN2) polypeptide. In some embodiments, the ERN2 polypeptide comprises or consists of: RQQQPQVVEKQQETPLAPADFAHISQDAQSLHSGASRRSQKRLQSPSKQAQPLDDPEAE QLTVVGKISFNPKDVLGRGAGGTFVFRGQFEGRAVAVKRLRECFGLVRREVQLLQES DRHPNVLRYFCTERGPQFHIALELCRASLQEYVENPDLDRGGLEPEVVLQQLMSGLAH LHSLHIVHRDLKPGNILITGPSQGLGRVVLSDFGLCCKLPAGRCFSLSHSGIPGTEGWM APELLQLLPDSPTS AVDIFSAGCVFYVLSGGSHPFGDSL YRQANILTGAPCLAHLEEEV HDKVVARDLVGAMLSPLPQPRPSAPQVLAHPFFWSRAKQLQFFQDVSDWLEKESEQEP LVRAL EAGGCAVVRDNWHEHISMP LQTDLRKFRSYKGT SVRDLLRAVRNKKHHYREL PVEVRQALGQVPDGFVQYFTNRFPRLLLHTHRAMRSCASESLFLPYPPDSEARRPCPG ATGR (SEQ ID NO: 131).

[0146] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a pelota mRNA surveillance and ribosome rescue factor (PELO) polypeptide. In some embodiments, the PELO polypeptide comprises or consists of: KLVRKNIEKDNAGQVTLVPEEPEDMWHTYNLVQVGDSLRASTIRKVQTESSTGSVGSN RVRTTLTLCVEAIDFDSQACQLRVKGTNIQEN EYVKMGAYHTIELEPNRQFTLAKKQW DSVVLERIEQACDPAWSADVA AVVMQEGLAHICLVTPSMTLTRAKVEVNIPKRKGNC

SQHDRALERFYEQVVQAIQRHIFDVVKCILVASPGFVREQFCDYLFQQAVKTDNKLLL
ENRSKFLQVHASSGHKYSYLKEALCDPTVASRLSDTKAAGEVKALDDFYKMLQHEPDRA
FYGLKQVEKANEAMAIDTLLISDELFRHQDVATRSRYVRLVDSVKENAGTVRIFSSLHV
SGEQLSQLTGVAAILRFPVPELSDQEGDSSSEED (SEQ ID NO: 132).

[0147] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a YBEY metalloproteinase (YBEY) polypeptide. In some embodiments, the YBEY polypeptide comprises or consists of:

SLVIRNLQRVIPRRAPLRSKIEIVRRILGVQKFDLGHCVDNKNIQHINRIYRDRNVPTDVL
SFPFHEHLKAGEFPQPDFDDYNLGDIFLGVEYIFHQCKENEDYNDVLTVTATHGLCHLL
GFTHGTEAEWQQMFQKEKAVLDELGRRTGTRLQPLTRGLFGGS (SEQ ID NO: 133).

[0148] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a cleavage and polyadenylation specific factor 4 like (CPSF4L) polypeptide. In some embodiments, the CPSF4L comprises or consists of:

QEVIAGLERFTFAFEKDVEMQKGTGLLPFQGMDKSASAVCNFFTKGLCEKGKLCPRH
DRGEKMOVCKHWLRGLCKKGDHCKFLHQYDLTRMPECYFYFSKFGDCSNKECSFLHV
PAFKSQDCPWYDQGFCKDGPLCKYRHVPRIMCLNYLVGFCPEGPKCQFAQKIREFKLLP
GSKI (SEQ ID NO: 134).

[0149] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an hCG_2002731 polypeptide. In some embodiments, the hCG_2002731 polypeptide comprises or consists of:

KLVRKNIEKDNAGQVTLVPEEPEDMWHTYNLVQVGDSLRASTIRKVQTESSTGSGVGSN
RVRTTLTLCVEAIDFDSQACQLRVKGTNIQENEYVKMGAYHTIELEPNRQFTLAKKQW
DSVVLERIEQACDPAWSADVAAVVMQEGLAHICLVTPSMTLTRAKVEVNIPRKRKGNC
SQHDRALERFYEQVVQAIQRHIFDVVKCILVASPGFVREQFCDYMFQQAVKTDNKLLL
ENRSKFLQVHASSGHKYSYLKEALCDPTVASRLSDTKAAGEVKALDDFYKMLQHEPDRA
FYGLKQVEKANEAMAIDTLLISDELFRHQDVATRSRYVRLVDSVKENAGTVRIFSSLHV
SGEQLSQLTGVAAILRFPVPELSDQEGDSSSEED (SEQ ID NO: 135). In some embodiments,

the hCG_2002731 polypeptide comprises or consists of:

DPAWSADVAAVVMQEGLAHICLVTPSMTLTRAKVEVNIPRKRKGNC SQHDRALERFYEQVVQAIQRHIFDVVKCILVASPGFVREQFCDYMFQQAVKTDNKLLLENRSKFLQVHAS
SGHKYSYLKEALCDPTVASRLSDTKAAGEVKALDDFYKMLQHEPDRAFYGLKQVEKAN

EAMAIIDTLLISDELFRHQDVATRSRYVRLVDSVKENAGTVRIFSSLHVSQEQLSQLTGVA
AILRFPVPELSDQEGDSSSEED (SEQ ID NO: 136).

[0150] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an Excision Repair Cross-Complementation Group 1 (ERCC1) polypeptide. In some embodiments, the ERCC1 polypeptide comprises or consists of:

MDPGKDKEGVPQPSGPPARKKFVIPLDEDEVPPGVRGNPVLKFVRNVPWEFGDVIPDYV
LGQSTCALFLSLRYHNLHPDYIHGRLQSLGKNFALRVLLVQVDVKDPQQALKELAKMC
ILADCTLILAWSPEEAGRYLETYKAYEQKPADLLMEKLEQDFVSRVTECLTTVKSVNKT
DSQTLLTTFGSLEQLIAASREDLALCPGLGPQK (SEQ ID NO: 137).

[0151] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a ras-related C3 botulinum toxin substrate 1 isoform (RAC1) polypeptide. In some embodiments, the RAC1 polypeptide comprises or consists of:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCKPVNTFVHEPLVDVQNV
CFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACEGSPYV
PVHFDASVEDST (SEQ ID NO: 138).

[0152] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a Ribonuclease A A1 (RAA1) polypeptide. In some embodiments, the RAA1 polypeptide comprises or consists of:

QDNSRYTHFLTQHYDAKPQGRDDRYCESIMRRRGLTSPCKDINTFIHGKRSIKAICENK
NGNPHRENLRISKSSFQVTCKLHGGSPWPPCQYRATAGFRNVVVACENGLPVHLDQSI
FRRP (SEQ ID NO: 139).

[0153] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a Ras Related Protein (RAB1) polypeptide. In some embodiments, the RAB1 polypeptide comprises or consists of:

GLGLVQPSYGQDGMVQRFLRQHVHPEETGGSDRYCNLMMQRRKMTLYHCKRFNTFIH
EDIWNIRSICSTTNIQCKNGKMNCHGVVKTDCRDTGSSRAPNCRYRAIASTRRVVIAC
EGNPQVPVHFDG (SEQ ID NO: 140).

[0154] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a DNA Replication Helicase/Nuclease 2 (DNA2) polypeptide. In some embodiments, the DNA2 polypeptide comprises or consists of:

XSAVDNILLKLAKFKIGFLRLGQIQKVHPAIQFTEQEICRSKSIKSLALLEELYNSQLIVA

TTCMGINHPIFSRKIFDFCIVDEASQISQPICLGPLFFSRRFVLVGDHQQLPPLVLNREARA
LGMSESLFKRLEQNKSASVVQLTVQYRMNSKIMSLSNKLTIEGKLECGSDKVANAVINL
RHFKDVKLELEFYADYSDNPWLMGVFEPNNPVCFLNTDKVPAPEQVEKGGVSNVTEA
KLIVFLTSIFVKAGCSPSDIGIIPYRQQLKIINDLLARSIGMVEVNTVDKYQGRDKSIVLV
SFVRSNKDGTVGELLKDWRRLNVAITRAKHKLILLGCVPSLNCYPPLEKLLNHLNSEKLI
SFFFCIWSHLIAL (SEQ ID NO: 141).

[0155] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a FLJ35220 polypeptide. In some embodiments, the FLJ35220 polypeptide comprises or consists of:

MALRSHDRSTRPLYISVGHRMSLEAAVRLTCCCCRFRIPEPVRQADICSREHIRKSLGLP
GPPTPRSPKAQRPVACPKGDSGESSALC (SEQ ID NO: 142).

[0156] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a FLJ13173 polypeptide. In some embodiments, the FLJ13173 polypeptide comprises or consists of:

CYTNHALSYDQAKRVPRWVLEHISKSKIMGDADRKHCKFKPDPNIPPTFSAFNEDYVGS
GWSRGHMAPAGNNKFSSKAMAETFYLSNIVPQDFDNNSGYWNRIEMYCRELTERFEDV
WVVSGLTLPQTRGDGKKIVSYQVIGEDNVAVPSHLYKVILARRSSVSTEPLALGAFVV
PNEAIGFQPQLTEFQVSLQDLEKLSGLVFFPHLDRT (SEQ ID NO: 143).

[0157] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of Teneurin Transmembrane Protein (TENM) polypeptide. In some embodiments, the second RNA binding protein comprises or consists of Teneurin Transmembrane Protein 1 (TENM1) polypeptide. In some embodiments, the TENM1 polypeptide comprises or consists of:

VTVSQMTSVLNGKTRRFADIQLQHGAFCFNIRYGTTVVEEKNHVLEIARQRAVAQAWT
KEQRRLQEGEEGIRAWTEGEKQQLSTGRVQGYDGYFVLSVEQYLELSDSANNIHFMR
QSEIGRR (SEQ ID NO: 144). In some embodiments, the second RNA binding protein

comprises or consists of Teneurin Transmembrane Protein 2 (TENM2) polypeptide. In some embodiments, the TENM2 polypeptide comprises or consists of:

TVSQPTLLVNGKTRRFTNIEFQYSTLLLSIRYGLTPDTLDEEKARVLDQARQRALGTAW
AKEQQKARDGREGSRLWTEGEKQQLSTGRVQGYEGYYVLPVEQYPELADSSSNIQFL
RQNEMGKR (SEQ ID NO: 145).

In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of Ribonuclease Kappa (RNaseK) polypeptide. In some embodiments, the RNaseK polypeptide comprises or consists of:

MGWLRPGPRPLCPPARASWAFSHRFPSPAPRRSPTPFFMASLLCCGPKLAACGIVLSA
WGVIMLIMLGIFNVHSAVLIEDVPFTEKDFENGQPQNIYNLYEQVSYNCFIAAGLYLLLG
GFSFCQVRLNKRKEYMVR (SEQ ID NO: 204).

In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a transcription activator-like effector nuclease (TALEN) polypeptide or a nuclease domain thereof.

In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists a zinc finger nuclease polypeptide or a nuclease domain thereof. In some embodiments, the second RNA binding protein comprises or consists of a ZNF638 polypeptide or a nuclease domain thereof.

[0158] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a PIN domain derived from the human SMG6 protein, also commonly known as telomerase-binding protein EST1A isoform 3, NCBI Reference Sequence: NP_001243756.1. In some embodiments, the PIN from hSMG6 is used herein in the form of a Cas fusion protein and as an internal control.

Guide RNA

[0159] The terms guide RNA (gRNA) and single guide RNA (sgRNA) are used interchangeably throughout the disclosure.

[0160] Guide RNAs (gRNAs) of the disclosure may comprise of a spacer sequence and a scaffolding sequence. In some embodiments, a guide RNA is a single guide RNA (sgRNA) comprising a contiguous spacer sequence and scaffolding sequence. In some embodiments, the spacer sequence and the scaffolding sequence are contiguous. In some embodiments, a scaffold sequence comprises a “direct repeat” (DR) sequence. DR sequences refer to the repetitive sequences in the CRISPR locus (naturally-occurring in a bacterial genome or plasmid) that are interspersed with the spacer sequences. It is well known that one would be able to infer the DR sequence of a corresponding Cas protein if the sequence of the associated CRISPR locus is known. In some embodiments, the spacer sequence and the scaffolding sequence are not contiguous. In some embodiments, a sequence encoding a guide RNA of the disclosure

comprises or consists of a spacer sequence and a scaffolding sequence, that are separated by a linker sequence. In some embodiments, the linker sequence may comprise or consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or any number of nucleotides in between. In some embodiments, the linker sequence may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or any number of nucleotides in between.

[0161] Guide RNAs (gRNAs) of the disclosure may comprise non-naturally occurring nucleotides. In some embodiments, a guide RNA of the disclosure or a sequence encoding the guide RNA comprises or consists of modified or synthetic RNA nucleotides. Exemplary modified RNA nucleotides include, but are not limited to, pseudouridine (Ψ), dihydrouridine (D), inosine (I), and 7-methylguanosine (m7G), hypoxanthine, xanthine, xanthosine, 7-methylguanine, 5, 6-Dihydrouracil, 5-methylcytosine, 5-methylcytidine, 5-hydroxymethylcytosine, isoguanine, and isocytosine.

[0162] Guide RNAs (gRNAs) of the disclosure may bind modified RNA within a target sequence. Within a target sequence, guide RNAs (gRNAs) of the disclosure may bind modified RNA. Exemplary epigenetically or post-transcriptionally modified RNA include, but are not limited to, 2'-O-Methylation (2'-OMe) (2'-O-methylation occurs on the oxygen of the free 2'-OH of the ribose moiety), N6-methyladenosine (m6A), and 5-methylcytosine (m5C).

[0163] In some embodiments of the compositions of the disclosure, a guide RNA of the disclosure comprises at least one sequence encoding a non-coding C/D box small nucleolar RNA (snoRNA) sequence. In some embodiments, the snoRNA sequence comprises at least one sequence that is complementary to the target RNA, wherein the target sequence of the RNA molecule comprises at least one 2'-OMe. In some embodiments, the snoRNA sequence comprises at least one sequence that is complementary to the target RNA, wherein the at least one sequence that is complementary to the target RNA comprises a box C motif (RUGAUGA) and a box D motif (CUGA).

[0164] Spacer sequences of the disclosure bind to the target sequence of an RNA molecule. Spacer sequences of the disclosure may comprise a CRISPR RNA (crRNA). Spacer sequences of the disclosure comprise or consist of a sequence having sufficient complementarity to a target sequence of an RNA molecule to bind selectively to the target sequence. Upon binding to a target sequence of an RNA molecule, the spacer sequence may guide one or more of a scaffolding sequence and a fusion protein to the RNA molecule. In some embodiments, a

sequence having sufficient complementarity to a target sequence of an RNA molecule to bind selectively to the target sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96, 97%, 98%, 99%, or any percentage identity in between to the target sequence. In some embodiments, a sequence having sufficient complementarity to a target sequence of an RNA molecule to bind selectively to the target sequence has 100% identity the target sequence.

[0165] Scaffolding sequences of the disclosure bind the first RNA-binding polypeptide of the disclosure. Scaffolding sequences of the disclosure may comprise a trans acting RNA (tracrRNA). Scaffolding sequences of the disclosure comprise or consist of a sequence having sufficient complementarity to a target sequence of an RNA molecule to bind selectively to the target sequence. Upon binding to a target sequence of an RNA molecule, the scaffolding sequence may guide a fusion protein to the RNA molecule. In some embodiments, a sequence having sufficient complementarity to a target sequence of an RNA molecule to bind selectively to the target sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96, 97%, 98%, 99%, or any percentage identity in between to the target sequence. In some embodiments, a sequence having sufficient complementarity to a target sequence of an RNA molecule to bind selectively to the target sequence has 100% identity the target sequence. Alternatively, or in addition, in some embodiments, scaffolding sequences of the disclosure comprise or consist of a sequence that binds to a first RNA binding protein or a second RNA binding protein of a fusion protein of the disclosure. In some embodiments, scaffolding sequences of the disclosure comprise a secondary structure or a tertiary structure. Exemplary secondary structures include, but are not limited to, a helix, a stem loop, a bulge, a tetraloop and a pseudoknot. Exemplary tertiary structures include, but are not limited to, an A-form of a helix, a B-form of a helix, and a Z-form of a helix. Exemplary tertiary structures include, but are not limited to, a twisted or helicized stem loop. Exemplary tertiary structures include, but are not limited to, a twisted or helicized pseudoknot. In some embodiments, scaffolding sequences of the disclosure comprise at least one secondary structure or at least one tertiary structure. In some embodiments, scaffolding sequences of the disclosure comprise one or more secondary structure(s) or one or more tertiary structure(s).

[0166] In some embodiments of the compositions of the disclosure, a guide RNA or a portion thereof selectively binds to a tetraloop motif in an RNA molecule of the disclosure. In some embodiments, a target sequence of an RNA molecule comprises a tetraloop motif. In some

embodiments, the tetraloop motif is a “GRNA” motif comprising or consisting of one or more of the sequences of GAAA, GUGA, GCAA or GAGA.

[0167] In some embodiments of the compositions of the disclosure, a guide RNA or a portion thereof that binds to a target sequence of an RNA molecule hybridizes to the target sequence of the RNA molecule. In some embodiments, a guide RNA or a portion thereof that binds to a first RNA binding protein or to a second RNA binding protein covalently binds to the first RNA binding protein or to the second RNA binding protein. In some embodiments, a guide RNA or a portion thereof that binds to a first RNA binding protein or to a second RNA binding protein non-covalently binds to the first RNA binding protein or to the second RNA binding protein.

[0168] In some embodiments of the compositions of the disclosure, a guide RNA or a portion thereof comprises or consists of between 10 and 100 nucleotides, inclusive of the endpoints. In some embodiments, a spacer sequence of the disclosure comprises or consists of between 10 and 30 nucleotides, inclusive of the endpoints. In some embodiments, a scaffold sequence of the disclosure comprises or consists of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides. In some embodiments, the spacer sequence of the disclosure comprises or consists of 20 nucleotides. In some embodiments, the spacer sequence of the disclosure comprises or consists of 21 nucleotides. In some embodiments, a scaffold sequence of the disclosure comprises or consists of between 10 and 100 nucleotides, inclusive of the endpoints. In some embodiments, a scaffold sequence of the disclosure comprises or consists of 30, 35, 40, 45, 50, 55, 60, 65, 70, 76, 80, 87, 90, 95, 100 or any number of nucleotides in between. In some embodiments, the scaffold sequence of the disclosure comprises or consists of between 85 and 95 nucleotides, inclusive of the endpoints. In some embodiments, the scaffold sequence of the disclosure comprises or consists of 85 nucleotides. In some embodiments, the scaffold sequence of the disclosure comprises or consists of 90 nucleotides. In some embodiments, the scaffold sequence of the disclosure comprises or consists of 93 nucleotides.

[0169] In some embodiments of the compositions of the disclosure, a guide RNA or a portion thereof not comprise a nuclear localization sequence (NLS).

[0170] In some embodiments of the compositions of the disclosure, a guide RNA or a portion thereof not comprise a sequence complementary to a protospacer adjacent motif (PAM).

[0171] Therapeutic or pharmaceutical compositions of the disclosure do not comprise a PAMmer oligonucleotide. In other embodiments, optionally, non-therapeutic or non-

pharmaceutical compositions may comprise a PAMmer oligonucleotide. The term “PAMmer” refers to an oligonucleotide comprising a PAM sequence that is capable of interacting with a guide nucleotide sequence-programmable RNA binding protein. Non-limiting examples of PAMmers are described in O’Connell *et al. Nature* 516, pages 263–266 (2014), incorporated herein by reference. A PAM sequence refers to a protospacer adjacent motif comprising about 2 to about 10 nucleotides. PAM sequences are specific to the guide nucleotide sequence-programmable RNA binding protein with which they interact and are known in the art. For example, *Streptococcus pyogenes* PAM has the sequence 5’-NGG-3’, where “N” is any nucleobase followed by two guanine (“G”) nucleobases. Cas9 of *Francisella novicida* recognizes the canonical PAM sequence 5’-NGG-3’, but has been engineered to recognize the PAM 5’-YG-3’ (where “Y” is a pyrimidine), thus adding to the range of possible Cas9 targets. The Cpf1 nuclease of *Francisella novicida* recognizes the PAM 5’-TTTN-3’ or 5’-YTN-3’.

[0172] In some embodiments of the compositions of the disclosure, a guide RNA or a portion thereof comprises a sequence complementary to a protospacer flanking sequence (PFS). In some embodiments, including those wherein a guide RNA or a portion thereof comprises a sequence complementary to a PFS, the first RNA binding protein may comprise a sequence isolated or derived from a Cas13 protein. In some embodiments, including those wherein a guide RNA or a portion thereof comprises a sequence complementary to a PFS, the first RNA binding protein may comprise a sequence encoding a Cas13 protein or an RNA-binding portion thereof. In some embodiments, the guide RNA or a portion thereof does not comprise a sequence complementary to a PFS.

[0173] In some embodiments of the compositions of the disclosure, a guide RNA sequence of the disclosure comprises a promoter to drive expression of the guide RNA. In some embodiments, a vector comprising a guide RNA sequence of the disclosure comprises a promoter to drive expression of the guide RNA. In some embodiments, the promoter is a constitutive promoter. In some embodiments, a promoter is a tissue-specific and/or cell-type specific promoter. In some embodiments, a promoter is an inducible promoter. In some embodiments, a promoter is a hybrid or a recombinant promoter. In some embodiments, a promoter is a promoter capable of driving expression in a mammalian cell. In some embodiments, a promoter is a promoter capable of expression in a human cell. In some embodiments, a promoter is a promoter capable of expressing the guide RNA sequence and

restricting the expression to the nucleus of the cell. In some embodiments, a promoter is a human RNA polymerase promoter or a promoter sequence isolated or derived from a human RNA polymerase promoter. In some embodiments, a promoter is a U6 promoter or a sequence isolated or derived from a sequence encoding a U6 promoter. In some embodiments, a promoter is a human tRNA promoter or a promoter sequence isolated or derived from a human tRNA promoter. In some embodiments, a promoter is a human valine tRNA promoter or a promoter sequence isolated or derived from a human valine tRNA promoter.

[0174] In some embodiments of the compositions of the disclosure, a promoter further comprises a regulatory element. In some embodiments, a vector comprising a promoter which further comprises a regulatory element. In some embodiments, a regulatory element enhances expression of the guide RNA. Exemplary regulatory elements include, but are not limited to, an enhancer element, an intron, an exon, or a combination thereof.

[0175] In some embodiments of the compositions of the disclosure, a vector of the disclosure comprises one or more of a guide RNA sequence, a promoter to drive expression of the guide RNA and a regulatory element to enhance expression of the guide RNA. In some embodiments of the compositions of the disclosure, the vector further comprises a nucleic acid sequence encoding a fusion protein of the disclosure.

Fusion Proteins

[0176] Fusion proteins of the disclosure comprise a first RNA binding protein and a second RNA binding protein. In some embodiments, along a sequence encoding the fusion protein, the sequence encoding the first RNA binding protein is positioned 5' of the sequence encoding the second RNA binding protein. In some embodiments, along a sequence encoding the fusion protein, the sequence encoding the first RNA binding protein is positioned 3' of the sequence encoding the second RNA binding protein.

[0177] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a protein capable of binding an RNA molecule. In some embodiments, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a protein capable of selectively binding an RNA molecule and not binding a DNA molecule, a mammalian DNA molecule or any DNA molecule. In some embodiments, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a protein capable of binding an RNA molecule and inducing

a break in the RNA molecule. In some embodiments, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a protein capable of binding an RNA molecule, inducing a break in the RNA molecule, and not binding a DNA molecule, a mammalian DNA molecule or any DNA molecule. In some embodiments, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a protein capable of binding an RNA molecule, inducing a break in the RNA molecule, and neither binding nor inducing a break in a DNA molecule, a mammalian DNA molecule or any DNA molecule.

[0178] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a protein with no DNA nuclease activity.

[0179] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a protein having DNA nuclease activity, wherein the DNA nuclease activity does not induce a break in a DNA molecule, a mammalian DNA molecule or any DNA molecule when a composition of the disclosure is contacted to an RNA molecule or introduced into a cell or into a subject of the disclosure.

[0180] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a protein having DNA nuclease activity, wherein the DNA nuclease activity is inactivated and wherein the DNA nuclease activity does not induce a break in a DNA molecule, a mammalian DNA molecule or any DNA molecule when a composition of the disclosure is contacted to an RNA molecule or introduced into a cell or into a subject of the disclosure. In some embodiments, the sequence encoding the first RNA binding protein comprises a mutation that inactivates or decreases the DNA nuclease activity to a level at which the DNA nuclease activity does not induce a break in a DNA molecule, a mammalian DNA molecule or any DNA molecule when a composition of the disclosure is contacted to an RNA molecule or introduced into a cell or into a subject of the disclosure. In some embodiments, the sequence encoding the first RNA binding protein comprises a mutation that inactivates or decreases the DNA nuclease activity and the mutation comprises one or more of a substitution, inversion, transposition, insertion, deletion, or any

combination thereof to a nucleic acid sequence or amino acid sequence encoding the first RNA binding protein or a nuclease domain thereof.

[0181] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein of an RNA-guided fusion protein disclosed herein comprises a sequence isolated or derived from a CRISPR Cas protein. In some embodiments, the CRISPR Cas protein comprises a Type II CRISPR Cas protein. In some embodiments, the Type II CRISPR Cas protein comprises a Cas9 protein. Exemplary Cas9 proteins of the disclosure may be isolated or derived from any species, including, but not limited to, a bacteria or an archaea. Exemplary Cas9 proteins of the disclosure may be isolated or derived from any species, including, but not limited to, *Streptococcus pyogenes*, *Haloferax mediteranii*, *Mycobacterium tuberculosis*, *Francisella tularensis subsp. novicida*, *Pasteurella multocida*, *Neisseria meningitidis*, *Campylobacter jejune*, *Streptococcus thermophilus*, *Campylobacter lari* CF89-12, *Mycoplasma gallisepticum str. F*, *Nitratifactor salsuginis str. DSM 16511*, *Parvibaculum lavamentivorans*, *Roseburia intestinalis*, *Neisseria cinerea*, *Gluconacetobacter diazotrophicus*, *an Azospirillum B510*, *a Sphaerochaeta globus str. Buddy*, *Flavobacterium columnare*, *Fluviicola taffensis*, *Bacteroides coprophilus*, *Mycoplasma mobile*, *Lactobacillus farciminis*, *Streptococcus pasteurianus*, *Lactobacillus johnsonii*, *Staphylococcus pseudintermedius*, *Filifactor alocis*, *Treponema denticola*, *Legionella pneumophila str. Paris*, *Sutterella wadsworthensis*, *Corynebacter diphtherias*, *Streptococcus aureus*, and *Francisella novicida*.

[0182] Exemplary wild type *S. pyogenes* Cas9 proteins of the disclosure may comprise or consist of the amino acid sequence:

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1  MDKKYSIGLD  IGTNSVGWAV  ITDEYKVPSK  KFKVLGNTDR  HSIKKNLIGA  LLFDSGETAE
61  ATRLKRTARR  RYTRRKNRIC  YLQEIFSNEM  AKVDDSFHR  LEESFLVEED  KKHERHPIFG
121 NIVDEVAYHE  KYPTIYHLRK  KLV DSTKAD  LR LIYLALAH  MIKFRGHFLI  EGD LNPDNSD
181 VDKLFIQLVQ  TYNQLFEENP  INASGVDAKA  ILSARLSKSR  RLENLIAQLP  GEKKNGLFGN
241 LIALSLGLTP  NFKSNFDLAE  DAKLQLSKDT  YDDDLNLLA  QIGDQYADLF  LAAKNLSDAI
301 LLSDILRVNT  EITKAPLSAS  MIKRYDEHHQ  DL TLLKALVR  QQLPEKYKEI  FFDQSKNGYA
361 GYIDGGASQE  EFYKFIKPIL  EKMDGTEELL  VKLNREDLLR  KQRTFDNGSI  PHQIHLGELH
421 AILRRQEDFY  PFLKDNREKI  EKILTRIPY  YVGPLARGNS  RFAWMTRKSE  ETITPNFEE
481 VVDKGASAQS  FIERMTNFDK  NLPNEKVLPK  HSLLYEYFTV  YNELTKVKYV  TEGMRKPAFL
541 SGEQKKAIVD  LLFKTNRKVT  VKQLKEDYFK  KIECFDSVEI  SGVEDRFNAS  LGTYHDL LKI
601 IKDKDFLDNE  ENEDILEDIV  LTLTFLFEDRE  MIEERLKTYA  HLFDDKVMKQ  LKRRRYTGWG
661 RLSRKLINGI  RDKQSGKTIL  DFLKSDGFAN  RNFMQLIHDD  SLTFKEDIQK  AQVSGQGDSL

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721 HEHIANLAGS PAIKKGILQT VKVDELVKV MGRHKPENIV IEMARENQTT QKGQKNSRER
 781 MKRIEEGIKE LGSQILKEHP VENTQLQNEK LYLYYLQNGR DMYVDQELDI NRLSDYDVDH
 841 IVPQSFLKDD SIDNKVLTRS DKNRGKSDNV PSEEVVKKMK NYWRQLLNAK LITQRKFDNL
 901 TKAERGGLSE LDKAGFIKRQ LVETRQITKH VAQILDSRMN TKYDENDKLI REVKVITLKS
 961 KLVSDFRKDF QFYKREINN YHHAHDAYLN AVVGTAIIKK YPKLESEFVY GDYKVYDVRK
 1021 MIAKSEQEIG KATAKYFFYS NIMNFFKTEI TLANGEIRKR PLIETNGETG EIVWDKGRDF
 1081 ATVRKVLSP QVNIVKKTEV QTGGFSKESI LPKRNSDKLI ARKKDWDPKK YGGFDSPTVA
 1141 YSVLVVAKVE KGKSKKLKSV KELLGITIME RSSFEKNPID FLEAKGYKEV KKDIIKLKPK
 1201 YSLFELENGR KRMLASAGEL QKGNELALPS KYVNFLYLAS HYEKLKGSPE DNEQKQLFVE
 1261 QHKHYLDEII EQISEFSKRV ILADANLDKV LSAYNKHRRD PIREQAENII HLFITLTNLGA
 1321 PAAFKYFDTT IDRKYRSTK EVLDATLIHQ SITGLYETRI DLSQLGGD (SEQ ID NO: 147).

[0183] Nuclease inactivated *S. pyogenes* Cas9 proteins may comprise a substitution of an Alanine (A) for a Aspartic Acid (D) at position 10 and an alanine (A) for a Histidine (H) at position 840. Exemplary nuclease inactivated *S. pyogenes* Cas9 proteins of the disclosure may comprise or consist of the amino acid sequence (D10A and H840A bolded and underlined):

1 MDKKYSIG**LA** IGTNSVGWAV ITDEYKVPSK KFKVLGNTDR HSIKKNLIGA LLFDSGETAE
 61 ATRLKRTARR RYTRRKNRIC YLQEIFSNEM AKVDDSFHR LEESFLVEED KKHERHPIFG
 121 NIVDEVAYHE KYPTIYHLRK KLVDSTDKAD LRLLIYLALAH MIKFRGHFLI EGDLPNDNSD
 181 VDKLFIQLVQ TYNQLFEENP INASGVDAKA ILSARLSKSR RLENLIAQLP GEKKNGLFGN
 241 LIALSLGLTP NFKSNFDLAE DAKLQLSKDT YDDDLNLLA QIGDQYADLF LAAKNLSDAI
 301 LLSDILRVNT EITKAPLSAS MIKRYDEHHQ DLTLLKALVR QQLPEKYKEI FFDQSKNGYA
 361 GYIDGGASQE EFYKFIKPIL EKMDGTEELL VKLNREDLLR KQRTFDNGSI PHQIHLGELH
 421 AILRRQEDFY PFLKDNREKI EKILTRIPY YVGPLARGNS RFAWMTRKSE ETITPNWFEE
 481 VVDKGASQS FIERMTNFDK NLPNEKVLPK HSLLEYFTV YNELTKVKYV TEGMRKPAFL
 541 SGEQKKAIVD LLFKTNRKVT VKQLKEDYFK KIECFDSVEI SGVEDRFNAS LGTYHDLKLI
 601 IKDKDFLDNE ENEDILEDIV LTTLTFEDRE MIEERLKTYA HLFDDKVMKQ LKRRRYTGWG
 661 RLSRKLINGI RDKQSGKTIL DFLKSDGFAN RNFMQLIHDD SLTFKEDIQK AQVSGQGDSD
 721 HEHIANLAGS PAIKKGILQT VKVDELVKV MGRHKPENIV IEMARENQTT QKGQKNSRER
 781 MKRIEEGIKE LGSQILKEHP VENTQLQNEK LYLYYLQNGR DMYVDQELDI NRLSDYDVD**A**
 841 IVPQSFLKDD SIDNKVLTRS DKNRGKSDNV PSEEVVKKMK NYWRQLLNAK LITQRKFDNL
 901 TKAERGGLSE LDKAGFIKRQ LVETRQITKH VAQILDSRMN TKYDENDKLI REVKVITLKS
 961 KLVSDFRKDF QFYKREINN YHHAHDAYLN AVVGTAIIKK YPKLESEFVY GDYKVYDVRK
 1021 MIAKSEQEIG KATAKYFFYS NIMNFFKTEI TLANGEIRKR PLIETNGETG EIVWDKGRDF
 1081 ATVRKVLSP QVNIVKKTEV QTGGFSKESI LPKRNSDKLI ARKKDWDPKK YGGFDSPTVA
 1141 YSVLVVAKVE KGKSKKLKSV KELLGITIME RSSFEKNPID FLEAKGYKEV KKDIIKLKPK
 1201 YSLFELENGR KRMLASAGEL QKGNELALPS KYVNFLYLAS HYEKLKGSPE DNEQKQLFVE
 1261 QHKHYLDEII EQISEFSKRV ILADANLDKV LSAYNKHRRD PIREQAENII HLFITLTNLGA

1321 PAAFKYFDTT IDRKRYTSTK EVLDATLIHQ SITGLYETRI DLSQLGGD (SEQ ID NO: 148).

[0184] Nuclease inactivated *S. pyogenes* Cas9 proteins may comprise deletion of a RuvC nuclease domain or a portion thereof, an HNH domain, a DNase active site, a $\beta\beta\alpha$ -metal fold or a portion thereof comprising a DNase active site or any combination thereof.

[0185] Other exemplary Cas9 proteins or portions thereof may comprise or consist of the following amino acid sequences.

[0186] In some embodiments the Cas9 protein can be *S. pyogenes* Cas9 and may comprise or consist of the amino acid sequence:

MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRR
YTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV
DSTDKADLRILIYALAHMIKFRGHFLIEGDLNPDNSDVDFKFIQLVQTYNQLFEENPINASGVDAKAILSAR
LSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQY
ADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG
YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYF
FLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGGASQSFIERMTNFDKNLP
NEKVLPKHSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIE
CFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDK
VMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIIKKGILQTVKVVDDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIIEG
IKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLT
RSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQIT
KHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVGITALI
KKYPKLESEFVYGDYKVDYVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRLIETNGETG
EIVWDKGRDFATVRKVLSPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVA
YSVLVVAKEVGKSKKLKSVKELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIIKLPKYSLELENGRKR
MLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
ANLDKVL SAYNKHDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO: 149)

[0187] In some embodiments the Cas9 protein can be *S. aureus* Cas9 and may comprise or consist of the amino acid sequence:

MKRNILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHRIQRVKLLF
DYNLLTDHSELGINPYEARVKGLSQKLSEEFSAALLHLAKRRGVHNVNEVEDTGNELSTKEQISRNSK
ALEEKYVAELQLERLKKDGEVRGSINRFKTSYVKEAKQLLKVQKAYHQLDQSFIDTYIDLLETRRTTYEG
PGEGPSFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYEYEFQII

ENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIIEAELLDDQIAKILTIY
 QSSEDIQEELTNLNSLTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVKKVDLS
 QQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIKKYGLPNDIIELAREKNSKDAQKMINEMQKRNQRQTNERIE
 EIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHIIPRSVSFDNSFNKVLVKQEE
 NSKKGNRTPFQYLSSSDSKISYETFKKHILNLAAGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRYA
 TRGLMNLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHAEDALIINANADFIFKEWKKLD
 KAKKVMENQMFEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKNRELINDTLYSTRKD
 DKGNTLIVNNLNGLYDKDNDKLLKLINKSPEKLLMYHHDPPQTYQKLKLIMEQYGDENPLYKYEEETGN
 YLTKYSKKDNGPVIKKIKYYGNKLNALHDITDDYPNSRNKVVKLSLKPYPFDVYLDNGVYKFVTVKNLD
 VIKKENYYEVNSKCYEEAKKLLKISNQAEFASFYNNDLIKINGELRVIGVNNDLLNRIEVMIDITYREYL
 ENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG (SEQ ID NO:150)

[0188] In some embodiments the Cas9 protein can be *S. thermophiles* CRISPR1 Cas9 and may comprise or consist of the amino acid sequence:

MSDLVLGLDIGISVGVGILNKVTGEIIHKNSRIFPAAQAENNLVRRTNRQGRRLARRKKHRRVRLNRLFEE
 SGLITDFTKISINLNPYQLRVKGLTDELSNEELFIALKNMVKHRGISYLDASDDGNSSVGDYAQIVKENS
 QLETKTPGQIQLERYQTYGQLRGDFTVEKDGGKHRLINVFPTSAYRSEALRILQTQQEFNPQITDEFINRYLE
 ILTGKRKYHGPNEKSRTDYGRYRTSGETLDNIFGILIGKCTFYDEFRAAKASYTAQEFNLLNDLNNLTV
 PTETKKSKEQKNQIINYVKNEKAMGPAKLKFKYIAKLLSCDVADIKGYRIDKSGKAEIHTFEAYRKMKTLE
 TLDIEQMDRETLDKLAAYVLTNTEREGIQEALHEFADGSFSQKQVDELVQFRKANSSIFGKGWHNFSVKL
 MMELIPELYETSEEQMTILTRLGKQKTTSSSNKTKYIDEKLLTEIYNPVVAKSVRQAIKIVNAAIKEYGDFD
 NIVIAMARETNEDDEKKAIQIKQKANKDEKDAAMLKAANQYNGKAELPHSVFHGHKQLATKIRLWHQQG
 ERCLYTGTISIHDLINNSNQFEVDHILPLSITFDDSLANKVLVYATANQEKGQRTPYQALDSMDDAWSFRE
 LKAFVRESKTLNKKKEYLLTEEDISKFDVRKKFIERNLVDTRYASRVVLNALQEHFRAHKIDTKVSVVRG
 QFTSQLRRHWGIEKTRDITYHHHAVDALIAASSQLNLWKKQKNTLVSYSEDQLLDIETGELISDDEYKESVF
 KAPYQHFDVTLKSKEFEDSILFSYQVDSKFNKRKISDATIYATRQAKVGKDKADETYVLGKIKDIYTQDGYD
 AFMKIYKKDKSKFLMYRHDPQTFEKVIEPILENYPNKQINDKGKEVPCNPFLKYKEEHGYIRKYSKKGNP
 EIKSLKYYDSKLGHNHIDITPKDSNNKVVQLQSVSPWRADVFNKTTGKYEILGLKYADLQFDKGTGTYKISQ
 EKYNDIKKKEGVSDSEFKFTLYKNDLLLVKDTETKEQQLFRFLSRTMPKQKHVELKPYDKQKFEGGEA
 LIKVLGNVANSQGCKKGLGKSNISYKVRTDVLGNQHIIKNEGDKPKLDF (SEQ ID NO: 151).

[0189] In some embodiments the Cas9 protein can be *N. meningitidis* Cas9 and may comprise or consist of the amino acid sequence:

MAAFKPNPINYILGLDIGIASVGWAMVEIDEDENPICLIDLGVRVFERAEVPKTGDSLAMARRLARSVRRLT
 RRRRAHRLLRARRLLKREGVLQAADFDEGLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLS
 QRKNEGETADKELGALLKGVADNAHALQTGDFRTPAELALNKFEEKSGHIRNQRGDYSHTFSRKDLQAE
 ILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHCTFEPAEPKAAKNITYAERFIWTKL
 NNLRIEQGSERPLTDTERATLMDEPYRKSCLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMKAY

HAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLKDRIQPEILEALLKHISFDKQVQISLKAL
 RRIVPLMEQKGKRYDEACAEIYGDHYGKKNTTEKIYLPPIPADEIRNPVVLRLALSQARKVINGVVRRYGSPAR
 IHIETAREVGKSKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPSKDILKLRLYEQQHGKCLYSGKE
 INLGRLENEKGYVEIDHALPFSRTWDDSFNNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETS
 RFPRSKKQRILLQKFDEDEGFKERNLNDTRYVNRFLCQFVADRMRLTGKGKKRVFASNGQITNLLRGFWGL
 RKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGEVLHQKTHFPQPWEFFAQ
 EVMIRVFGKPDGKPEFEEADTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHEMETVKSAGR
 LDEGVSVLRVPLTQLKLKDLEKMNVREREPKLYEALKARLEAHKDDPAKAFAPFYKYDKAGNRTQQVK
 AVRVEQVQKTGVWVRNHNGIADNATMVRVDVFEGDKYYLVPIYSWQVAKGILPDRAVVQKGDEEDW
 QLIDDSFNFKFSLHPNDLVEVITKKARMGFYFASCHRGTTGNINIRIHDLDHKIGKNGILEGIGVKTALSFQKY
 QIDELGKEIRPCRLKKRPPVR (SEQ ID NO: 152).

[0190] In some embodiments the Cas9 protein can be *Parvibaculum. lavamentivorans* Cas9 and may comprise or consist of the amino acid sequence:

MERIFGFDIGTTSIGFSVIDYSSTQSAGNIQRLGVRIPEARDPDGTPLNQRRQKRMMRRQLRRRRIRRKAL
 NETLHEAGFLPAYGSADWPVVMADPEYELRRRGLEEGLSAYEFGRAIYHLAQHRHFKGRELEESDTPDPD
 VDDEKEAANERAATLKALKNEQTTLGAWLARRPPSDRKRGIHAHRNVVAEEFERLWEVQSKFHPALKSE
 EMRARISDTIFAQRPVFWRKNTLGEGRFMPGPEPLCPKGSWLSQRRMLEKLNNLAAGGNARPLDAEERD
 AILSKLQQQASMSWPGVRSALKALYKQRGEPGAEKSLKFNLELGESKLLGNALAEKLADMFPGDPWAH
 PRKQEIRHAVHERLWAADYGETPDKKRVIILSEKDRKAHREAAANSFVADFGITGEQAAQLQALKLPTGW
 EPYSIPALNLFALAELEKGERFGALVNGPDWEGWRRTNFPHRNQPTGEILDKLPSPASKEERERISQLRNPTV
 VRTQNELRKVVNNLIGLYGKPDRIEIVGRDVGKSKREREIEQSGIRRNEKQRKKATEDLIKNGIANPSRDD
 VEKWILWKEGQERCPYTGDQIGFNALFREGRYEVEHIWPRSRSFDNSPRNKTLCKRDVNIEKGNRMPFEAF
 GHDEDRWSAIQIRLQGMVSAKGGTGMSPGKVKRFLAKTMPEDFAARQLNDTRYAAKQILAQLKRLWPD
 MGPEAPVKVEAVTGQVTAQLRKLWTLNNILADDGEKTRADHRHHAIDALTVACTHPGMTNKLSTRYWQL
 RDDPRAEKPALTPPWDITRADAERKAVSEIVVSHRVRKKVSGPLHKETTYGDTGTDIKTKSGTYRQFVTRKK
 IESLSKGELDEIRDPRIKEIVAHHVAGRGGDPKKAFFPYPCVSPGGPEIRKVRLTSKQQLNLMAQTGNGYAD
 LGSNHHIAIYRLPDGKADFEIVSLFDASRRLAQRNPVQRTRADGASFVMSLAAGEAIMIPEGSKKGIWIVQ
 GVWASGQVVLERDTHADHSTTTTRPMPNPILKDDAKKVSIDPIGRVRPSND (SEQ ID NO: 153).

[0191] In some embodiments the Cas9 protein can be *Corynebacter diphtheria* Cas9 and may comprise or consist of the amino acid sequence:

MKYHVGIDVGTFVGLAAIEVDDAGMPIKTLVSHIHDSGLDPDEIKSAVTRLASSGIARRTRRLYRRKRR
 RLQQLDKFIQRQGWPIELEDYSPLYPWKVRaelaAASYIADEKERGEKLSVALRHIARHRGWRNPYAKV
 SSLYLPDGPSDAFKAIREEIKRASGQVPETATVGQMVTLCELGTLKLRGEGGVL SARLQQSDYAREIQEIC
 RMQEIGQELYRKIIDVVF AAESP KGSASSRVGKDPLQPGKNRALKASDAFQRYRIAALIGNLRVRVDGEKRI
 LSVEEKNLVFDHLVNLTPKKEPEWVTIAEILGIDRGQLIGTATMTDDGERAGARPPHTDNRNRSIVNSRIAPL
 VDWWKTASALEQHAMVKALSNAEVDDFDSPEGAKVQAFFADLDDDVHAKLDSLHLPVGRAAYSEDTLV

RLTRRMLSDGVDLYTARLQEFGIEPSWTPPTPRIGEPVGNPAVDRVLKTVSRWLESATKTWGAPERVIIIEHV
REGFVTEKRAREMDGDMRRRAARNAKLQEMQEKLNVQGKPSRADLWRYQSVQRQNCQACAYCGSPITF
SNSEMDHIVPRAGQGSTNTRENLVAVCHRCNQSKGNTPFAIWAKNTSIEGVSVEAVERTRHWVTDGTGM
RSTDFKKFTKAVVERFQRA TMDEEIDARSMESVAWMANELRSRVAQHFA SHGTTVRVYRGS LTAEARRA
SGISGKLKFFDGVGKSRLDRRHHIDA AVIAFTSDYVAETLAVRSNLKQSQAHRQEAPQWREFTGKDAEH
RAAWRVWCQKMEKLSALLTEDLRDDR VVMSNVRLRLGNGSAHKETIGKLSKVKLSSQLSVSDIDKASS
EALWCALTREP GFDPK EGLPANPERHIRVNGTHVYAGDNIGLFPVSAGSIALRGGY AELGSSFH HARVYKI
TSGKKPAFAMLRVYTIDLLPYRNQDLFSVELKPQTMSMRQAEKKLRDALATGNAEYLGWLVDDELVDV
TSKIATDQVKAVEAELGTIRRWVRVDGFFSPSKLRLRPLQMSKEGIKKESAPELSKIIDRPGWLPAVNKLFSD
GNVTVVRRDSLGRVRLESTAHLPVTWKVQ (SEQ ID NO: 154).

[0192] In some embodiments the Cas9 protein can be *Streptococcus pasteurianus* Cas9 and may comprise or consist of the amino acid sequence:

MTNGKILGLDIGIASVGVGII EAKTGKVVHANSRLFSAANAENNAERRGFRGSRRLNRRKKHRVKRVRDLF
EKYGIVTDFRNLNLNPYELRVKGLTEQLKNEELFAALRTISKRRGISYLD DAEDDSTGSTDYAKSIDENRRL
LKNKTPGQIQLEKYLEKYGQLRGNTVYDENG EAHRLINVFSTSDYEKEARKILETQADYNKKITA EFIDYV
EILTQKRKYHYHGP GNEKSRTDYGRFR TDGTTLENIFGILIGKCNFY PDEYRASKASYTAQEYNFLNDLNNLK
VSTETGKLSTEQKESLVEFAKNTATLGPAKLLKEIAKILDCKVDEIKGYREDDKGKPD LHTFEPYRKLKFNL
ESINIDDL SREVIDKLADILTLNTEREGIEDAIKRNLPNQFTEEQISEI KVRKSQSTAFNKGWHSFS AKLMNE
LIPELYATSDEQMTILTRLEKFKVNKKSSKNTKTIDEKEVTDEIYNPVVAKSVRQTIKIINA AVKKGDFDKI
VIEMPRDKNADDEKKFIDKR NKENKEKDDALKRAAYLYNSSDKLPDEVFHGNKQLET KIRLWYQQGER
CLYSGKPISIQELVHNSNNFEIDHILPLSLSFDDSLANKVLVYAWTNQEK GQKTPYQVIDSMDAAWSFREM
KDYVLKQKGLGKKKRDYLLTTENIDKIEVKKKFIERNLVDTRYASRVVLNSLQSALREL GKDTKVSVVRG
QFTSQLRRKWKIDKSRETYHHHA VD ALIIAASSQLKLWEKQDNPMFVDY GKNQVVDKQTGEILSVSDDEY
KELVFQPPYQGFVNTISSKGFED EILFSYQVDSKYNRKVSDAT IYSTRKAKIGKDKKEETYVLGKIKDIYSQ
NGFDTFIKKYNKDKTQFLMYQKDSL TWENVIEVILRDYPTTKK SEDGKNDVKCNPFEEYRRENG LICKYSK
KGKGTPIKSLKYDYDKLGNCIDITPEESRNK VILQSINPWRADVFN PETLKYELMGLKYS DLSFEKGTGNY
HISQEKYDAIKEKEGIGKKSEFKFTLYRNDLILIKDIASGEQE IYRFLSRTMPNVNHYVELKPYDKEKFDNVQ
ELVEALGEADKVGRCIKGLNKPNI SIYKVRTDVLGNKYFVKKKGDKPKLDFKNNKK (SEQ ID NO: 155).

[0193] In some embodiments the Cas9 protein can be *Neisseria cinerea* Cas9 and may comprise or consist of the amino acid sequence:

MAAFKPNPMNYILGLDIGIASVGWAIVEIDEEENPIRLIDLGV RVFERAEV PKTGDSLAAARRLARSVRRLT
RRRAHRLLRARRLLKREGVLQAADF DENGLIKSLPNTPWQLRAAALDRKLT PLEWSAVLLHLIKHRGYLS
QRKNEGETADKELGALLKG VADNTHALQTGDFRTPAELALNKFEKESGHIRNQRGDY SHTFNRKDLQAEL
NLLFEKQKEFGNPHVSDGLKEGIETLLMTQRPALSGDAVQKMLGHCTFEPT EPKAAKNTYTAERFVWLTK
LNNLRILEQGSERPLTDTERATLMDEPYRKSLTYAQARKLLDLD DTAFFKGLRYGKDN AEASTLMEMKA
YHAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLKDRVQPEILEALLKHISFDK FVQISLK

ALRRIVPLMEQGNRYDEACTEIIYGDHYGKKNTTEKIYLPPIPADEIRNPVVLRLALSQARKVINGVVRRYGSP
 ARIHIETAREVGKSFKDRKEIEKRQEENRKDREKSAKFREYFPNFVGEPKSKDILKLRLYEQQHKGKCLYSG
 KEINLGRLLNEKGYVEIDHALPFSRTWDDSFNNKVLALGSENQNKGNQTPYEYFNGKDNSREWQEFKARVE
 TSRFRPRSKKQRILLQKFDEDDGFKERNLNDTRYINRFLCQFVADHMLLTGKGKRRVFASNGQITNLLRGFWG
 LRKVRAENDRRHHALDAVVVACSTIAMQQKITRFVRYKEMNAFDGKTIDKETGEVLHQKAHFPQPWEFFA
 QEVMIRVFGKPDGKPEFEEADTPEKLRTLLAEKLSSRPEAVHKYVTPLFISRAPNRKMSGQGHMETVKS
 RLDEGISVLRVPLTQLKLKDLEKMVNREREPPLYEALKARLEAHKDDPAKAFAPFYKYDKAGNRTQQV
 KAVRVEQVQKTGVVWHNHNGIADNATIVRVDVFEGGKYYLVPIYSWQVAKGILPDRAVVQKGDEEDW
 TVMDDSFEFKFLYANDLIKLTAKKNEFLGYFVSLNRATGAIDIRTHDSTDSTKGKNGIFQSVGVKTALS
 FQKYQIDELGKEIRPCRLKKRPPVR (SEQ ID NO: 156).

[0194] In some embodiments the Cas9 protein can be *Campylobacter lari* Cas9 and may comprise or consist of the amino acid sequence:

MRILGFDIGINSIGWAFVENDELKDCGVRIPTKAENPKNKESLALPRRNARSSRRRLKRRKARLIAIKRILAK
 ELKLNKYDYYAADGELPKAYEGSLASVYELRYKALTQNLETKDLARVILHIAKHRGYMKNKNEKKSNDK
 KGKILSALKNNALKLENYQSVGEYFYKEFFQKYKKNNTKNFIKIRNTKDNYNVNCVLSSDLEKELKLILEKQK
 EFGYNYSEDFINEILKVAFFQRPLKDFSHLVGACTFFEEEEKRACKNSYSAWEFVALTKIINEIKSLEKISGEIV
 PTQTINEVLNLILDKGSITYKKFRSCINLHESISFKSLKYDKENAENAKLIDFRKLVEFKKALGVHLSRQEL
 DQISTHITLIKDNVKKLTVLEKYNLSNEQINNLEIEFNDYINLSFKALGMILPLMREGKRYDEACEIANLKP
 KTVDEKKDFLPAFCDSIFAHELSNPVVNRAISEYRKVLNALLKKYGVHKKHLELARDVGLSKKAREKIEK
 EQKENQAVNAWALKECENIGLKASAKNILKLKLWKEQKEICIYSGNKISIEHLKDEKALEVDHIYPYSRSD
 DSFINKVLVFTKENQEKLNTPFEEAFGKNIEKWSKIQTALQNLKYKKKNKILDENFKDKQQEDFISRLNDT
 RYIATLIAKYTKEYLNFLLLSENENANLKSGEKSGKIHVQTISGMLTSVLRHTWGFDDKDRNNHLHHALDA
 IIVAYSTNSIIKAFSDFRKNQELLKARFYAKELTSDNYKHQVKFFEPFKSFREKILSKIDEIFVSKPPRRARR
 ALHKDTFHSENKIIDKCSYNSKEGLQIALSCGRVRKIGTKYVENDTIVRVDIFKKQNKFYAIPYAMDFA
 LGLPNKIVITGKDKNNPKQWQTIDESYEFCFSLYKNDLILLQKKNMQEPEFAYYNDFSISTSSICVEKHDNKF
 ENLTSNQKLLFSNAKEGSKVESLGIQNLKVFEKYIITPLGDKIKADFQPRENISLKTSSKKYGLR (SEQ ID
 NO: 157).

[0195] In some embodiments the Cas9 protein can be *T. denticola* Cas9 and may comprise or consist of the amino acid sequence:

MKKEIKDYFLGLDVGTVGSVGWAVTDDTYKLLKANRKDLWGMRCFETAETAEVRRRLHRGARRRIERRKK
 RIKLLQELFSQEIAKTDEGFFQRMKESPFYAEDKTILQENTLFNDKDFADKTYHKAYPTINHLIKAWIENKV
 KPDPRLLYLACHNIIKKRGHFLFEGDFDSENQFDTSIQALFEYLREDMEVDIDADSQKVKEILKDS
 SLKNSKQSRNLNKLGLKPSDKQKKAITNLISGNKINFADLYDNPDLKDAEKNSSFSKDDFDALSDDL
 ASILGDSFELLLKAKAVYNCSVLSKVIGDEQYLSFAKVKIYEKHKTDLTCLKNVIKKHFPKDYKKVFGYNK
 NEKNNNNYSGYVGVCKTKSKKLIINNSVNQEDFYKFLKTILSAKSEIKEVNDILTEIETGTFLPKQISKS
 NAEIPYQLRKMELEKILSNAEKHFSFLKQKDEKGLSHSEKIIMLLTFKIPYYIGPINDNHKKFFPDRCWV
 VKKEKSPSGKTTTPWN

FFDHIDKEKTAEAFITSRTNFCTYL VGESVLPKSSLLYSEYTVLNEINNLIQIIDGKNICDIKQKIYEDLFKK
YKKITQKQISTFIKHEGICNKTDEVIILGIDKECTSSLKSYIELKNIFGKQVDEISTKNMLEEIRWATIYDEGE
GKTILKTKIKA EYGKYCSDEQIKILNLKFSWGRLSRKFLETVTSEMPGFSEPVNIITAMRETQNNLMELLS
SEFTFTENIKKINSGFEDA EKQFSYDGLVKPLFLSPSVKKMLWQTLKL VKEISHITQAPPKIFIEMAKGAEL
EPARTKTRLKILQDL YNNCKNDADAFSSEIKDL SGKIENEDNLRLRSDKLYLYYTQLGKCMYCGKPIEIGH
VFDTSNYDIDHIYPQSKIKDD SISNRVLVCSSCNKNKEDKYPLKSEIQSKQRGFWNFLQRNNFISLEKLNRLT
RATPISDDETAKFARQLVETRQATKVA AKVLEKMF PETKIVYSKAETVSMFRNKF DIVKCREINDFHHAH
DAYLNIVVGNVYNTKFTNPNWFIKEKRDNPKIADTYNYKYVFDYDVKRNNITAW EKGKTIITVKDMLKR
NTPIYTRQAACKKGELFNQTIMKKGLGQHPLKKEGPF SNISKYGGYNKVSAAYYTLIEYEEKGNKIRSLETI
PLYLVKDIQKDQDVLKSYLTDLLGKKEFKILVPKIKINSLKINGFPCHITGKTNDSFLLRPAVQFCCSNEV
LYFKKIIRFSEIRSQREKIGKTISPYEDLSFRSYIKENLWKKTKNDEIGEKEFYDLLQKKNLEIYDMLLTKHKD
TIYKKRPNSATIDILVKGKEKFKSLIENQFEVILEILKLF SATRNVS DLQHIGGSKYSGVAKIGNKISSLDNCI
LIYQSITGIFEKRIDLKLV (SEQ ID NO: 158).

[0196] In some embodiments the Cas9 protein can be *S. mutans* Cas9 and may comprise or consist of the amino acid sequence:

MKKPYSIGLDIGTNSVGWAVVTDDYKVP AKKMKVLGNTDKSHIEKNLLGALLFDSGNTAEDRRLKRTAR
RRYTRRRNRILYLQEIFSEEMGKVDDSFHRL EDSFLVTE DKRGERHPIFGNLEEEVKYHENFPTIYHLRQYL
ADNPEKVDLRLVYLALAHIIKFRGHFLIEGKF DTRNNDVQRLFQEFLAVYDNTFENSSLQE QNVQVEEILTD
KISSAKKDRVLKLPNEKSNGRFAEFLKLIVGNQADFKKHFELEEKAPLQFSKDTYEEEELEVLLAQIGDNY
AELFLSAKKLYDSILLSGILTVTDVGTKAPLSASMIQRYNEHQMDLAQLKQFIRQKLSDKYNEVFSDVSKD
GYAGYIDGKTNQEA FYKYLKGLLNKIEGSGYFLDKIEREDFLRKQRTFDNGSIPHQIHLQEMRAIIRQA EF
YPFLADNQDRIEKL LTFRIPYYVGPLARGKSDFAWLSRKSADKITPWNFDEIVDKESSAEAFINRMTNYDLY
LPNQKVLPKHSLLYEKFTVYNELTKVKYKTEQGKTAFFDANMKQEIFDGVFKVYRKVTKDKLMDFLEKE
FDEFRIVDLTGLDKENKVFNASYGTYHDLCKILDKDFLDNSKNEKILEDIVLTLTLFEDREMIRKRL ENYSD
LLTKEQVKKLERRHYTGWGRLSAELIHGIRNKESRKTILDYLI DDGNSNRNFMQLINDDALSFKEEI AKAQV
IGETDNLNQVVS DIAGSPA IKGILQSLKIVDELVKIMGHQPENIVVEMARENQFTNQGRRNSQQRLKGLTD
SIKEFGSQILKEHPVENSQ LQNDRLFLYYLQNGRDMYTGEELDIDYLSQYDIDHIIPQAFIKDNSIDNRVLTSS
KENRGKSDDVPSKDVVRKMKSYSKLLSAKLITQRKFDNLTKAERGGLTDDDKAGFIKRQLVETRQITKH
VARILDERFNTETDENNKKIRQVKIVTLKSNLVS NFRKEFELYK VREINDYHHAHDAYLNAVIGKALLGVY
PQLEPEFVYGDYPHFHGHKENKATAKFFYSNIMNFFKKDDVRTDKNGEIIWKKDEHISNIKKVLSYPQVN
IVKKVEEQTGGFSKESILPKGNSDKLIPRKTKKFYWDTKKYGGFDSPIVAYSILVIADIEKGKSKKLKTVKAL
VGVTIMEKMTFERDPVAFLERKGYRNVQEENI KLPKYSLFKLENGRKRL LASARELQKGNEIVLPNHLGT
LLYHAKNIHKVDEPKHLDYVDKHKDEFKELLDVVS NFSKKYTLAEGNLEKIKELYAQNNGEDLKELASSFI
NLLTFTAIGAPATFKFFDKNIDRKRYTSTTEILNATLIHQ SITGLYETRIDLNKLGGD (SEQ ID NO: 159)

[0197] In some embodiments the Cas9 protein can be *S. thermophilus* CRISPR 3 Cas9 and may comprise or consist of the amino acid sequence:

MTKPYSIGLDIGTNSVGWAVTTDNYKVPSKKMKVLGNTSKKYIKKNLLGVLLFDSGITAEGRRLLKRTARR
 RYTRRRNRILYLQEIFSTEMATLDDAFFQRLDDSFLVPDDKRDSKYPIFGNLVEEKAYHDEFPTIYHLRKYL
 ADSTKKADRLVYLAALAHMIKYRGHFLIEGEFNSKNNDIQKNFQDFLDTYNAIFESDLSLENSKQLEEIVKD
 KISKLEKKDRILKLPGEKNSGIFSEFLKLIVGNQADFRKCFNLDEKASLHFSKESYDEDETLTLLGYIGDDYS
 DVFLKAKKLYDAILLSGFLTVTDNETEAPLSSAMIKRYNEHKEDLALLKEYIRNISLKTYNVFKDDTKNG
 YAGYIDGKTNQEDFYVYLKLLAEFEGADYFLEKIDREDFLRKQRTFDNGSIPYQIHLQEMRAILDKQAKF
 YPFLAKNKERIEKILTFRIPYYVGPLARGNSDFAWSIRKRNEKITPWNFEDVIDKESSAEAFINRMTSFDLYL
 PEEKVLPKHSLLYETFNVYNELTKVRFIAESMRDYQFLDSKQKKDIVRLYFKDKRKVTDKDIIYELHAIYGY
 DGIELKGIEKQFNSSLSTYHDLNINIDKEFLDDSSNEAIIIEIHTLTIFEDREMIKQRLSKFENIFDKSVLKKL
 SRRHYTGWGLSASLINGIRDEKSGNTILDYLDGSGNRNFMQLIHDDALSFKKKIQKAQIIGDEDEKGNIE
 VVKSLPGSPAIAKKGILQSIKIVDELVKVMGGRKPESIVVEMARENQYTNQGKSNSQQRLKRLEKSLKELGS
 KILKENIPAKLSKIDNNALQNDRLYLYYLQNGKDMYTGDDLDIDRLSNYDIDHIIPQAFLKDNSIDNKVLVS
 SASNRGKSDDVPSLEVVKRRTFWYQLLKSGLISQRKFDNLTKAERGGSPEDKAGFIQRQLVETRQITKH
 VARLLDEKFNNKKDENNRAVRTVKIITLSTLVSQFRKDFELYKREINDFHHAHDAYLNAVVASALLKK
 YPKLEPEFVYGDYPKYNSFRERKSATEKVYFYSNIMNIFKKSISLADGRVIERPLIEVNEETGESVWNKESDL
 ATVRRVLSYPQVNVVKKVEEQNHGLDRGPKGLFNANLSSKPKPNSNENLVGAKEYLDPKKYGGYAGIS
 NSFTVLVKGTIEKGAKKKITNVLEFQGISILDRINYRKDKLNFLEKGYKDIELIIEPKYSLFELSDGSRRML
 ASILSTNNKRGEIHKGNQIFLSQKFVKLLYHAKRISNTINENHRKYVENHKKKEFEELFYIILEFNENYVGAK
 KNGKLLNSAFQSWQNHSIDELCSSFIGPTGSEKGLFELTSRGSAADEFELGVKIPRYRDYTPSSLLKDATLI
 HQSVTGLYETRIDLAKLGEG (SEQ ID NO: 160)

[0198] In some embodiments the Cas9 protein can be *C. jejuni* Cas9 and may comprise or consist of the amino acid sequence:

MARILAFDIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRLARSARKRLARRKARLNHLKHLI
 ANEFKLNIEDYQSFDESLAKAYKGLISPYELRFRALNELLSKQDFARVILHIAKRRGYDDIKNSDDKEKG
 AILKAIKQNEEKLANYQSVGEYLYKEYFQKFENSKEFTNVRNKKESYERCIAQSFLKDELKLIFKKQREFG
 FSFSKKFEEEVLSVAFYKRALKDFSHLVGNCSFFTDEKRAPKNSPLAFMFVALTRIINLLNNLKNTEGILYTK
 DDLNALLNEVLKNGTLTYKQTKKLLGLSDDYEFKGEKGTIFYEFKKYKEFIKALGEHNLSQDDLNEIAKDI
 TLIKDEIKLKKALAKYDLNQNQIDSLSKLEFKDHLNISFKALKLVTPLMLEGKKYDEACNELNLKVAINED
 KKDFLPAFNETYYKDEVTPVVLRAIKEYRKVLNALLKKYGVHKINIELAREVGKNHSQRAKIEKEQNE
 NYKAKKDAELECEKLGLKINSKNILKRLRFKEQKEFCAYSGEKIKISDLQDEKMLEIDHIYPYSRSFDDSYM
 NKVLVFTKQNQEKLNQTPFEAFGNDSAKWQKIEVLAKNLPTKKQKRILDKNYKDKEQKNFKDRNLNDTR
 YIARLVNNTKDYLDLPLSDDENTKLNLTQKGSKVHVEAKSGMLTSALRHTWGFSAKDRNNHLHHAID
 AVIIAYANNSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFFEPFSGFRQKVLDKIDEIFVSKPERKKP
 SGALHEETFRKEEFYQSYGGKEGVKALELGKIRKVNKGIVKNGDMFRVDIFKHKKTNKFYAVPIYTMD
 FALKVLPNKAVARSKKGEIKDWILMDENYEFCSLYKDSLILIQTKDMQEPEFVYYNAFTSSTVSLIVSKHD
 NKFETLSKNQKILFKNANEKEVIAKSIGIQNLKVFEKYIVSALGEVTKAEFRQREDFKK (SEQ ID NO: 161)

[0199] In some embodiments the Cas9 protein can be *P. multocida* Cas9 and may comprise or consist of the amino acid sequence:

MQTTNLSYILGLDLGIASVGWAVVEINENEDPIGLIDVGVRIFERAEVPKTGESLALSRRRLARSTRRLIRRA
HRLLLAKRFLKREGILSTIDLEKGLPNQAWELRVAGLERRLSAIEWGAVLLHLIKHRGYLSKRKNESQTN
KELGALLSGVAQNHQLQSDDYRTPAELALKKFAKEEGHIRNQRGAYTHTFNRLDLLAELNLLFAQQHQF
GNPHCKEHIQQYMTTELLMWQKPALSGEAILKMLGKCTHEKNEFKAAKHTYSAERFVWLTCLNNLRILED
GAERALNEEERQLLINHPYEKSKLTYAQVRKLLGLSEQAIFKHLRYSKENAESATFMELKAHWAIKALEN
QGLKDTWQDLAKKPDLLDEIGTAFSLYKTDEDIQQYLTNKVPNSVINALLVSLNFDKFIELSLKSLRKILPL
MEQGKRYDQACREIYGHYGEANQKTSQLLPAIPAQEIRNPVVLRTLSQARKVINAIIRQYGSPARVHIETG
RELGKSFKERREIQKQQEDNRTKRESAVQKFELFSDFSSEPKSKDILKFRLYEQQHKGKCLYSGKEINIRL
NEKGYVEIDHALPFSRTWDDSFNNKVLVLASENQKGNQTPYEWLQGKINSERWKNFVALVLGSQCSAA
KKQRLLTQVIDDNKFIDRNLDTRYIARFLSNYIQENLLL VGKNKKNVFTPNGQITALLRSRWGLIKARENN
NRHHALDAIVVACATPSMQQKITRFRFKEVHPYKIENRYEMVDQESGEIISPHFPEPWAYFRQEVNIRVFD
NHPD TVLKEMLPDRPQANHQFVQPLFVSRAPTRKMSGQGHMETIKSAKRLAEGISVLRIPLTQLKPNLLEN
MVNKEREPALYAGLKARLAEFNQDPAKAFATPFYKQGGQVKAIRVEQVQKSGVLVRENNGVADNASIV
RTDVFINKNNKFFLVPIYTWQVAKGILPNKAIVAHKNEDEWEEMDEGAKFKFSLFPNDLVELKTKKEYFFGY
YIGLDRATGNISLKEHDGEISKGKDGVRVGVKLALSFEKYQVDELGKNRQICRPQQRQPVR (SEQ ID NO:
162)

[0200] In some embodiments the Cas9 protein can be *F. novicida* Cas9 and may comprise or consist of the amino acid sequence:

MNFKILPIAIDLGVKNTGVFSAFYQKGTSLERLDNKNKGK VYELSKDSYTLLMNNRTARRHQRRGIDRKQL
VKRLFKLIWTEQLNLEWDKDTQQAISFLFNRRGFSFITDGY SPEYLNIVPEQVKAILMDIFDDYNGEDDLDS
YLKLATEQESKISEIYNKLMQKILEFKLMKLCTDIKDDKVSTKTLKEITSYEFELLADYLANYESLKTQKFS
YTDKQGNLKELSYHHDKYNIQEFLKRHATINDRILDTLLTDDLDIWNFNFEKFDKNEEKLQNQEDKD
HIQAHLHHFVFAVNKIKSEMASGGRHRSQYFQEITNVLDENNHQEGYLKNFCENLHNKKYSNLSVKNLVN
LIGNLSNLELKPLRKYFNDKIHAKADHWDEQKFTETYCHWILGEWRVGVKDQDKKDGAKYSYKDLCLNEL
KQKVTKAGLVDFLLELDPCRTIPPYLDNNNRKPPKCQSLILNPKFLDNQYPNWQQYLQELKKLQSIQNYLD
SFETDLKVLKSSKDQPYFVEYKSSNQQIASGQRDYKDL DARILQFIFDRVKASDELLNEIYFQAKKLKQKA
SSELEKLESSKKLDEVANSQLSQILKSQHTNGIFEQGTFLHLVCKYYKQQRARARSRLYIMPEYRYDKKLH
KYNNTGRFDDDNQLLTYCNHKPRQKRYQLLNDLAGVLQVSPNFLKDKIGSDDDLFISKWLVEHIRGFKKA
CEDSLKIQKDNRGLLNHNKINIARNTKGKCEKEIFNLICKIEGSEDKKGNKHLAYELGVLLFGEPNEASKP
EFDRIKKFNSIYSFAQIQQIAFAERKGNANTCAVCSADNAHRMQQIKITEPVEDNNDKIILSAKAQRLPAIP
TRIVDGA VVKMATILAKNIVDDNWQNIKQVLSAKHQLHIPITESNAFEFEPALADVKGKSLKDRRKKALE
RISPENIFKDKNNRIKEFAKGISAYSGANLTDGDFDGAKEELDHIIPRSHKKYGTLNDEANLICVTRGDKN
KGNRIFCLRDADNYKLKQFETDDLEIEKKIADTIWDANKKDFKFGNYRSFINLTPQEQA FRHALFLADE
NPIKQAVIRAINNRNRTFVNGTQRYFAEVLANNIYLRAKKENLNTDKISFDYFGIPTIGNRGIAEIRQLYEK

VDSDIQAYAKGDKPQASYSHLIDAMLAFCIAADEHRNDGSIGLEIDKNYSLYPLDKNTGEVFTKDIFSQIKIT
DNEFSDKKLVKRKAIEGFNTHRQMTDGIYAENYLPILHKELNEVRKGYTWKNSEEIKIFKGKKYDIQQL
NNLVYCLKFVDKPISIDIQISTLEELRNILTTNNIAATAEYYYINLKTQKLHEYYIENYNTALGYKKYSKEME
FLRSLAYRSERVKIKSIDDVKQVLDKDSNFIIGKITLPFKKEWQRLYREWQNTTIKDDYEFLKSFFNVKSITK
LHKKVRKDFSLPISTNEGKFLVKKRTWDNNFIYQILNDSRSDRADGTPFIPAFDISKNEIVEAIIDSFTSKNIF
WLPKNIELQKVDNKNIFAIDTSKWFEVETPSDLRDIGIATIQYKIDNNSRPKVRVKLDYVIDDDSKINYFMN
HSLKSRYPDKVLEILKQSTIIEFESSGFNKTIKEMLGMKLAGIYNETSNN (SEQ ID NO: 163)

[0201] In some embodiments the Cas9 protein can be *Lactobacillus buchneri* Cas9 and may comprise or consist of the amino acid sequence:

MKVNNYHIGLDIGTSSIGWVAIGKDGKPLRVKGKTAIGARLFQEGNPAADRRMFRTTRRRLSRRKWRLKL
LEEIFDPYITPVDSTFFARLKQSNLSPKDSRKEFKGSMLFPDLTDMQYHKNYPTIYHLRHALMTQDKKFDIR
MVYLAIHIVKYRGNFLNSTPVDSFKASKVDFVDQFKKLNELYAAINPEESFKINLANSEDIGHQFLDPSIRK
FDKKKQIPKIVPMMNDKVTDRLNGKIASEIIHAILGYKAKLDVVLQCTPVDSKPWALKFDDDEDIDAKLEK
ILPEMDENQQSIVAILQNLYSQVTLNQIVPNGMSLSESMIEKYNDHHDHLKLYKKLIDQLADPKKKA VLKK
AYSQYVGDDGKVIEQAEFWSSVKKNLDDSELSKQIMDLIDAEKFMPKQRTSQNGVIPHQLHQRELDEIIH
QSKYYPWLVEINPNKHDHLAKYKIEQLVAFRVPYVVGPMITPKDQAESAETVFSWMERKGTETGQITPW
NFDEKVDRKASANRFIKRMTTKDTYLGEDVLPDESLLYEKFKVLNELNMVRVNGKLLKVADKQAIFQDL
FENYKHVSVKKLQNYIAKTGLPSDPEISGLSDPEHFNNSLGTYNDFKKLFGSKVDEPDQLQDDFEKIVEWST
VFEDKKILREKLNEITWLSDDQKDVLESSRYQGWGRLSKLLTGIVNDQGERIIDKLWNTNKNFMQIQSDD
DFAKRIHEANADQMQA VDVEDVLADAYTSPQNKKAIRQVVKVVDIQAAMGGVAPKYISIEFTRSED RNP
RRTISRQRQLENTLKDTAKSLAKSINPELLSELDNAAKSKKGLTDRLYLFTQLGKDIYTGEPINIDELNKYD
IDHILPQAFIKDNSLDNRVLVLTAVNNGKSDNVPLRMFGAKMGHFWKQLAEAGLISKRKLKNLQTDPTIS
KYAMHGFIRRLVETSQVIKLVANILGDKYRNDTKIIEITARMNHQMRDEFGFIKNREINDYHHAFDAYL
TAFLGRYLYHRYIKLRPYFVYGDFKKFREDKVTMRNFNHLHDLTDDTQEKIADAETGEVIWDRENSIQQLK
DVYHYKFMLISHEVYTLRGAMFNQTVYPASDAGKRKLIPVKADRPVNVYGGYSGSADAYMAIVRIHNKK
GDKYRVVGVPMRALDRLDAAKNVSDADFDRAKDV LAPQLTKTKKSRKTGEITQVIEDFEIVLGKVMYR
QLMIDGDKKFMLGSSTYQYNAKQLVLSQSVKTLASKGRLDPLQESMDYNNVYTEILDKNVQYFSLYDM
NKFRHKLNLGFSKFISFPNHNVLGDNTKVSSGKREILQEILNGLHANPTFGNLKDVGITTPFGQLQQPNIGILL
SDETKIRYQSPTGLFERTVSLKDL (SEQ ID NO: 164)

[0202] In some embodiments the Cas9 protein can be *Listeria innocua* Cas9 and may comprise or consist of the amino acid sequence:

MKKPYTIGLDIGTNSVGWAVLTDQYDLVKKRMKIAGDSEKKQIKKNFWGVRLFDEGQTAADRRMARTA
RRRIERRRNRISYLQGIFAEEMSKTDANFFCRLSDSFYVDNEKRNSRHPFFATIEEEVEYHKNYPTIYHLREE
LVNSSEKADLRLVYLALAHIIKYRGNFLIEGALDTQNTSVDGIYKQFIQTYNQVFASGIEDGSLKKLEDNKD
VAKILVEKVTRKEKLERILKLYPGEKSAGMFAQFISLIVGSKGNFQKPFDLIEKSDIECAKDSYEEDLESLLA
LIGDEYAELFVAAKNAYSAVVLSSIITVAETETNAKLSASMIERFDTHEEDLGELKAFIKLHLPKHYYEIFS

TEKHGYAGYIDGKTKQADFYKYMKMTLENIEGADYFIAKIEKENFLRKQRTFDNGAIPHQLHLEEELEAILH
 QQAKYYPFLKENYDKIKSLVTFRIPYFVGPLANGQSEFAWLTRKADGEIRPWNIEEKVDFGKSAVDFIEKM
 TNKDTYLPKENVLPKHSLCYQKYL VYNELTKVRYINDQGKTSYFSGQEKEQIFNDLFKQKRKVKKKDLEL
 FLRNMSHVESPTIEGLEDSFNSSYSTYHDLLKVGIKQEILDNPVNTEMLNIVKILTVFEDKRMIKEQLQQFS
 DVLDGVVLKKLERRHYTGWGRLSAKLLMGIRDKQSHLTILDYLMNDDGLNRNLMQLINDSNLSFKSIEK
 EQVTTADKDIQSIVADLAGSPAIIKKGILQSLKIVDELVSVMGYPPQTIVVEMARENQTTGKGKNNSRPRYKS
 LEKAIKEFGSQILKEHPTDNQELRNNRLYYLQNGKDMYTGOQLDIHNL SNYDIDHIVPQSFITDNSIDNL
 VLTSSAGNREKGDVPPLEIVRKRKVFWEKLYQGNLMSKRKFDYLTKAERGGLTEADKARFIHRQLVETR
 QITKNVANILHQRFNYEKDDHGNTMKQVRIVTLKSALVSQFRKQFQLYKVRDVNDYHHAHDAYLNGVV
 ANTLLKVYPQLEPEFVYGDYHQFDWFKANKATAKKQFYTNIMLFFAQKDRIIDENGEILWDDKYLDTVKK
 VMSYRQMNIIVKKTEIQKGEFSKATIKPKGNSSKLIPRKTNWDPMKYGGLDSPNMAYAVVIEYAKGKNKL
 FEKKIIRVTIMERKAFAEKDEKAFLEEQGYRQPKVLAKLPKYTLYECEEGRRRMLASANEAKGNQQVLPN
 HLVTLLHHAANCEVSDGKSLDYIESNREMF AELLAHVSEFAKRYTLAEANLNKINQLFEQNKEGDIKAIQAQ
 SFVDLMAFNAMGAPASFKFFETTIERKRYNNLKELLNSTIYQSITGLYESRKRLDD (SEQ ID NO: 165)

[0203] In some embodiments the Cas9 protein can be *L. pneumophila* Cas9 and may comprise or consist of the amino acid sequence:

MESSQILSPIGIDLGKFTGVCLSHLEAFAELPNHANTKYSVILIDHNNFQLSQAQRRA TRHRVRNKKRNQF
 VKRVALQLFQHILSRDLNAKEETALCHYLNNGYTYVDTDLDEYIKDETTINLLKELLPSESEHNFDWFLQ
 KMQSSEFRKILVSKVEKKDDKELKNAVKNKNFITGFEKNSVEGHRHRKVYFENIKSDITKDNQLDSIKKK
 IPSVCLSNLLGHLNLQWKNLHRYLAKNPQKQFDEQTFGNEFLRMLKNFRHLKGSQESLAVRNLIQQLEQSQ
 DYISILEKTPPEITIPPYEARTNTGMEKDQSLLLNPEKLNLYPNWRNLIPGIIDAHFPLEKDLEHTKLDRKR
 IISPSKQDEKRDSYILQRYLDLNNKIDKFKIKKQLSFLGQKQLPANLIETQKEMETHFNSSLVSVLIQIASAY
 NKEREDAAQGIWFDNAFSLCELSNINPPRKQKILPLL VGAILSEDFINNKKDWAKFKIFWNTHKIGRTSLKS
 KCKEIEEARKNSGNAFKIDYEEALNHPEHSNNKALIKIQTIPDIIQAIQSHLGHNDSQALIYHNPFSLSQLYTI
 LETKRDGFHKNCVAVTCENYWRSQKTEIDPEISYASRLPADSVRPFDGVLARMMQRLAYEIAMAKWEQIK
 HIPDNSSLLIPIYLEQNRFEFEESFKKIKGSSSDKTLEQAIEKQNIQWEEKFQRIINASMNICPYKGASIGGQGE
 IDHIYPRSLSKKHFGVIFNSEVNLIYCSSQGNREKKEEHYLLHLSPLYLKHQFGTDNVSDIKNFISQNVANI
 KKYISFHLLTPEQQAARHALFLDYDDEAFKTITKFLMSQKARVNGTQKFLGKQIMEFLSTLADSKQLQL
 EFSIKQITAEVVDHRELLSKQEPKLVKSRQQSFPSHAIDATLTMSIGLKEFPQFSQELDNSWFINHLMPEV
 HLNVPVRSKEKYNKPNISSSTPLFKDSL YAEFIPVWVKGETFAIGFSEKDLFEIKPSNKEKLFTLLKTYSTKNP
 GESLQELQAKSKAKWL YFPINKTLALEFLHHYFHKEIVTPDDTTVCHFINSRLYYTKKESITVKILKEPMPVL
 SVKFESSKKNVLGSFKHTIALPATKDWERLFNHPNFLALKANPAPNPKEFNEFIRKYFLSDNNPNSDIPNNG
 HNIKPQKHKA VRKVFSPLVIPGNAGTMMRIRRKDNKGQPLYQLQTIDDTSPMGIQINEDRLVKQEVLMDA
 YKTRNLSTIDGINNSEGQAYATFDNWL TLPVSTFKPEIKLEMKPHSKTRRYIRITQSLADFIKTIDEALMIKP
 SDSIDDLNMPNEIVCKNKLFGNELKPRDGKMKIVSTGKIVTYEFESDSTPQWIQTL YVTQLKKQP (SEQ ID
 NO: 166)

[0204] In some embodiments the Cas9 protein can be *N. lactamica* Cas9 and may comprise or consist of the amino acid sequence:

MAAFKPNPMNYILGLDIGIASVGWAMVEVDEEENPIRLIDLGVRFERAEVPKTGDSLAMARRLARSVRRL
TRRAHRLRLRARLLKREGVLQDADFENGLVKSLPNTPWQLRAAALDRKLTCLWSAVLLHLVKHRGY
LSQRKNEGETADKELGALLKGVADNAHALQTGDFRTPAELALNKFEEKESGHIRNQRGDYSHTFSRKDLQA
ELNLLFEKQKEFGNPHVSDGLKEDIETLLMAQRPALSGDAVQKMLGHCTFEPAPKAANKNTYTAEFIWL
TKLNNLRILEQGSERPLTDTERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEM
KAYHAISRALEKEGLKDKKSPLNLSLTELQDEIGTAFSLFKTDKIDTGRLLKDRVQPEILEALLKHISFDKVFQIS
LKALRRIVPLMEQGKRYDEACAEIYGDHYCKKNAEKKIYLPPIPADEIRNPVLRALSQARKVINCVRVRY
GSPARIHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPSKDKILKLRLYEQQHGKCL
YSGKEINLVRLEKGYVEIDHALPFSRTWDDSFNNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKA
RVETSRFPRSKKQRILLQKFDEEGFKERNLNDTRYVNRFLCQFVADHILLTGKGKRRVFASNGQITNLLRGF
WGLRKVRTENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGEVLHQKAHFPQPWE
FFAQEVMIRVFGKPDGKPEFEEADTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVK
SAKRLDEGISVLRVPLTQLKLKLEKMMVNREREPKLYDALKAQLETHKDDPAKAFAPFYKYDKAGSRTQ
QVKAVRIEQVQKTGVWVRNHNGIADNATMVRVDVFEEKGGKYYLVPIYSWQVAKGILPDRAVVAFKDEE
DWTVMDDSFEFRLFVLYANDLIKLTAKKNEFLGYFVSLNRATGAIDIRTHDSTDSTKGKNGIFQSVGVKTALS
FQKNQIDELGKEIRPCRLKKRPPVR (SEQ ID NO: 167)

[0205] In some embodiments the Cas9 protein can be *N. meningitidis* Cas9 and may comprise or consist of the amino acid sequence:

MAAFKPNPINYILGLDIGIASVGWAMVEIDEDENPICLIDLGVRFERAEVPKTGDSLAMARRLARSVRRLT
RRRAHRLRLRARLLKREGVLQAADFENGLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLS
QRKNEGETADKELGALLKGVADNAHALQTGDFRTPAELALNKFEEKESGHIRNQRGDYSHTFSRKDLQAE
ILLFEKQKEFGNPHVSGGLKEGIEETLLMTQRPALSGDAVQKMLGHCTFEPAPKAANKNTYTAEFIWLTKL
NNLRILEQGSERPLTDTERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMKAY
HAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLLKDRIQPEILEALLKHISFDKVFQISL
KALRRIVPLMEQGKRYDEACAEIYGDHYGKKNTTEKIYLPPIPADEIRNPVLRALSQARKVINGVRVRYGSPAR
IHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPSKDKILKLRLYEQQHGKCLYSGKE
INLGRLEKGYVEIDHALPFSRTWDDSFNNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETS
RFPRSKKQRILLQKFDEEDGFKERNLNDTRYVNRFLCQFVADRMRLTGKGKRRVFASNGQITNLLRGFWGL
RKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGEVLHQKTHFPQPWEFFAQ
EVMIRVFGKPDGKPEFEEADTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSAR
LDEGVSVLRVPLTQLKLKLEKMMVNREREPKLYEALKARLEAHKDDPAKAFAPFYKYDKAGNRTQQVK
AVRVEQVQKTGVWVRNHNGIADNATMVRVDVFEEKGDKYYLVPIYSWQVAKGILPDRAVVQKDEEDW
QLIDDSFNFKFSLHPNDLVEVITKKARMGFYFASCHRG TGNNIRIHDLHDHKIGKNGILEGIGVKTALS
FQKNQIDELGKEIRPCRLKKRPPVR (SEQ ID NO: 168)

[0206] In some embodiments the Cas9 protein can be *B. longum* Cas9 and may comprise or consist of the amino acid sequence:

MLSRQLLGASHLARPVSYSYNVQDNDVHCSYGERCFMRGKRYRIGIDVGLNSVGLAAVEVSDENSPVRLL
NAQSVIHDGGVDPQKNKEAITRKNMSGVARRTRRMRRRKRRERLHKLDMLLGKFGYPVIEPESLDKPFEEW
HVRAELATRYIEDDELRRRESISIALRHMARHRGWRNPYRQVDSLISDNPYSKQYGELKEKAKAYNDDATA
AEEESTPAQLVVAMLDAGYAEAPRLRWRTGSKKPDAEGYLPVRLMQEDNANELKQIFRVQRPADSEWKP
LFRSVFYAVSPKGSAEQRVGQDPLAPEQARALKASLAFQEYRIANVITNLRKIDASAE LRKLTVDEKQSIYD
QLVSPSSEDITWSDLCDFLGFKRSQKGVGSLTEDGEERISSRPRLTSVQRIYESDNKIRKPLVAWWKSAS
DNEHEAMIRLLSNTVDIDKVREDVAYASAIEFIDGLDDDALTKLDSVDLPSGRAAYSVELTQKLTRQMLTT
DDDLHEARKTLFNVTD SWRPPADPIGEPLGNPSVDRVLKNVNRYLMNCQQRWGNPVSVNIEHVRSSFSSV
AFARKDKREYEKNNEKRSIFRSSLSEQLRADEQMEKVRESDLRRLLEAIQRQNGQCLYCGRTITFRTCEMDH
IVPRKGVGSTNTRTNFAAVCAECNRMKSNTPF AIWARSEDAQTRGVSLAEAKKRVMTFTFNPKSYAPREV
KAFKQAVIARLQQTEDDAAIDNRSIESVAWMADDELHRRIDWYFNAKQYVNSASIDDAEAEATMKTTVSVFQ
GRVTASARRAAGIEGKIHFIGQQSKTRLD RRRHHAVDASVIAMMNTAAAQTLMERESLRESQR LIGLMPGER
SWKEYPYEGTSRYESFHLWLDNMDVLELLNDALDNDRIAVMQSQRYVLGNSIAHDATIHPLEKVPLGSA
MSADLIRRASTPALWCALTRLPDYDEKEGLPEDSHREIRVHDTRYSA DDEMGGFFASQAAQIAVQEGSADIG
SAIHHARVYRCWKTNAKGVRKYFYGMIRVFQTDLLRACHDDLFTVPLPPQSISMRYGEPRVVQALQSGNA
QYLGSLVVGDEIEMDFSSLDVDGQIGEYLQFFSQFSGGNLAWKHWVVDGFFNQTLRLRIPRYLAAEGLAK
AFSDDVVPDGVQKIVTKQGWLPVNTASKTAVRIVRRNAFGEPRLSSAHMPCSWQWRHE (SEQ ID NO:
169)

[0207] In some embodiments the Cas9 protein can be *A. muciniphila* Cas9 and may comprise or consist of the amino acid sequence:

MSRSLTFSFDIGYASIGWAVIASASHDDADPSVCGCGTVLFPKDDCQAFKRREYRRLRRNIRSRRVRIERIG
RLLVQAQIITPEMKETSGHPAPFYLA SEALKGHRTLAPIELWHVLRWYAHNRGYDNNASWSNSLSEGGN
GEDTERVKHAQDLMDKHGTATMAETICRELKLEEGKADAPMEVSTPAYKNLNTAFPR LIVEKEVRRILELS
APLIPGLTAEIIELIAQHHP LTTEQRGVLLQHGIKLARRYRGSLLFGQLIPRFDNR IISRCPVTWAQVYEAELK
KGNSEQSARERAELSKVPTANCPEFYEYRMARILCNIRADGEPLSAEIRRELMNQARQEGKLT KASLEKAI
SSRLGKETETNVSNYFTLHPDSEEALYLNPAVEVLQRSIGIGILSPSVYRIANRLRRGKSVTPNYLLNLLKS
RGESGEALEKKIEKESKKKEADYADTPLKPKYATGRAPYARTVLKKVVEEILDGEDPTRPARGEAHPDGEL
KAHDGCLYCLLDTSSVNQH QKERRLD TMTNNHLVRHRMLILDRLKDLIQDFADGQKDRISRVCVEVG
KELTTFSAMD SKKIQRELTLRQKSHTDAVNRLKRKLPGKALSANLIRKCR IAMD MNWTCPTGTATYGDHE
LENLELEHIVPHSFRQSNALSSLVLTWPGVNRMKGQRTGYDFVEQE QENPVDPKPNLHICSLNNYRELVEK
LDDKKGHEDDRRRKKKRKALLMVRGLSHKHQSQNHEAMKEIGMTEGMMTQSSHLMKLACKSIKTS LPD
AHIDMIPGAVTA EVRKAWDVFGVFKELCPEAADPD SGKILKENLRSLTHLHHALDACVLGLIPYIIPAH HN
GLLRRLVAMRRIPEKLIPQVRPVANQRHYVLNDDGRMMLRDL SASLKENIREQLMEQRVIQHVPADMGG
ALLKETMQRVLSVDGSGEDAMVSLSKKKDGKKEKNQVKASKLVGVFPEGPSKLKALKAAIEIDGNYGVA

LDPKPVVIRHIKVFKRIMALKEQNGGKPVRLKKGMLIHLTSSKDPKHAGVWRIESIQDSKGGVKLDLQRA
HCAVPKNKTHECNWREVDLISLLKKYQMKRYPTSYTGTTPR (SEQ ID NO: 170)

[0208] In some embodiments the Cas9 protein can be *O. laneus* Cas9 and may comprise or consist of the amino acid sequence:

METTLGIDLTNSIGLALVDQEEHQILYSGVRIFPEGINKDTIGLGEKEESRNATRRAKRQMRRQYFRKKLR
KAKLLELLIAYDMCPLKPEDVRRWKNWDKQQKSTVRQFPDTPAFREWLLKQNPYELRKQAVTEDVTRPEL
GRILYQMIQRRGFLSSRKGKEEGKIFTGKDRMVGIDETRNKLNKQTLGAYLYDIAPKNGEKYRFRTERVRA
RYTLRDMYIREFEIHWQRQAGHLGLAHEQATRKKNIFLEGSATNVNRNSKLITHLQAKYGRGHVLIEDTRITV
TFQLPLKEVLGGKIEIEEEQLKFKSNEVLFWQRPLRSQKSLLSKCVFEGRNIFYDPVHQQWIIAGPTAPLSH
PEFEEFRAVYQFINNIYGKNEHLTAIQREAVFELMCTESKDFNFEEKIPKHLKLFEKFNFD DTTKVPACTTISQL
RKLFPHPVWEEKREEIWHCFYFYDDNTLLFEKLQKDYALQTNDELEKIKIRLSESYGNVSLKAIRRNIPYLK
KGYAYSTAVLLGGIRNSFGKRFEYFKEYEPEIEKAVCRILKEKNAEGEVIRKIKDYLVHNRFGFAKNDRAFQ
KLYHHSQAITTQAQKERLPETGNLRNPVQQGLNELRRTVNKLLATCREKYGPSFKFDHIHVEMGRELRSS
KTEREKQSRQIRENEKKNEAAKVLAEYGLKAYRDNQKYLKYKEIEEKGTVCCPYTGKTLNISHTLGSD
NSVQIEHIIPYSISLDDSLANKTLCDATFNREKGETPYDFYQKDPSPEKWGASSWEEIEDRAFRLPYAKAQ
RFIRRKQESNEFISRQLNDTRYISKKA VEYLSAICSDVKAFPGQLTAELRHLWGLNNILQSAPDITFPLPVA
TENHREYYVITNEQNEVIRLFPKQGETPRTEKGELLLTGEVERKVFRCKGMQEFQTDVSDGKYWRIKLSS
SVTWSPLFAPKPISADGQIVLKGRIEKGVFVCNQLKQKLTGLPDGSYWISLPVISQTFKEGESVNNSKLT SQ
QVQLFGRVREGIFRCHNYQCPASGADGNFWCTLDTDTAQPAFTPIKNAPPGVGGGQIILTG DVDDKGIFHA
DDDLHYELPASLPKGKYGIFTVESCDPTLPIELSAPKTSKGENLIEGNIWVDEHTGEVRFDPKKNREDQR
HHAIDAIVIALSSQSLFQRLSTYNARRENKKRGLDSTEHPSPWPGFAQDVRQSVVPLLVS YKQNPKTLCKI
SKTLYKDGGKIHSCGNAV RGQLHKETVYGQRTAPGATEKSYHIRKDIRELKT SKHIGKVVDITIRQMLLKH
LQENYHIDITQEFNIPSNAFFKEGVYRIFLPNKHGEPVPIKKIRMKEELGNAERLKDINQYVNPRNNHHVMI
YQDADGNLKEEIVSFWSVIERQNQGQPIYQLPREGRNIVSILQINDTFLIGLKEEPEVYRNDLSTLSKHL YR
VQKLSGMYYTFRHHLASTLNNEREEFRIQSLEAWKRANPVKVQIDEIGRITFLNGPLC (SEQ ID NO: 171).

[0209] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a CRISPR Cas protein. In some embodiments, the CRISPR Cas protein comprises a Type V CRISPR Cas protein. In some embodiments, the Type V CRISPR Cas protein comprises a Cpf1 protein. Exemplary Cpf1 proteins of the disclosure may be isolated or derived from any species, including, but not limited to, a bacteria or an archaea. Exemplary Cpf1 proteins of the disclosure may be isolated or derived from any species, including, but not limited to, *Francisella tularensis subsp. novicida*, *Acidaminococcus sp. BV3L6* and *Lachnospiraceae bacterium sp. ND2006*. Exemplary Cpf1 proteins of the disclosure may be nuclease inactivated.

[0210] Exemplary wild type *Francisella tularensis subsp. Novicida* Cpf1 (FnCpf1) proteins of the disclosure may comprise or consist of the amino acid sequence:

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1 MSIIYQEFVNK YSLSKTLRFE LIPQOGKTLEN IKARGLILDD EKRAKDYKKA KQIIDKYHQF
61 FIEEILSSVC ISEDLLQNY S DVYFKLKKSD DDNLQKDFKS AKDTIKKQIS EYIKDSEKFK
121 NLFNQNLIDA KKGQESDLIL WLKQSKDNGI ELFKANS DIT DIDEALEIIK SFKGWTTYFK
181 GFHENRKNVY SSNDIPTSII YRIVDDNLPK FLENKAKYES LKDKAPEAIN YEQIKKDLAE
241 ELTFDIDYKT SEVNQRVFSL DEVFEIANFN NYLNQSGITK FNTIIGGKFV NGENTKRKGI
301 NEYINLYSQQ INDKTLKKYK MSVLFKQILS DTESKSFVID KLEDDSDVVT TMQSFYEQIA
361 AFKTVEEKSI KETLSLLFDD LKAQKLDLSK IYFKNDKSLT DLSQQVFDDY SVIGTAVLEY
421 ITQQIAPKNL DNPSKKEQEL IAKKTEKAKY LSLETIKLAL EEFNKHRDID KQCRFEEILA
481 NFAAIPMIFD EIAQNKDNL QISIKYQNGG KKDLLQASAE DDVKAIKDLL DQTNLLHLKL
541 KIFHISQSED KANILDKDEH FYLVFEECYF ELANIVPLYN KIRNYITQKP YSDEKFKLNF
601 ENSTLANGWD KNKEPDNTAI LFIKDDKYLL GVMNKKNNKI FDDKAIKENK GEGYKKIVYK
661 LLPGANKMLP KVFFSAKSIK FYNPSEDILR IRNHSTHTKN GSPQKGYEKF EFNIEDCRKF
721 IDFYKQSISK HPEWKDFGFR FSDTQRYNSI DEFYREVENQ GYKLTFFENIS ESYIDSVVNQ
781 GKLYLFQIYN KDFSAYS KGR PNLHTLYWKA LFDERNLQDV VYKLNGEAE L FYRKQSIPKK
841 ITHPAKEAIA NKNKDNPKKE SVFEYDLIKD KRFTEDKFFF HCPITINFKS SGANKFNDEI
901 NLLLKEKAND VHILSIDRGE RHLAYYTLVD GKGNIIKQDT FNIIGNDRMK TNYHDKLAAI
961 EKDRDSARKD WKKINNIIKEM KEGYLSQVHV EIAKLIVIEYN AIVVFEDLNF GFKRGRFPKVE
1021 KQVYQKLEKM LIEKLNLYLV KDNEFDKTTGG VLRAYQLTAP FETFKMGKQ TGIIYYVPAG
1081 FTSKICPVTG FVNQLYPKYE SVSKSEFFS KFDKICYNLD KGYFEFSFDY KNFGDKAAKG
1141 KWTIASFGSR LINFRNSDKN HNWDTREVPY TKELEKLLKD YSIEYGHGEC IKAACGESD
1201 KKFFAKLTSV LNTILQMRNS KTGTELDYL I SPVADVNGNF FDSRQAPKNM PQDADANGAY
1261 HIGLKGLMLL GRIKNNQEGK KLNLVIKNEE YFEFVQNRNN (SEQ ID NO: 172).

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[0211] Exemplary wild type *Lachnospiraceae bacterium sp. ND2006* Cpf1 (LbCpf1) proteins of the disclosure may comprise or consist of the amino acid sequence:

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1 AASKLEKFTN CYLSKTLRFE KAIPVGKTQE NIDNKRLLE DEKRAEDYKG VKKLLDRYYL
61 SFINDVLHSI KLKLNLYIS LFRKKTRTEK ENKELENLEI NLRKEIAKAF KGAAGYKSLF
121 KKDIIETILP EAADDKDEIA LVNSFNGFTT AFTGFFDNRE NMFSEEAKST SIAFRGINEN
181 LTRYISNMDI FEKVDAIFDK HEVQEIKEKI LNSDYDVEDF FEGEFFNFVL TQEGIDVYNA
241 IIGGFVTESE EKIKGLNEYI NLYNAKTKQA LPKFKPLYKQ VLSDRESLSF YGEGYTSDEE
301 VLEVFRTNLN KNSEIFSSIK KLEKLFKNFD EYSSAGIFVK NGPAISTISK DIFGEWNLR
361 DKWNAEYDDI HLKKKAVVTE KYEDDRKSF KKIGSFSLEQ LQEYADADLS VVEKLKEIII
421 QKVDEIYKVY GSSEKLFAD FVLEKSLKKN DAVVAIMKDL LDSVKS FENY IKAFFGEGKE
481 TNRDES FYGD FVLAYDILLK VDHIYDAIRN YVTQKPYSK KFKLYFQNPQ FMGGWDKDK
541 TDYRATILRY GSKYYLAIMD KKYAKCLQKI DKDDVNGNYE KINYKLLPGP NKMLPKVFFS
601 KKW MAYNPS EDIQKIYKNG TFKKGDMFNL NDCHKLIDFF KDSISRYPKW SNAYDFNFSE
661 TEKYKDIAGF YREVEEQGYK VSFESASKKE VDKLVEEGKL YMFQIYNKDF SDKSHGTPNL
721 HTMYFKLLFD ENNHGQIRLS GGAELFMRR SLKKEELVVH PANSPIANKN PDNPKKTTTL
781 SYDVYKDKRF SEDQYELHIP IAINKCPKNI FKINTEVRVL LKHDDNPYVI GIDRGERNLL
841 YIVVDGKGN IVEQYSLNEI INNFNIRIK TDYHSLDDK EKERFEARQN WTSIENIKEL
901 KAGYISQVH KICELVEKYD AVIALEDLNS GFKNRVRKVE KQVYQKFEKM LIDKLNMYVD
961 KKS NPCATGG ALKGYQITNK FESFKSMSTQ NGFIFYIPAW LTSKIDPSTG FVNLLKTKYT
1021 SIADSKKFIS SFDRIMYVPE EDLFEFALDY KNFSRTDADY IKKWKLYSYG NRIRIFAAAK
1081 KNNVFAWEEV CLTSAYKELF NKYGINYQQG DIRALLCEQS DKAIFYSSFMA LMSLMLQMRN
1141 SITGR TDVDF LISPVKNSDG IFYDSRNYEA QENAILPKNA DANGAYNIAR KVLWAIQQFK
1201 KAEDKELDKV KIAISNKEWL EYAQTSVK (SEQ ID NO: 173).

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[0212] Exemplary wild type *Acidaminococcus sp. BV3L6* Cpf1 (AsCpf1) proteins of the disclosure may comprise or consist of the amino acid sequence:

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1 MTQFEGFTNL YQVSKTLRFE LIPQGKTLKH IQEQGFIEED KARNDHYKEL KPIIDRIYKT
61 YADQCLQLVQ LDWENLSAAI DSYRKEKTEE TRNALIEEQA TYRNAIHDFY IGRTDNLTD
121 INKRHAEIYK GLFKAELFNG KVLKQLGTVT TTEHENALLR SFDKFTTYFS GFYENRKNVF
181 SAEDISTAIP HRIVQDNFPP FKENCHIFTR LITAVPSLRE HFENVKKAIG IFVSTSIEEV
241 FSFPFYNQLL TQTQIDLYNQ LLGGISREAG TEKIKGLNEV LNLAIQKNDE TAHIIASLPH
301 RFIPLFKQIL SDRNTLSFIL EEFKSDEEVI QSFCKYKTLL RNVNLETAE ALFNELNSID
361 LTHIFISHKK LETISSALCD HWDTLRNALY ERRISELTGK ITKSAKEKVQ RSLKHEDINL
421 QEIIISAAGKE LSEAFKQKTS EILSHAAAL DQPLPTTLKK QEEKEILKSQ LDSLLGLYHL
481 LDWFAVDEN EVDPEFSARL TGIKLEMEPS LSFYNKARNY ATKPYVSVEK FKLNFQMPTL
541 ASGWDVNKEK NNGAILFVKN GLYYLGIMPK QKGRYKALSF EPTEKTSEGF DKMYDYFPD
601 AAKMIPKCST QLKAVTAHFQ THTTPILLSN NFIEPLEITK EIYDLNNPEK EPKKFQTAYA
661 KKTGDQKGYR EALCKWIDFT RDFLSKYTKT TSIDLSSLRP SSQYKDLGEY YAEINPLLYH
721 ISFQRIAEKE IMDAVETGKL YLFQIYNKDF AKGHHGKPNL HTLYWTGLFS PENLAKTSIK
781 LNGQAEFYR PKSRMKRMAH RLGEKMLNKK LKDQKTPIPD TLYQELYDYV NHRLSHDLSD
841 EARALLPNVI TKEVSHEIHK DRRFTSDKFF FHPITLNYQ AANSPSKFNQ RVNAYLKEHP
901 ETPIIGIDRG ERNLIYITVI DSTGKILEQR SLNTIQQFDY QKKLDNREKE RVAARQAWSV
961 VGTIKDLKQG YLSQVIHEIV DLMHYQAVV VLENLNFQFK SKRTGIAEKA VYQQFEKMLI
1021 DKLNCVLKLD YPAEKVGGVL NPYQLTDQFT SFAKMGTSQ FLFYVPAPYT SKIDPLTGFV
1081 DPFVWKTIKN HESRKHFLG FDFLHYDVKT GDFILHFKMN RNLSFQRLP GFMPAWDIVF
1141 EKNETQFQAK GTPFIAGKRI VPVIENHRFT GRYRDLYPAN ELIALLEEKG IVFRDGSNIL
1201 PKLLENDSSH AIDTMVALIR SVLQMRNSNA ATGEDYINSP VRDLNGVCFD SRFQNPWPM
1261 DADANGAYHI ALKGQLLLNH LKESKDLKLQ NGISNQDWLA YIQELRN (SEQ ID NO:
174) .

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[0213] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein comprises a sequence isolated or derived from a CRISPR Cas protein. In some embodiments, the CRISPR Cas protein comprises a Type VI CRISPR Cas protein or portion thereof. In some embodiments, the Type VI CRISPR Cas protein comprises a Cas13 protein or portion thereof. Exemplary Cas13 proteins of the disclosure may be isolated or derived from any species, including, but not limited to, a bacteria or an archaea. Exemplary Cas13 proteins of the disclosure may be isolated or derived from any species, including, but not limited to, *Leptotrichia wadei*, *Listeria seeligeri* serovar 1/2b (strain ATCC 35967 / DSM 20751 / CIP 100100 / SLCC 3954), *Lachnospiraceae* bacterium, *Clostridium aminophilum* DSM 10710, *Carnobacterium gallinarum* DSM 4847, *Paludibacter propionigenes* WB4, *Listeria weihenstephanensis* FSL R9-0317, *Listeria weihenstephanensis* FSL R9-0317, bacterium FSL M6-0635 (*Listeria newyorkensis*), *Leptotrichia wadei* F0279, *Rhodobacter capsulatus* SB 1003, *Rhodobacter capsulatus* R121, *Rhodobacter capsulatus* DE442 and *Corynebacterium ulcerans*. Exemplary Cas13 proteins of the disclosure may be DNA nuclease inactivated. Exemplary Cas13 proteins of the disclosure include, but are not limited to, Cas13a, Cas13b, Cas13c, Cas13d and orthologs thereof. Exemplary Cas13b proteins of the disclosure include, but are not limited to, subtypes 1 and 2 referred to herein as Csx27 and Csx28, respectively.

[0214] Exemplary Cas13a proteins include, but are not limited to:

Cas13a number	Cas13a abbreviation	Organism name	Accession number	Direct Repeat sequence
Cas13a1	LshCas13a	Leptotrichia shahii	WP_018451595.1	CCACCCCAATATCGAAGGGGACTAA AAC (SEQ ID NO: 175)
Cas13a2	LwaCas13a	Leptotrichia wadei	WP_021746774.1	GATTTAGACTACCCCAAAAACGAAG GGGACTAAAAC (SEQ ID NO: 176)
Cas13a3	LseCas13a	Listeria seeligeri	WP_012985477.1	GTAAGAGACTACCTCTATATGAAAG AGGACTAAAAC (SEQ ID NO: 177)
Cas13a4	LbmCas13a	Lachnospiraceae bacterium MA2020	WP_044921188.1	GTATTGAGAAAAGCCAGATATAGTT GGCAATAGAC (SEQ ID NO: 178)
Cas13a5	LbnCas13a	Lachnospiraceae bacterium NK4A179	WP_022785443.1	GTTGATGAGAAGAGCCCAAGATAG AGGGCAATAAC (SEQ ID NO: 179)
Cas13a6	CamCas13a	[Clostridium] aminophilum DSM 10710	WP_031473346.1	GTCTATTGCCCTCTATATCGGGCTGT TCTCCAAAC (SEQ ID NO: 180)
Cas13a7	CgaCas13a	Carnobacterium gallinarum DSM 4847	WP_034560163.1	ATTAAAGACTACCTCTAAATGTAAG AGGACTATAAC (SEQ ID NO: 181)
Cas13a8	Cga2Cas13a	Carnobacterium gallinarum DSM 4847	WP_034563842.1	AATATAAACTACCTCTAAATGTAAG AGGACTATAAC (SEQ ID NO: 182)
Cas13a9	Pprcas13a	Paludibacter propionigenes WB4	WP_013443710.1	CTTGTGGATTATCCCAAAATTGAAG GGAAGTACAAC (SEQ ID NO: 183)
Cas13a10	LweCas13a	Listeria weihenstephanensis FSL R9-0317	WP_036059185.1	GATTTAGAGTACCTCAAAATAGAAG AGGTCTAAAAC (SEQ ID NO: 184)
Cas13a11	LbfCas13a	Listeriaceae bacterium FSL M6-0635 (Listeria newyorkensis)	WP_036091002.1	GATTTAGAGTACCTCAAAACAAAAG AGGACTAAAAC (SEQ ID NO: 185)
Cas13a12	Lwa2cas13a	Leptotrichia wadei F0279	WP_021746774.1	GATATAGATAACCCCAAAAACGAA GGGATCTAAAAC (SEQ ID NO: 186)
Cas13a13	RcsCas13a	Rhodobacter capsulatus SB 1003	WP_013067728.1	GCCTCACATCACCGCCAAGACGACG GCGGACTGAAC (SEQ ID NO: 187)
Cas13a14	RcrCas13a	Rhodobacter capsulatus R121	WP_023911507.1	GCCTCACATCACCGCCAAGACGACG GCGGACTGAAC (SEQ ID NO: 188)

Cas13a15	RcdCas13a	Rhodobacter capsulatus DE442	WP_023911507.1	GCCTCACATCACCGCCAAGACGACG GCGGACTGAAC (SEQ ID NO: 189)
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[0215] Exemplary wild type Cas13a proteins of the disclosure may comprise or consist of the amino acid sequence:

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1  MGNLFGHKRW YEVRDKKDFK IKRKVKVKRN YDGNKYILNI NENNNKEKID NNKFIRKYIN
61 YKKNDNILKE FTRKFHAGNI LFKLKGKEGI IRIENDDDFL ETEEVVLYIE AYGKSEKLKA
121 LGITKKKIID EAIRQGITKD DKKIEIKRQE NEEIEIDIR DEYTNKTLND CSIILRIEN
181 DELETKKSIY EIFKNINMSL YKIIIEKIIEN ETEKVFENRY YEEHLREKLL KDDKIDVILT
241 NFMEIREKIK SNLEILGFVK FYLNVGGDKK KSKNKKMLVE KILNINVDLT VEDIADFKIK
301 ELEFWNITKR IEKVKKVNE FLEKRRNRTY IKSIVLLDKH EKFKIERENK KDKIVKFFVE
361 NIKNSIIEK IEKILAEFKI DELIKKLEKE LKKGNCDEI FGIFKKHYKV NFDSKKFSKK
421 SDEEKELYKI IYRYLKGRIE KILVNEQKVR LKMEKIEIE KILNESILSE KILKRVKQYT
481 LEHIMYLGKL RHNDIDMTTV NTDDFSRLHA KEELDLELIT FFASTNMELN KIFSRENINN
541 DENIDFFGGD REKNYVLDKK ILNSKIKIIR DLDFIDNKN ITNNFIRKFT KIGTNERNRI
601 LHAISKERDL QGTQDDYNKV INIIQNLKIS DEEVSKALNL DVVFKDKKNI ITKINDIKIS
661 EENNNDIKYL PSFSKVLPEI LNLRYNNPKN EPFDTIETEK IVLNALIYVN KELYKKLILE
721 DDLEENESKN IFLQELKKTL GNIDEIDENI IENYKNAQI SASGNNKAI KKYQKKVIEC
781 YIGYLRKNYE ELDFDSDFKM NIQEIKKQIK DINDNKTYER ITVKTSDKTI VINDDFEYII
841 SIFALLNSNA VINKIRNRFF ATSVWLNTSE YQNIIDILDE IMQLNLTNRNE CITENWNLNL
901 EEFIQMKKEI EKDFDDFKIQ TKKEIFNNY EDIKNNILTE FKDDINGCDV LEKKLEKIVI
961 FDDETKFEID KKSNIQDEQ RKLSNINKKD LKKKVDQYIK DKDQEIYSKI LCRIIFNSDF
1021 LKKYKKEIDN LIEDMESENE NKFQEIYYPK ERKNELYIYK KNLFNLNIGP NFDKIYGLIS
1081 NDIKMADAKF LFNIDGKNIR KNKISEIDAI LKNLNDKLNG YSKEYKEYI KKLKENDDFE
1141 AKNIQNKNYK SFEKDYNRV EYKKIRDLVE FNYLNKIESY LIDINWKLAI QMARFERDMH
1201 YIVNGLRELG IIKLSGYNTG ISRAYPKRNG SDGFYTTTAY YKFFDEESYK KFEKICYGFG
1261 IDLSENSEIN KPENESIRNY ISHFYIVRNP FADYSIAEQI DRVSNLLSYS TRYNNSTYAS
1321 VFEVFKKDVN LDYDELKKKF KLIGNNDILE RLMKPKKVSV LELESYNSDY IKNLIIELLT
1381 KIENTNDTL (SEQ ID NO: 190)

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[0216] Exemplary Cas13b proteins include, but are not limited to:

Species	Cas13b Accession	Cas13b Size (aa)
Paludibacter propionigenes WB4	WP_013446107.1	1155
Prevotella sp. P5-60	WP_044074780.1	1091
Prevotella sp. P4-76	WP_044072147.1	1091
Prevotella sp. P5-125	WP_044065294.1	1091
Prevotella sp. P5-119	WP_042518169.1	1091
Capnocytophaga canimorsus Cc5	WP_013997271.1	1200
Phaeodactylibacter xiamenensis	WP_044218239.1	1132
Porphyromonas gingivalis W83	WP_005873511.1	1136
Porphyromonas gingivalis F0570	WP_021665475.1	1136
Porphyromonas gingivalis ATCC 33277	WP_012458151.1	1136
Porphyromonas gingivalis F0185	ERJ81987.1	1136
Porphyromonas gingivalis F0185	WP_021677657.1	1136
Porphyromonas gingivalis SJD2	WP_023846767.1	1136
Porphyromonas gingivalis F0568	ERJ65637.1	1136
Porphyromonas gingivalis W4087	ERJ87335.1	1136
Porphyromonas gingivalis W4087	WP_021680012.1	1136

Porphyromonas gingivalis F0568	WP_021663197.1	1136
Porphyromonas gingivalis	WP_061156637.1	1136
Porphyromonas gulae	WP_039445055.1	1136
Bacteroides pyogenes F0041	ERI81700.1	1116
Bacteroides pyogenes JCM 10003	WP_034542281.1	1116
Alistipes sp. ZOR0009	WP_047447901.1	954
Flavobacterium branchiophilum FL-15	WP_014084666.1	1151
Prevotella sp. MA2016	WP_036929175.1	1323
Myroides odoratimimus CCUG 10230	EHO06562.1	1160
Myroides odoratimimus CCUG 3837	EKB06014.1	1158
Myroides odoratimimus CCUG 3837	WP_006265509.1	1158
Myroides odoratimimus CCUG 12901	WP_006261414.1	1158
Myroides odoratimimus CCUG 12901	EHO08761.1	1158
Myroides odoratimimus (NZ_CP013690.1)	WP_058700060.1	1160
Bergeyella zoohelcum ATCC 43767	EKB54193.1	1225
Capnocytophaga cynodegmi	WP_041989581.1	1219
Bergeyella zoohelcum ATCC 43767	WP_002664492.1	1225
Flavobacterium sp. 316	WP_045968377.1	1156
Psychroflexus torquis ATCC 700755	WP_015024765.1	1146
Flavobacterium columnare ATCC 49512	WP_014165541.1	1180
Flavobacterium columnare	WP_060381855.1	1214
Flavobacterium columnare	WP_063744070.1	1214
Flavobacterium columnare	WP_065213424.1	1215
Chryseobacterium sp. YR477	WP_047431796.1	1146
Riemerella anatipestifer ATCC 11845 = DSM 15868	WP_004919755.1	1096
Riemerella anatipestifer RA-CH-2	WP_015345620.1	949
Riemerella anatipestifer	WP_049354263.1	949
Riemerella anatipestifer	WP_061710138.1	951
Riemerella anatipestifer	WP_064970887.1	1096
Prevotella saccharolytica F0055	EKY00089.1	1151
Prevotella saccharolytica JCM 17484	WP_051522484.1	1152
Prevotella buccae ATCC 33574	EFU31981.1	1128
Prevotella buccae ATCC 33574	WP_004343973.1	1128
Prevotella buccae D17	WP_004343581.1	1128
Prevotella sp. MSX73	WP_007412163.1	1128
Prevotella pallens ATCC 700821	EGQ18444.1	1126
Prevotella pallens ATCC 700821	WP_006044833.1	1126
Prevotella intermedia ATCC 25611 = DSM 20706	WP_036860899.1	1127
Prevotella intermedia	WP_061868553.1	1121
Prevotella intermedia 17	AFJ07523.1	1135
Prevotella intermedia	WP_050955369.1	1133
Prevotella intermedia	BAU18623.1	1134
Prevotella intermedia ZT	KJJ86756.1	1126
Prevotella aurantiaca JCM 15754	WP_025000926.1	1125
Prevotella pleuritidis F0068	WP_021584635.1	1140
Prevotella pleuritidis JCM 14110	WP_036931485.1	1117
Prevotella falsenii DSM 22864 = JCM 15124	WP_036884929.1	1134

Porphyromonas gulae	WP_039418912.1	1176
Porphyromonas sp. COT-052 OH4946	WP_039428968.1	1176
Porphyromonas gulae	WP_039442171.1	1175
Porphyromonas gulae	WP_039431778.1	1176
Porphyromonas gulae	WP_046201018.1	1176
Porphyromonas gulae	WP_039434803.1	1176
Porphyromonas gulae	WP_039419792.1	1120
Porphyromonas gulae	WP_039426176.1	1120
Porphyromonas gulae	WP_039437199.1	1120
Porphyromonas gingivalis TDC60	WP_013816155.1	1120
Porphyromonas gingivalis ATCC 33277	WP_012458414.1	1120
Porphyromonas gingivalis A7A1-28	WP_058019250.1	1176
Porphyromonas gingivalis JCVI SC001	EOA10535.1	1176
Porphyromonas gingivalis W50	WP_005874195.1	1176
Porphyromonas gingivalis	WP_052912312.1	1176
Porphyromonas gingivalis AJW4	WP_053444417.1	1120
Porphyromonas gingivalis	WP_039417390.1	1120
Porphyromonas gingivalis	WP_061156470.1	1120

[0217] Exemplary wild type *Bergeyella zoohelcum* ATCC 43767 Cas13b (BzCas13b) proteins of the disclosure may comprise or consist of the amino acid sequence:

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1 menktslgnn iyympfkpqq ksyfagyfna amentdsvfr elgkrlkgke ytsenffdai
61 fkenislvey eryvklldsy fpmarlldkk evpikerken fknfnkgiik avrdlnrfyt
121 hkehgeveit deifgvldem lkstvlvtvkk kkvktktke ilkksiekql dilcqkley
181 lrdtarkiee krnrqrege kelvapfkys dkrddliaai yndafdvaid kkkdskless
241 kakyntksdp qqeegdlkip isknngvfl1 slfltkqeih afkskiagfk atvideatvs
301 eatvshgkns icfmatheif shlaykklkr kvrtaeinyg eaenaeglsv yaketlmmqm
361 ldelskvpdv vyqnlsevdq ktfiedwney lkenngdvgt meeeqvihpv irkryedkfn
421 yfairfldef aqfptlrfqv hlgnylhdsr pkenlisdr ikekitvfgr lselehkkal
481 fikntetned rehyweifpn pnydfpkeni svndkdfpia gsildrekqp vagkigikvk
541 llnqqyvsev dkavkahqlk qrkaskpsiq niieivpin esnpkeavf ggqptaylsm
601 ndihsilyef fdkwekkkek lekkgelkr keigkelekk ivgkiqagiq qiidkdnak
661 ilkpyqdgns taidkeklik dlkqeqnilq klkdeqtre keyndfiayq dknreinkvr
721 drnhkqylkd nlkrkypeap arkevlyyre kgkvavwlan dikrfmptdf knewkgeqhs
781 llqkslayye qckeelknll pekvfqhlpf klgyfyqqky lyqfytcyld krleyisglv
841 qqaenfxsen kvfkkvenec fkflkkqnyt hkeldarvqs ilgypifler gfmdekptii
901 kgktfkgnea lfadwfryyk eyqnfqtfyd tenyplvele kkgadrkrkt kiyqqkndv
961 ftllmakhif ksvfkqdsid qfsledlyqs reerlgnqr arqtgerntn yiwnktvdlk
1021 lcdgkitven vlnknvgdfi kyeydqrva flkyeeniew qaflikeske eenypyvver
1081 eieqyekvrr eellkevhli eeyilekvkd keilkkgdnq nfkyylngl lkqlknedve
1141 sykvfnlnte pedvninqlk qeatdleqa fvltyirnkf ahnqlpkkef wdycqekyqk
1201 iekektyaey faevfkkeke alik (SEQ ID NO: 191).

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[0218] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein, or RNA-guided target RNA binding protein, comprises a sequence isolated or derived from a CasRX/Cas13d protein. CasRX/Cas13d is an effector of the type VI-D CRISPR-Cas systems. In some embodiments, the CasRX/Cas13d protein is an RNA-guided

RNA endonuclease enzyme that can cut or bind RNA. In some embodiments, the CasRX/Cas13d protein can include one or more higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domains. In some embodiments, the CasRX/Cas13d protein can include either a wild-type or mutated HEPN domain. In some embodiments, the CasRX/Cas13d protein includes a mutated HEPN domain that cannot cut RNA but can process guide RNA. In some embodiments, the CasRX/Cas13d protein does not require a protospacer flanking sequence.

[0219] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig6049000251:

LYLTSFGKGN AAVIEQKIEP ENGYRVTGMQ ITPSITVNKA TDESVRFRVK RKIAQKDEFI	60
ADNPMHEGRH RIEPSAGSDM LGLKTKLEKY YFGKEFDDNL HIQIIYNILD IEKILAVYST	120
NITA	124

(SEQ ID NO: 54).

[0220] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig546000275:

MDSYRPKLYK LIDFCIFKHY HEYTEISEKN VDTLRAAVSE EQKESFYADE AKRLWGIFDK	60
QFLGFCKKIN VWVNGSHEKE ILGYIDKDAY RKKSDVSYFS KFLYAMSFFL DGKEINDLLT	120
TLINKFDNIA SFISTAKELD AEIDRILEKK LDPVTGKPLK GKNSFRNFIA NNVIENKRFI	180
YVIKFCNPKN VLKLVKNTKV TEFVLKRMPE SQIDRYYS SC IDTEKNPSVD KKISDLAEMI	240
KKIAFDDFRN VRQKTRTREE SLEKERFKAV IGLYLTVVYL LIKNLVNVS RYVMAFHCLC	300
RDALYGINI GKNYIELTED LCRENENSRS AYLARNKRLR DCVKQIDNA KNMKSKEK	358

(SEQ ID NO: 57).

[0221] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig4114000374:

DTKINPQTWL YQLENTPDLD NEYRDTLDHF FDERFNEINE HFVTQATNL CIMKEVFPDE	60
DFKSIADLYY DFIVVKS YKN IGFSIKKLE KMLELPEAKR VTSTEMDSVR SKLYKLIDFC	120
IFKHYHEKPE TVEMIVSMLR AYTSED MKE	149

(SEQ ID NO: 61).

[0222] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig721000619:

KEGSTMAKNE KKKSTAKALG LKSSFVVNND IYMTSFGKGN KAVLEKKITE NTIENKSDTT	60
YFDVINRDPK GFTLEGRRIA DMTAFSNDPK YHVN VNGKF LEDQLGARSE LEKKVFGRTF	120
DDNVHIQLIH NILDIEKIMA QYVSDIVYLL HNTIKRDMND DIMGYISIRN SFDDFCHPER	180
IPDRKAKDNL QKQHDIFDE ILKCGRLAYF GNAFFEDGSD NKEIAKLKRY KEIYHIIALM	240
GSLRQSYFHG ENSDKNFQGP TWAYTLESNL TGKYKEFKDT LDKTFDERYE MISKDFGSTN	300
MVNLQILEEL LKMLYGNVSP	320

(SEQ ID NO: 67).

[0223] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig2002000411:

EKQNKAKYQA IISLYLMVMY QIVKNMIYVN SRYVIAFHCL ERDSNQLLGR FNSRDASMYN	60
KLTQKFITDK YLNDGAQGCS KKVGNLYSHN ITCCSDELK EYRNQVDHFA VVRMIGKYAA	120
DIGKFSTWFE LYHYVMQRII FDKRNPLSET ERTYKQLIAK HHTYCKDLVK ALNTPFGYNL	180
ARYKNLSIGE LFDRNNYNAK TKET	204

(SEQ ID NO: 69).

[0224] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig13552000311:

LIDFLIYDLY YNRKPARIEE IVDKLRESVN DEEKESIYSA ETKYVYEALG KVLVRSLLKKY	60
LNGATIRDLK NRYDAKTANR IWDISEHSKS GHVNCFCCLI YMTLMMLDGK EINDLLTTLV	120
NKFDNIASFI DVMDLGLLEH SFTDNYKMFA DSKAICLDLQ FINSFARMSK IDDEKSKRQL	180
FRDALVVDI GDNEDWIEK YLTSDIFKRD ENGNKIDGK RDFRNFIANN VIKSARFKYL	240
VKYSSADGMI KKKNEKLIS FVLEQLPETQ IDRYYESCGI DCAVADRKVR IEKLTGLIRD	300
MRFDNFRGVN YSNDACKKDK QAKAKYQAI SLYLMVLYQI VKNMIYVNSR YVIAFHCLER	360
DLLFFNIELD NSYQYSNCNE LTEKFIKDKY MKEGALGFNM KAGRYLTKNI GNCSNELRKI	420
YRNQVDHFAV VRKIGNYAAD IASVGSWFE	449

(SEQ ID NO: 71).

[0225] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig10037000527:

YMDQNFANS DAWAHVYRNK IQHLDVVRHA DMYIGDIREF HSWFELYHYI IQRRIIDQYA	60
YESTPGSSRD GSAIIDEERL NPATRRYFRL ITTYKT	96

(SEQ ID NO: 72).

[0226] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig238000329:

RYDKDRSKIY TMMDFVIYRY YIDNNNSID FINKLRSSID EKSKEKLYNE EANRLWNKLK	60
EYMLYIKEFN GKLSRTPDR DGNISEFVES LPKIHRLPR GQKISNFSKL MYLLTMFLDG	120
KEINDLLTTL INKFENIQGF LDIMPEINVN AKFEPEYVFF NKSHEIAGEL KLIKGAQMG	180
EPAATLKLEM TADAIKILGT EKEDAELIKL AESLFKDENG KLLGNKQHG M RNFIGNNVIK	240
SKRFHYLIRY GDPAHLHKIA TNKNVVRFLV GRIADMQKKQ GQKGKNQIDR YYEVCVGNKD	300
IKKTIEEKID ALTDIIVNMN YDQFEKKAV IENQNRGKTF EEKNKYKRDN AEREKFKKII	360
SLYLTVIYHI LKNIVNVNSR YILGFHCLER DKQLYIEKYN KDKLDGFVAL TKFCLGDEER	420
YEDLKAKAQA SIQALETANP KLYAKYMNYS DEEKKEEFKK QLNREVRKNA RNAYLKNIKN	480
YIMIRLQLRD QTDSSGYLCG EFRDKVAHLE VARHAHEYI	519

(SEQ ID NO: 73).

[0227] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig2643000492:

NGEIVSLAEK EAFSAKIADK NIGCKIENKQ FRHPKGYDVI ADNPIYKGS RQDMLGLKET	60
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LEKRYFSPSD SIDNVRVQVA HNILDIEKIL AEYITNAVYS FDNIAGFGKD IIGDDFSPVY	120
TYDKFEKSDR YEYFKNLLNN SRLGYGQAF FECDDSKENK KKKDAIKCYN IIALLSGLRH	180
W	181

(SEQ ID NO: 84).

[0228] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig874000057:

MSKNKESYAK GMGLKSALVS GSKVYMTSFE GGNDKLEKV VENSEIVSLA EKESFSAEIF	60
KKNIGCKIEN KKFHKPKRYD VIADNPLYKG SVRQDMLGLK ETLEKRYFNS ADGTDNVCIQ	120
VIHNILDIEK ILAEYITNAV YSFDNIAGFG EDIIGMGGFK PIYTYKQFKE PDKYNKKFDD	180
ILNNSRLGYY GKAFFEKNDL KHNPNNKKRD KNPYILKYDN ECYIIALLS GLRHWNIHSH	240
AKDDLVSRYW LYNLDSILNR EYISTLNYLY DDIADELTS FSKNSSANVN YIAETLNIDP	300
SEFAQQYFRF SIMKEQKNMG FNVSKLREIM LDRKELSDIR DNHRVFDISIR SKLYTMDFV	360
IYRYYIEEAA KTEAENRNLP ENEKKISEKD FFVINLRGSF DENQKEKLYI EEAERLWEKL	420
KDIMLKIKEF RGEKVKEYKK	440

(SEQ ID NO: 85).

[0229] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig4781000489:

LDKQLDYEYI RTLNMFNDI ADELTRTFESK NSAANVNYIA ETLNIDPNKF AEQYFRFSIM	60
KEQKNLGFNL TKLRESMLDR RELSDIRDNH NVFDSIRPKL YTMDFVIYK HYIDEAKKTE	120
AENKSLPDDR KNLSEKD	137

(SEQ ID NO: 86).

[0230] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig12144000352:

RMGEPVANTK RVMMIDAVKI LGTDLSDEL KEMADSFVKD SDGNLLKKGK HGMRNFITNN	60
VIKNKRFHYL IRYGDPALH EIAKNEA	87

(SEQ ID NO: 87).

[0231] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig5590000448:

VHNNEEKDLI KYTWLYNLDK YLDAEYITTL NYMYNDIGDE LTDSEFSKNSA ANINYIAETL	60
GIDPKTFAEQ YFRFSIMKEQ KNLGFNLTKL REVMLDRKDM SEIRENHND FDSIRAKVYTM	120
MDFVIYRYI EEAQVNAAN KSLPDNEKSL SEKDFVISL RGSFNEDQKD RLYYDEAQRL	180
WSKVGKMLK IKKFRGKDTR KYKNMGTPRI RRLIPEGRDI STFSKLMYAL TMFLDGKEIN	240
DLTTLINKF DNIQSFLKVM PLIGVNAKFA EEYSFFNNSE KIADELRLIK SFARMGEPVA	300
DARAMYIDA IRILGTDLS DELKALADSF SLDENGKLG KGKHGMRNFI INNVITNKRF	360
HYLIRYGNPV HLHEIAKNEA VVKFVLGRIA DIQKKQGQNG KNQIDRYET CIGK	414

(SEQ ID NO: 88).

[0232] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig525000349:

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MSKKENRKSYS VKGLGLKSTL VSDSKVYLTT FADGSNAKLE KCVENNKIIC ISNDKEAFAA      60
SIANKNVGYK IKNDEKFRHP KGYDIISNNP LLHNSVQQD MLGLKNVLEK RYFGKSSGGD      120
NNLCIQIIHN IIDIEKILSE YIPNVVYAFN NIAGFKDEHN NIIDIIGTQT YNSSYTYADF      180
SKDKSDKKYI EFQKLLKNKR LGYWGKAFFT GQGNNAKVRQ ENQCFHIIAL LISLRNWATH      240
SNELDKHTR TWLYKLDDTN ILNAEYVKTL NYLYDTIADE LTKSESKNGA VNVNVLAKKY      300
NIKDDLPGFS EQYFRFSIMK EQKNLGFNIS KLRENMLDFK DMSVI                      345

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(SEQ ID NO: 89).

[0233] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig7229000302:

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KKISSLTKFC LGESDEKKLK ALAKKSLEEL KTTNSKLYEN YIKYSDERKA EEAKRQINRE      60
RAKTAMNAHL RNTKWNIDIMY GQLKDLADSK SRICSEFRNK AAHLEVARYA HMYINDISEV      120
KSYFRLYHYI MQRRIIDVIE NNPKAKEYEGK VKVYFEDVKK NKKYNKNLLK LMCVPFGYCI      180
PRFKNLSIEQ MFDNETDNS DKKKEK                      206

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(SEQ ID NO: 90).

[0234] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig3227000343:

```

IGDISEVNSY FQLYHYIMQR ILIDKIGSKT TGKAKEYFDS VIVNKKYDDR LLKLLCSPLG      60
YCLTRYKDLS IEALFDMNEA AKYDKLNKER KNKKK                      95

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(SEQ ID NO: 91).

[0235] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Gut_metagenome_contig7030000469:

```

SIRSKLYTMM DFVIYRYIE ESAKAAENK PSEDSFVIR LRGSFNENQK EELYIEEAER      60
LWKKFGEIML KIKEFRGEKV KEYKKEVPRI ERILPHGKDI SAFSKLMYML SMFLD          115

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(SEQ ID NO: 92).

[0236] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d gut_metagenome_P17E0k2120140920_c87000043:

```

MYFSKMIYML TYFLDGKEIN DLLTTLISKF DNIKEFLKIM KSSAVDVECE LTAGYKLFND      60
SQRITNELFI VKNIASMRKP AASAKLTMFR DALITLGIDD KITDDRISI LKLKEKGKGI      120
HGLRNFITNN VIESSRFVYL IKYANAQKIR EVAKNEKVV FVLGGIPDTQ IERYKSCVE      180
FPDMNSSLEA KRSELARMIK NISFDDFKNV KQQAAGRENV AKERAKAVIG LYLT          234

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(SEQ ID NO: 93).

[0237] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OBVH01003037.1, human gut metagenome sequence (also found in WGS contigs emb|OBXZ01000094.1| and emb|OBJF01000033.1|):

```

MAKKKRITAK ERQNHRRELL MKKADSNAEK EKAKKPVVEN KPDTAISKDN TPKPNKEIKK      60

```

SKAKLAGVKW	VIKANDDVAY	ISSFGKGNN	VLEKRIMGDV	SSNVNKDSHM	YVNPKYTKKN	120
YEIKNGFSSG	SSLVTYPNKP	DKNSGMDALC	LKPYFEKDFE	GHIFTDNMHI	QAIYNIFDIE	180
KILAKHITNI	IYTVNSFDRN	YNQSGNDTIG	FGLNYRVPYS	EYGGGKDSNG	EPKNQSKWEK	240
RDNFIKFYNE	SKPHLGYYEN	IFYDHGEPIS	EEKFYNYLNI	LNFI RNNTFH	YKDDDIELYS	300
ENYSEEFVFI	NCLNKFVKNK	FKNVNKNFIS	NEKNLYIIL	NAYGKDTENV	EVVKKYSKEL	360
YKLSVLKTNK	NLGVNVKKLR	ESAIEGYGCP	LPYDKEKEVA	KLSSVKHKLY	KTYDFVITHY	420
LNSNDKLLLE	IVETLRLSKN	DDEKENVYKK	YAEKLFKADD	VINPIKAISK	LFARKGNKLF	480
KEKIIIKKEY	IEDVSDKNI	YDFTKVIFFM	TCFLDGKEIN	DLTNIISKL	QVIEDHNNVI	540
KFISNNKDAV	YKDYSKYAI	FRNAGKIAE	LEAIKSIARM	ENKIENAPQE	PLLKDALLSL	600
GVSDDTKVLE	NTYNKYFDSK	EKTDKQSQKV	STFLMNNVIN	NNRFKYVIKY	INPADINGLA	660
KNRYLVKFVL	SKIPPEQIDS	YYKLSNEEE	PGCEEKIKLL	TKKISKLNQ	TLFENNKIPN	720
VEKEKKKAI	TYFTIVYIL	VKNLVNINGL	YTLALYFVER	DGYFYKDICG	KKDKKSYND	780
VDYLLLEIF	SGSKYREETK	NLKLPEKDR	DIMKKYLPND	KDREKYNKFF	TAYRNNIVHL	840
NIIAKLSELT	KNIDKDINSY	FDIHYCTQR	VMFNYCKEKN	DVVLAKMKDL	AHIKSDCNEF	900
SSKHTYFSS	AVLRFMNLPF	AYNVPRFKNL	SYKKFFDKQ			939

(SEQ ID NO: 94).

[0238] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig tpg|DJXD01000002.1|

(uncultivated Ruminococcus assembly, UBA7013, from sheep gut metagenome):

MKKQKSKKTV	SKTSGLKEAL	SVQGTVIMTS	FGKGNMANLS	YKIPSSQKPQ	NLSSAGLKN	60
VEVSGKKIKF	QGRHPKIATT	DNPLFKPQPG	MDLLCLKDKL	EMHYFGKTFD	DNIHIQLIYQ	120
ILDIEKILAV	HVNNIVFTLD	NVLHPQKEEL	TEDFIGAGGW	RINLDYQTLR	GQTNKYDRFK	180
NYIKRKELLY	FGEAFYHENE	RRYEEDIFAI	LTLLSALRQF	CFHSDLSSDE	SDHVNSEFWLY	240
QLEDQLSDEF	KETLSILWEE	VTERIDSEFL	KTNTVNLHIL	CHVFPKESKE	TIVRAYYEFL	300
IKKSFKNMGF	SIKKLREIML	EQSDLKSFEK	DKYNSVRAKL	YKLDFDIITY	YYDHHAFEKE	360
ALVSSLRSSL	TEENKEEIIY	KTARTLASAL	GADFKKAAAD	VNAKNIRDYQ	KKANDYRISF	420
EDIKIGNTGI	GYFSELIYML	TLLLDGKEIN	DLTTLINKF	DNIISFIDIL	KKLNLEFKFK	480
PEYADFFNMT	NCRYTLEELR	VINSIARMQK	PSADARKIMY	RDALRILGMD	NRPDEEIDRE	540
LERTMPVGAD	GKFIKKGQGF	RNFIA SNVIE	SSRFHYLVRY	NNPHKTRTLV	KNPNVVKFVL	600
EGIPETQIKR	YFDVCKGQEI	PPTSDKSAQI	DVLARIISV	DYKIFEDVPQ	SAKINKDDPS	660
RNFSDALKKQ	RYQAI VSLYL	TVMYLITKNL	VYVNSRYVIA	FHCLERDAFL	HGVTLPKMNK	720
KIVYSQLTTH	LLTDKNYTTY	GHLKNQKQHR	KWYVLVKNNL	QNSDITAVSS	FRNIVAHISV	780
VRNSNEYISG	IGELHSYFEL	YHYLVQSMIA	KNNWYDTSHQ	PKTAEYLNLL	KKHHTYCKDF	840
VKAYCIPFGY	VVPYKNLTI	NELFDRNNPN	PEPKEEV			877

(SEQ ID NO: 95).

[0239] An exemplary direct repeat sequence of CasRX/Cas13d Metagenomic hit (no protein accession): contig tpg|DJXD01000002.1| (uncultivated Ruminococcus assembly, UBA7013, from sheep gut metagenome) (SEQ ID NO: 95) comprises or consists of the nucleic acid sequence:

CasRX/Cas13d DR:

caactacaac cccgtaaaaa tacgggggttc tgaaac

36

[0240] (SEQ ID NO: 96).

[0241] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig OGZC01000639.1 (human gut metagenome assembly):

MKKKNIRATR	EALKAQKIKK	SQENEALKKQ	KLAEEAAQKR	REELEKKNLA	QWEETSAEGR	60
RSRVKAVGVK	SVFVVGDDLY	LATFGNGNET	VLEKKITPDG	KITTFPEEET	FTAKLKFAQT	120
EPTVATSIGI	SNGRIVLPEI	SVDNPLHTTM	QKNTIKRSAG	EDILQLKDV	ENRYFDRSFN	180
DDLHIRLIYN	ILDIEKILAE	YTTNAVFAID	NVSGCSDDFL	SNFSTRNQWD	EFQNPEQHRE	240
HFGNKDNVIC	SVKKQQDLFF	NFFKNNRIGY	FGKAFFHAES	ERKIVKKTEK	EVYHILTLIG	300
SLRQWITHST	EGGISRLWLY	QLEDALSREY	QETMNNCYNS	TIYGLQKDFE	KTNPANLNFL	360
AEILGKNASE	LAEPYFRFII	TKEYKNLGFS	IKTLREMLLD	QPDQLQEIREN	HNVDYSIRSK	420
LYKMIDFVLV	YAYSNERKSK	ADALASNLR	AITEDAKKRI	YQNEADQLWT	SYQELFKRIR	480
GFKGAQVKEY	SSKNMPIPIQ	KQIQNILKPA	EQVTYFTKLM	YLLTMFLDGK	EINDLLTTLI	540
NKFDNISSLL	KTMEQLELQT	TFKEDYTFFQ	QSSRLCKEIT	QLKSFARMGN	PISNLKEVMM	600
VDAIQILGTE	KSEQELQSMA	CFFFRDKNGK	KLNTGEHGM	NFIGNNVISN	TRFYLYIRYG	660
NPQLHLTSLQ	NETVVRVFLS	RIAKNQRVQG	MNGKNQIDRY	YETCGGTNSW	SVSEEEKINF	720
LCKILTNSMY	DQFQDVKQSG	AEITAEKRRK	KERYKAIISL	YLTVLYQLIK	NLVNINARIYI	780
IAFHCLERDA	ILYSSKFNTS	INLKKRYTAL	TEMILGYETD	EKARRKDTRT	VYEKAEAAKN	840
RHLKNVKNWC	KTRENLENAD	KNAIVAFRNI	VAHLWIIRDA	DRFITGMGAM	KRYFDCYHYL	900
LQRELGYILE	KSNQGSEYTK	KSLEKVQQYH	SYCKDFLHML	CLPFAYCIPR	YKNLSIAELF	960
DRHEPEAEFK	EEASSVNNSQ	FITT				984

(SEQ ID NO: 97).

[0242] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OHBM01000764.1 (human gut metagenome assembly):

XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	60
XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	120
XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	180
XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXHPLQKRYR	YLTSTNLKSF	240
ETYKNNLVNK	KKFDLDRVKK	IPQLAYFGSA	FYNTPEDTSA	KITKTKIKSN	EIIYYTFMLL	300
STARNFSAHY	LDRNRAKSSD	AEDFDGTSVI	MYNLDNEELY	KKLYNKKVHM	ALTGMKKVLD	360
ANFNKKVEHL	NNSFIKNSAK	DFVILCEVLG	IKSRDEKTKF	VKDYDFVVR	KNYKHLGFSV	420
KELRELLFAN	HDSNKYIKEF	DKISNKKFDS	VRSRLNRLAD	YIIYDYNNKN	NAKVSDLVKY	480
LRAAADDEQK	KKIYLNESIN	LVKSGILERI	KKILPKLNGK	IIGNMQPDST	ITASMLHNTG	540
KDWHPISENA	HYFTKWIYTL	TLFMDGKEIN	DLVTTLINKF	DNIASFIEVL	KSQSVCTHFS	600
EERKMFDISA	EICSELSAMN	SFARMEAPGA	SSKRAMFVEA	ARILGDNRSK	EELLEYFDTL	660
FDKSASKKEK	GFRNFIRNNV	VDSNRFKYLT	RYTDTSSVKA	FSNNKALVKF	AIKDIPQEIQI	720
LRYYNCSFGA	SERYNDGMS	DKLVEAIGKI	NLMQFNGVIQ	QADRNLPEE	KKKANAQKEK	780
YKSIIRLYLT	VCYLFFKNLV	YVNSRYYSAF	YNLEKDRSLF	EINGELKPTG	KFDEGHYTGL	840

VKLFI DNGWI	NPRASAYLTV	NLANSDETA I	RTFRNTAEHL	EALRNADKYL	NDLKQFDSYF	900
EIYHYITQRN	IKEKCEMLKE	QTVKYNNDDL	KYHGYSKDFV	KALCVFPGYN	LPRFKNLSID	960
ALFDKNDKRE	KLKKG FED					978

(SEQ ID NO: 98).

[0243] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OHCP01000044.1 (human gut metagenome assembly):

MAKKITAKQK	REEKERLNKQ	KWAKNDSVII	VPETKEEIKT	GEIQDNNRKR	SRQKSQAKAM	60
GLKAVLSFDN	KIAIASFVSS	KNAKSSHIER	ITDKEGTTIS	VNSKMFESSV	NKRDINIEKR	120
ITIEEPQQDG	TIKKEEGVK	STTCNPYFKV	GGKDYIGIKE	IAEEHFFGRA	FPNENLRVQI	180
AYNIFDVQKI	LGTFVNNIY	SFYNLRSDEV	QSDNDVIGML	YSISDYDRQK	ETETFLQAKS	240
LLKQTEAYYA	YFDDVFKNK	KPDKNKEGDN	SKQYQENLRH	NFNILRVLSF	LRQICMHAEV	300
HVSDDEGCTR	TQNYTDSLEA	LFNISKAFGK	KMPCLKTLID	NIYSGINAI	NDEFVKNGKN	360
NLYILSKVYP	NEKREVLRL	YYNFVCKEG	SNIGISTRKL	KETMIAQNMP	SLKEENTYRN	420
KLYTVMNFIL	VRELKNCATI	REQMIKELRA	NMDEEEGRDR	IYSKYAKEIY	LYVKDKLKL	480
LNVFKEEAEG	IIIPGKEDPV	KFSHGKLDKK	EIESFCLTK	NTEDITKVIY	FLCKFLDGKE	540
INELCCAMMN	KLDGISDLIE	TAKQCGEDVE	FVDQFKCLSK	CATMSNQIRI	VKNISRMKKE	600
MTIDNDTIFL	DALELLGRKI	EKYQDKNGD	YVKDEKGGKV	YTKDYNNFQD	MEFEGKNHRV	660
RNFVSNVVIK	SKWFSYVVRY	NKPAECQALM	RNSKLVKFAL	DELPDSQIEK	YYISVFGEKS	720
SSSNEEMRRE	LLKKLCDFSV	RGFLDEIVLL	SEDEMKQDK	FSEKEKKKSL	IRLYLTIVYL	780
ITKSMVKINT	RFSIACATYE	RDYILLCQSE	KAERAWEKGA	TAFALTRKFL	NHDKPTFEQY	840
YTREREISAM	PQEKRKELRK	ENDQLLKKTH	YSKHAYCYIV	DNVNNLTGAV	ANDNGRGLPC	900
LSEKNDNANL	FLEMRNKIVH	LNVDHDMVKY	INEIKNITSY	YAFFCYVLQR	MIIGNNSNEQ	960
NKFKAKYSKT	LQEFGTYSKD	LMWVLNLPFA	YNLPYKYNLS	NEQLFYDEEE	RMEKIVGRKN	1020
DSR						1023 (SEQ ID NO:

99).

[0244] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OGDF01008514.1| (human gut metagenome assembly):

MTETPKKRED	IAKTPAAKSR	SKAAGLKSTF	AVNGSVLLTS	FGRGNDVPE	KLITEKAVSE	60
INTVKPRFSV	EKPATSYSSS	FGIKSHISAT	ADNPLAGRAP	VGEDAIHAK	VLEQRVFGKT	120
FSDDNIHQIL	IYNILDIRKI	LSTYANNVVF	TINSMRRLDE	YDREQDYLG	LYTGNSYERL	180
LDIADKYAVD	GEDWRNTAAG	ISNDFEKKQF	QTINGFWDLL	DMIEPYMCYF	SEAFFCETTV	240
KDPDSGRIVP	CLEQRSDGDI	YNILRILSIV	RQTCMHNDAS	MRTVMFTLGQ	NSVRDRKNGF	300
DELAELLDYL	YDEKIDIVNR	DFLRNQKNNI	ELLSRIYGSS	ADSPERDLRV	QNFYDFRVLS	360
QDKNLGFSIK	KLREKLLDSP	ALSVVRSKKY	DTMRSKIYSL	IDFMIYRKFS	ENHVAVDDEV	420
EELRSLLTED	EKESAYS RWA	ETLINDGFAQ	EILVKLLPQT	DPAVIGKIKG	KKLLNDSIAG	480
IKLKKDASFF	TKIINVLCMF	QDGKEINELV	SSLVNKFANI	QSFVDVMRSQ	GIDSGFTADY	540
AMFAESGRIS	RELHILKGIA	RMQHSIAGLG	DKIYGSDDK	FHGVSRVYVT	DAAYILGFGE	600
RSEDNDGYVD	DYVSSKLLGG	ADKNLRNFIT	NNVIKNRRFL	YTVRYMNPKR	AKKLVQNDAL	660

VVLALSGIPE TQIDRYKSC IEKRSFNPDL NEKIAALSEM ITTLKIDDFE DVKQNPEKNA	720
NYEAKKNQRI SKERYKACIG LYLTVLYLIC KNLVKINARY SIAIGCLERD TQLHGVDFKG	780
AAYMTRDVFI AKGWINPKKP TVKSIKEQYA FLTPYIFTTY RNMIHLAAV TNAYKYIPQM	840
DRFKSWFHLV HTVIQHSILQ QYEYDRDYGR KGAPVVSERV LQLEQCREH SNYSRDLLHI	900
LNLPFGYNLP RYLNLSSEKY FDANAI	926

[0245] (SEQ ID NO: 100).

[0246] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OGPN01002610.1 (human gut metagenome assembly):

MAKKITAKQK REEKERLNKQ KWAKQDTPVV PKSKTEEEKPV AASDDKLLKT TQVKKVQTKS	60
KAKAMGLKTV LSFDDKIAIA SFVNDKKTCL PHIERITDKS GTTIHENARM FDSSVDEQNV	120
NIEKRMTIEE KQNDGTFKKD EKDVKATICN PYFKTCGKDY IGIKDVAEKY FFGKTFPNEN	180
LRVQIAYNVF DIQKILGTIV NNIIYSFYNL RRDGKSDVDI IGSLYAFADF DNQLKDKPAF	240
REAKDLLKNT EAYFSYFGDV FKSKKGGKD ENNEDYEKLN RHNFNVLRLV SFLRQICTHA	300
YVKCTGGAKN NGDSTKVEAE SLDALFNITE YFAKTAPELS KTINEIYKEG IDRINNDFVT	360
NGKNNLYILS KVYPDMQRNE LVKKYYQFVV CKEGNNVGIN TRKLKESIIS QHPWITTPQD	420
NNKANDYESC RHKLYTIMCF ILVAELDAHE SIRDNMVAEL RANMDGDDGR DAIYEKYAKD	480
IYHIVDKLL AMQKVFDEEL VPKVVEGKND PQQFTHGKLG KKEIESFCLS DKNTSDIAKV	540
VYFLCNFLDG KEINELCCAM MNKFDGIGDL IDTAKQCGEE VKFIEEFACL SNCRKITNDI	600
RVAKSISKMK NKVNIDNDII YLDAIELLGR KIEKYQKDEN GKILLGTDGK RLYTQEYKYF	660
NDMFFNAGNH KVRNFIANNV MQSKWFFYVV RYNKPAECQI IMRNKTLVKF TLDDLPMQI	720
QRYYSVFGD NNMPAVDEMR KRLDKINQF SVRGFLDELD EIVLMSDEES KRKSSEKEQ	780
KKSLIRLYLT IAYLITKSMV KINTRESIAC AMYERDYALL CQSEMKGGPW DGGALAVT	840
RKFLNHDREV FDRYCAREAE IARLPSEERK PLRKANDKLL KQTHYTNHSY TYIVNNLNSF	900
TDIDYCAKDV GLPAPNDKND NASILGEMRN DIAHLNIVHD MVKYIEELKD ISSYAFYCY	960
VLQRRLVGKD PNCQNKFKAK YAKELNDYGT YKNLMLWMLN LPFAYNLPRY KNLSSSEFLFY	1020
DMEYNKKDDE	1030

(SEQ ID NO: 101).

[0247] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): from contig emb|OBLI01020244 and emb|OBLI01038679 (from pig gut metagenome):

MAKKITAKQR REERERQNKQ KWAKQADAT AVFECEADIK PADSDEEDCT NIYIKREKKK	60
TQAKAMGLKT VLGFNDKIAI ASFMSSKDSK SSHIERITDP NGKTIREDDR MFDSNVDECS	120
INLEKRMTVE ERQKDGTIKK DEKDVKSTIC NPYSNECGKD YIGIKSVAEE LFFGRTPND	180
NLRVQIAYNI FDIQKILGTI INNIIYSFYN LSRDESQSDN DVIQGLYMLK DFDGQKETDT	240
FRQARALLER TEAYYSYFDN VFKKIDKNKK KSDDCKRERN EILRYNFNVL RVLFLRQIC	300
AHAQVKISNE HDREKGGGLV DSLDALFNIS RFFDAVAPEL NEVINSVYSK GIDDINDNFV	360
KNGKNNFYIL SKIYPEVARE DLLREYYYFV VSKEGNNIGI STKKLKEAII VQDMSYIKSE	420
DYDTYRNKLY TVLCFILVKE LNERTTIREQ MVADLRANMN GDIGREDIYS KYAKIIYAQV	480
KPRFDTMKSA FEEEAQDVIV PDKKKPVKFS HGKLDKNEIE RFCITSANTD SVAKIIYFLC	540
KFLDGKEINE LCCAMMNKLD GINDLIETAE QCGAKVEFVD KFSVLSNCET ISDQIRIVKS	600

ISKMKKEIAI	DNDTIFLDAL	ELLGRKIDKY	KKDATGKYLK	DENGKYLISK	EYDDFQYMEF	660
KDSHRVRNFI	SNSVIKSKWF	SYIVRYNQPS	ECRAIMKNKT	LVKFALDELP	DLQIQRYFVA	720
LYGDEDLPSY	GEMRKILLKK	LHDFSIGFL	DEIVLLSDLD	MESQDKYCEK	EQKKSFLRLY	780
LTIAYLITKS	MVKINTRFSI	ACATYERDYA	LLCASNKQER	AWSSGATALA	LTRRFLNQDK	840
LIFEKHYARE	GEISKLPKEE	RKAMRKVNDQ	LLKRTHFSKH	SYCYIVDNVN	RLTGGECDT	900
KRVLPVLENEK	NDNAGILLDF	RKTIAHLNVV	HKMVDYVDEI	KGITSYYAFF	CYVLQRMVLG	960
NNLNEKNAIK	EKYSATVKSF	GTYSKDFMWL	INLPFAYNLP	RYKNLSNEQL	FYDEEERNET	1020
EEQIDRL						1027

(SEQ ID NO: 102).

[0248] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig OIZX01000427.1:

MAKKKKTARQ	LREEMQQQRK	QAIQKQQEQR	QEKAARET	AAPEQPAAAP	VPKRQRKSLA	60
KAAGLKSNI	LDPQRRTTVM	TAFGQGSTAI	LEKQIVDRAI	SDLQPVQQFQ	VEPASAAYR	120
LKNSRVRFNP	VTADDPLYRR	KDGGFVPGMD	ALRRKNVLEQ	RFFGKSFADN	IHIQMIYSIL	180
DIHKILAAAS	GHIVHLLNIV	NGSKDRDFIG	MLAAHVLYNE	LNEEAKRSIA	DFCKSPRLIY	240
YSAAFYETLD	NGKSERRSNE	DIFNILALMT	CLRNFSHHS	IAIKVKDYSA	AGLYNLRLRG	300
PDMKKMLDTF	YTEAFIQLNQ	SFQDHNTNL	TCLFDILNIS	DSARQQLAE	EFYRYVVFKE	360
QKNLGFVSRK	LREEMLLLPD	AAVIADKRYD	TCSRKLNYLM	DFLILRVYRT	GRADRCCLKP	420
EALRAALTDE	EKAVVYHKEA	LSLWNEMRTL	ILDGLLPQMT	PENLSRLSGQ	KRKGELSDD	480
AMLKECLYEP	GPVPEDAAPE	EANAIFYCRM	IYLATLFMDG	KEINTLLTTL	ISKFENIAAF	540
LQTMELNIE	AELGPEYAMF	TRSRVAEQ	RVINSFALMK	KPQVNAKQQL	YRAAVTLTGT	600
EDPDGVTDEM	LCIDPVTGKM	LPPNQRRHGD	TGLRNFIANN	VVESRRFQYL	IRYSDPAQLH	660
QLASNNKLVR	FVLSSIPDTQ	INRYETCGQ	TRLAGRAAKV	EFLTDMIAAI	RFDQFRDVNQ	720
KERGANTQKE	RYKAMLGLYQ	TVLYLAVKNL	VNINARYVMA	FHCVERDMFL	YDGLTDPKG	780
ESVSAFLAVN	GKKGVPQYL	LLTQLFIRRD	YLKRSACEQI	QHNMENISDR	LLREYRNAVA	840
HLNVIAHLAD	YSADMREITS	YGLYHYLMQ	RHLFKRHAWQ	IRQPERPTEE	EQKLIEQEQK	900
QLAWEKALFD	KTLQYHSYNK	DLVKALNAPF	GYNLARYKNL	SIEPLFSKEA	APAAEIKATH	960
A						961

(SEQ ID NO: 103).

[0249] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig OCTW011587266.1:

MKQNDRENNN	KIKKSAKAV	GVKSLARLSD	GSTVVSSFSGK	GAAAELESIL	TGGEIRKLSD	60
KAILEITDDT	QKNAYNVKS	SRIPNLTART	DKLSDKSGMD	DLGFKRELEL	EVFGQCFDDS	120
IHIQIAHAVF	DIQKSLAAVI	PNVLYTLNNL	DRSYSTDNTS	DKKDIIGNTL	NYQHSYESFN	180
VEKRGEFTEY	YNAKDRFSY	FPDILCVLEK	VNGKDRYQPK	SEKDAFNVLS	SVNMLRNSLF	240
HFAPKSNQDG	ARIAVFNQF	DSDFSHITST	VNKIYSAKIA	GVNENFLNNE	GNNLYIILKA	300
TNWDIKKIVP	QLYRFVLSK	DKNMGFNMRK	LREFAVESKN	IDLSRLNDKF	LTNNRKKLYK	360
VIDFIIYYHL	NKVLKDSFVD	DFVAALRASQ	SEEEKEKLYA	QYSERLFADE	GLKSAIKKAV	420
DMISDTKSNI	FKMKTPLDKA	LIENIKVNSD	ASDFCKLIYV	FTRFLDGKEI	NILLNSLIKK	480
FQDIHSFNTT	VKKLSENNLI	INADYVDDYS	LFEQSGTVAR	ELMLIKSISK	MDFGLDNINL	540
SFMYDDALRT	LGVSDENLPE	VKREYFGKTK	NLSAYIRNNV	LENRRFKYVI	KYIHPSDVQK	600
IACNKAIAGF	VLNRMPDTQI	KRYYDSLINK	GATDIAQAK	ALLDCITGIS	FDAIKDDKHL	660

HKSKEKSPQR SADRERKKAM LTLYYTIVYI FVKQMLHINS LYTIGFFYLE RDQRFIYSRA 720
 KKENKNPSKN SYLNDFRSVT AYFIPSEIMK RIEKNENKGF LEDFEALWNS CGKTSRLRKE 780
 DVLLYARYIS PDHALKNYKM ILNSYRNKIA HINVIMSAGK YTGGIKRMDS YFSVFQHLVQ 840
 CDILSNPNK GKCFESESLK PLLLDMKFDG TDEKLYSKRL TRALNIPFGY NVPRYKNLTF 900
 EKIYLLKSSIN E 911 (SEQ ID NO: 104).

[0250] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OGNF01009141.1:

MADIDKKKSS AKAAGLKSTF VLENNKLLMT SFGNGNKAVI EKIIDEKVDS INEPEVFSVT 60
 PCDKKFELQP AKRGLAADSL VDNPLKSKKT AGDDAIHSRK FLERQFFDGN TFNDNIHIQL 120
 IYNILDIEKI LSVHVNDIVY SVNNILSRGE GMEYNDYIGT LNLKSFETYK NNLVNKKKFD 180
 LDRVKKIPQL AYFGSAFYNT PEDTSAKITK TKIKSNEEII YTFMLLSTAR NFSAHYLDRN 240
 RAKSSDAEDF DGTSMVIMYL DNEELYKKLY NKKVHMALTG MKKVLDANFN KKVEHLNNSF 300
 IKNSAKDFVI LCEVLGIKSR DEKTKFVKDY YDFVVRKNYK HLGFSVKELR ELLFANHDSN 360
 KYIKEFDKIS NKKFDSVRSR LNRLADYIIY DYNKNNAKV SDLVKYLRAA ADDEQKKKIY 420
 LNESINLVKS GILERIKKIL PKLNGKIIGN MQPDSTITAS MLHNTGKDWL PISENAHYFT 480
 KWIYTLTFLM DGKEINDLVT TLINKFDNIA SFIEVLKSQS VCTHFSEERK MFIDSAEICS 540
 ELSAMNSFAR MEAPGASSKR AMFVEAARIL GDNRSKEELE EYFDTLFDKS ASKKEKGFRN 600
 FIRNNVVDN RFKYLTRYTD TSSVKAFSNN KALVKFAIKD IPQEILRYY NSCFGASERY 660
 YNDGMSDKLV EAIGKINLMQ FNGVIQQADR NMLPEEKKKA NAQKEKYKSI IRLYLTVCYL 720
 FFKNLVYVNS RYSAFYNLE KDRSLFEING ELKPTGKFDE GHYTGVLVKLF IDNGWINPRA 780
 SAYLTVNLAN SDETAIRTFR NTAEHLEALR NADKYLNDLK QFDSYFEIYH YITQORNIKEK 840
 CEMLKEQTVK YNNDLLKYHG YSKDFVKALC VPFGYNLPRF KNLSIDALFD KNDKREKLKK 900
 GFED 904

(SEQ ID NO: 105).

[0251] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OIEN01002196.1:

MERQKRKMKS KSKMAGVKS FVIGDELLMT SFGDGDDAVL EKIDIDENG VV NDCRNPAAYD 60
 AVYGTDSIRV KKTNNNIRAK VNNPLAKSNI RSEESALFRT RVNEYKREQK DKYETLFFGK 120
 TFDDNIHIQL ISKILDIEKT FSVVIGNIVY AINNLSLEQS IDRPIDIFGD KNTQGISLRE 180
 DNDYLKTMLP RCEYLFHNIL NSDSNNNSKM NYNKNVKGKE EKDNRRNNENI EKLKKALEVI 240
 KIIRVDSFHG VDGIGKDQKF PRSKYNLAVN YNEEIQTIS EPPNRKVEEV QQDFYRNSCV 300
 NIDFLKEIMY GSNYTDRGSD SLECSYFNFA ILKQKNMGF SITSIRECLL DLYELNFESM 360
 QNLRPRANSF CDFLIYDYC KNESERANLV DCLRSAASEE EKKNIFYQTA ERVKEKFRNA 420
 ENRISRFDAS YIKNSREKNL SGGSSLPKYS FIEGFTKRSK KINDNDEKNA DLFCNMLYYL 480
 AQFLDGKEIN IFLTSIHNI FQNIDSFLKVM KEKGMECKEQ KDFKMFHSHAG HVAKKIEIVI 540
 SLAKMKKTL D FYNAQALKDA VTILGVSKKH QYLDMN SYLD FYMFDNRSGA TGKNAGKDHN 600
 LRNFLVSNVI RSRKFNYLSR YSNLAEVKKL AQNP SLVQFV LSRIEPLIC RYESSQGIS 660
 SEGITIDEQI KKLTGIIIVM NIDSFENINN GEIGMRYSKA TPQSIERRNQ MRVCVGLYLN 720
 VLYQIEKNLM NVNARYVLAF AFAERDALML NTFLECKKN KKRSSGGFSF IEMTQFFIDK 780
 KLFKVATEAI KKNVLKYNGN PESLNHIPGE YICKNMEGYH ENTVRNFRNM VAHLTAVARV 840

PLYISEVTQI DSYIALYHYC MQMNILQGIE QSGKILDNIK LKNALENARV HRTYSKDAVK 900
 YLCLPFAYNI SRYKALTIKD LFDWTEYSCK KDE 933

(SEQ ID NO: 106).

[0252] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Metagenomic hit (no protein accession): contig e-k87_11092736:

MKRQKTFAKR IGIKSTVAYG QGKYAITTFG KGSKAELIAVR SADPPEETLP TESDATLSIH 60
 AKFAKAGRDG REFKCGDVDE TRIHTSRSEY ESLISNPAES PREDYLGLKG TLERKFFGDE 120
 YPKDNLRIQI IYSILDIQKI LGLYVEDILH FVDGLQDEPE DLVGLGLGDE KMQKLLSKAL 180
 PYMGFFGSTD VFKVTKKREE RAAADEHNAK VFRALGAIRQ KLAHFKWKES LAIFGANANM 240
 PIRFFQGATG GRQLWNDVIA PLWKKRIERV RKSFLSNSAK NLWVLYQVFK DDTDEKKKAR 300
 ARQYYHFSVL KEGKNLGFNL TKTREYFLDK FFPIFHSSAP DVKRKVDTFR SKFYAILDFI 360
 IYEASVSAN SGQMKGKVPW KGAINNALVK LREAPDEEAK EKIYNVLAAS IRNDSLFLRL 420
 KSACDKFGAE QNRPFVFPNEL RNNRDIRNVR SEWLEATQDV DAAAFVQLIA FLCNFLEGKE 480
 INELVTALIK KFEGIQALID LLRNLEGVDS IRFENEALF NDDKGNMAGR IARQLRLLAS 540
 VGKMKPDMTD AKRVLYKSAL EILGAPPDEV SDEWLAENIL LDKSNNDYQK AKKTVNPFNR 600
 YIAKNVITSR SFYYLVRYAK PTAVRKLMSN PKIVRYVLKR LPEKQVASYY SAIWTQSESN 660
 SNEMVKLIEM IDRLTTEIAG FSFAVLKDKK DSIVSASRES RAVNLEVERL KKLTTLYMSI 720
 AYIAVKSIVK VNARYFIAYS ALERDLYFFN EKYGEEFRLH FIPYELNGKT CQFEYLAILK 780
 YYLARDEETL KRKCEICEEI KVGCEKHKKN ANPPYEYDQE WIDKKKALNS ERKACERRLH 840
 FSTHWAQYAT KRDNMAKHP QKWYDILASH YDELLALQAT GWLATQARND AEHLNPFVNEF 900
 DVYIEDLRRY PEGTPKNKDY HIGSYFEIYH YIRQRAYLEE VLAKRKEYRD SGSFTDEQLD 960
 KLQKILDDIR ARGSYDKNLL KLEYLPFAYN LPRYKNLTTE ALFDDDSVSG KKRVAEWRER 1020
 EKTREAEREQ RRQR 1034

(SEQ ID NO: 107).

[0253] An exemplary direct repeat sequence of CasRX/Cas13d Metagenomic hit (no protein accession): contig e-k87_11092736 (SEQ ID NO: 107) comprises or consists of the nucleic acid sequence:

CasRX/Cas13d Direct repeat 1: gtgagaagtc tccttatggg gagatgctac

30

(SEQ ID NO: 108).

[0254] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Ga0129306_1000735:

MQKQREQQTV TDESERKKKP LKSGAKAAGL KSVFVLSEK ELLTSFGRGN EAVPEKRVTG 60
 GTIANARTDN KEAFSAALQN KRFEVFGRTA GSSDDPLAVS RAPGQDLIGA KTALEERYFG 120
 RAFADNIHQ VIYAIQDINK ILAVHANNIV YTLNNDLREA DPETDDFIGS GYLTLKNTFE 180
 TYCDPAALNE REREKVTVSK QHFDAFMQNP RLAYYGNFAF RKLSKAERLA RGREIFDKES 240
 PERRQEILGS RGKNKSVDDE IRALAPEWVK REERDVYSEL VLMSELRQSC FHGQQKNSAR 300
 IFRLDNDLGP GVDGARELLD RLYAEKINDL RSFDKTSASS NFRLLFNAYH ADNEKKKELA 360
 QEFYRFVSLK VSKNTGFSIR TLREKIIEDH AAQYRDKIYD SMRKKLFSTF DFFLWRFYEE 420

REDEAEELRA	CLRAARSDEE	KEQIYAEAAA	SCWPSVKPFV	ESVAATLCDV	VKGRTKLNKL	480
KLSADESTLV	RNAIDGVRIS	PRASYFTKLI	YLMTLFLDGK	EINDLLTTLI	HAFENIDSFL	540
SVLGSERLER	TFDANYRIFA	DSGVIAQELR	AVNSFARMTT	EPFNSKLVMF	EDAAQLFGMS	600
GGLVEHAEEL	REYLDNKMLD	KTKLRLLPDG	KVDTGFRNFI	ISNVTESRRF	RYLVRYCEPR	660
AVRDYMSCRP	LIRLTLRDMP	DTILRRYYEQ	SVGAATVDRE	RILDTLADKL	LSLRFTDFEN	720
VNQRANAERN	REKQKMMGII	SLYLVNVAIQI	VKNLVYVNAR	YTMAYHCAER	DTELLLNAAAG	780
EGNLLRRDRS	WPARLHLPRR	ALARRRDRVE	VMERDVARGP	EAYNRDEWLG	LVRTLRRREKR	840
VCDNLHNNYA	YLCGADAEPG	DASLSLLFVY	RNKAHLSVL	NKGGRLSGDL	KEAKSWFYVY	900
HFLMQRVLEE	EFRTQALPE	RLRELLMAE	RYRGCSKDLI	KVLNLTFAYN	LPRYKNLSID	960
GRFDKNHPDP	SDE					973

(SEQ ID NO: 109).

[0255] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Ga0129317_1008067:

MKKQKKSIVK	AAGLKSAFVV	GDSVYLSFG	KGNAARLDTK	INPDNSTERY	VSDSEKHTLK	60
INSITDELR	LSGFPPKQAE	AKNPTHKKDN	EQKNTRQDML	GLKSTLEKFY	FGSTFDDNIH	120
IQIIHNIQDI	AKILAAHSNN	AGYALDNMLA	YQGVEFSDMI	GYMGTSRTFD	NYDPNHKNNK	180
DFFRFLKLPR	LGYFGSAFYS	QKGKDFEKR	DEEVYNICAL	MGQIRQCCFH	GKQEKYQLKW	240
LYNFHNFSN	KPFLDTLDKH	FDEMIDRINK	NFIKNNTPD	IILSGLYPDM	AKKELVRLFY	300
DFTTVKEYKN	MGFSVKKLRE	KMLESEEASD	FRDKDYDSVR	RKLYKLMDFC	IYYLYYSDSE	360
RNENLVSRRL	ESLTDENKDI	IYSKEAKIVW	NELRKKFSTI	LDNVKGSNIK	KLENVKEKFI	420
SEDEFDDIKL	DIDISYFSKL	MYVMCYFLDG	KEINDLLTTL	VSKFDNIGSI	IEAATQIGIN	480
IEFIDDFKFF	DRSKDISVEL	NIIRNFARMQ	APVPNAKRAM	QEDAIRILGG	SEEDIFSILD	540
DMTGYDKSGK	KLAQSKKGFR	NFIINNIVES	SREKYIVRYS	NPQKIRKLAN	NSVVVGFEVLG	600
KLPDAQIESY	FNSCLPNRVY	STPDKARESL	RDMLHNISFN	DFADVKQDDR	RATPEEKVEK	660
ERYKAIIGLY	LTVMYHLVKN	LVYVNSRYVM	AFHCLERDAM	HYDVSLDNYR	DLIRHLISEG	720
DSSCNHFISH	NRRMRDCIEE	NVKNSEQLIF	GKEDAVIRFR	NNVAHLSAIR	NANEYIGDIR	780
EITSYFALYH	YLMQRKLIDD	CKVNDTAHKY	FEQLTKYKTY	VMDMVKALCS	PFGYNLPREF	840
NLSIEGKFDM	HESK					854

(SEQ ID NO: 110).

[0256] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d Ga0224415_10048792:

MSKKENRKS	YVKGLGLKSTL	VSDSKVYLTT	FADGSNAKLE	KCVENNKIIC	ISNDKEAFAA	60
SIANKNVGYK	IKNDEKFRHP	KGYDIISNNP	LLHNNSVQQD	MLGLKNVLEK	RYFGKSSGGD	120
NNLCIQIHN	IIDIEKILSE	YIPNVVYAFN	NIAGFKDEHN	NIIDIIGTQT	YNSSYTYADF	180
SKDKSDKKYI	EFQKLLKNKR	LGYWGKAFFT	GQGNNAKVRQ	ENQCFHIAL	LISLRNWATH	240
SNELDKHTKR	TWLYKLDDTN	ILNAEYVKTL	NYLYDTIADE	LTKSFSKNGA	VNVNLYLAKKY	300
NIKDDLPGFS	EQYFRFSIMK	EQKNLGFNIS	KLRENMLDFK	DMSVIRDDHN	RYDKDRSKIY	360
TMMDFVIYRY	YIDNNNDSID	FINKLRSSID	EKSKEKLYNE	EANRLWNKLL	EYMLYIKEFN	420
GKLASRTPD	RDNISEFVES	LPKIHRLLPR	GQKISNFSKL	MYLLTMFLDG	KEINDLLTTL	480
INKFENIQGF	LDIMPEINVN	AKFEPEYVFF	NKSHEIAGEL	KLIKGAQMG	EPAATLKLEM	540
TADAIKILGT	EKEDAEIKL	AESLFKDENG	KLLGNKQHGM	RNFIGNNVIK	SKRFHYLIRY	600
GDPALHLKIA	TNKNVVRFVL	GRIADMQKKQ	GQKGKNQIDR	YYEVCVGNKD	IKKTIEEKID	660

ALTDIIIVNMN YDQFEKKKAV IENQNRGKTF EEKNKYKRDN AEREKFKKII SLYLTVIYHI 720
 LKNIVNVNSR YILGFHCLER DKQLYIEKYN KDKLDGFVAL TKFCLGDEER FEDLKAKAQA 780
 SIQALETANP KLYAKYMNYS DEEKKEEFKK QLNRRVKNR RNAYLKNIKN YIMIRLQLRD 840
 QTDSSGYLCG EFRDKVAHLE VARHAHEYIG NIKEVNSYFQ LYHYIMQCRL YDVLKNNTKA 900
 EAMVKGKAKE YFEALEKEGT YNDKLLKIAC VPFGYCIPRY KNLSMEELFD MNEKKFKKK 960
 APENT 965 (SEQ ID NO: 111).

[0257] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d 160582958 _gene49834:

MKNSVTFKLI QAQENKEAAR KKAKDIAEQ ARIAKRNGVVK KEENRINRIQ IEIQTQKKS 60
 TQNAYHLKSL AKAAGVKSVE AIGNDLLMTG FGPNGDATIE KRVFQNR AIE TLSSPEQYSA 120
 EFQNKQFKIK GNIKVLNHST QKMEEIQTEL QDNYNRPHFD LLGCKNVLEQ KYFGRTFSDN 180
 IHVQIAYNIM DIEKLLTPYI NNIIYTLNEL MRDNSKDDFF GCDSHFSVAY LYDELKAGYS 240
 DRLKTKPNLS KNIDRIWNMF CNYMNSDSGN TEARLAYFGE LFYKPKETGD AKSDYKTHLS 300
 NNQKEEWELK SDKEVYNIFA ILCDLRHFT HGESITPSGK PFPYNLEKNL FPEAKQVLNS 360
 LFEEKAESLG AEAFGKTAGK TDVSILKVF EKEQASQKEQ QALLKEYYDF KVQKTYKNMG 420
 FSIKKLREAI MEIPDAKFK DDLYSSLRHK LYGLDFDILV KHFLDTSDE NLQNNDFRQ 480
 LRACRCEEEK DQVRSIAVK VWEKVKKEL NMFQVQVVP SLSKDELKQM EMTKNTELLS 540
 SIETISTQAS LFSEMIFMMT YLLDGKEINL LCTSLIEKFE NIASFNEVLK SPQIGYETKY 600
 TEGYAFFKNA DKTAKELRQV NNMARMTKPL GGVNTKCMY NEAAKILGAK PMSKAELESV 660
 FNLDNHDTY SPSGKKIPNK NFRNFIINV ITSRRFLYLI RYGNPEKIRK IAINPSIISF 720
 VLKQIPDEQI KRYPPCIGK RTDDVTLMRD ELGKMLQSVN FEQFSRVNNK QNAKQNPNGE 780
 KARLQACVRL YLTPPYLFIK NMVNINARV LAFHCLERDH ALCFNSRKLN DDSYNEMANK 840
 FQMRKAKKE QYEKEYCKK QETGTAHTKK IEKLNQAIY IDKDIKNMHS YTCRNRYRLV 900
 AHLNVVSKLQ NYVSELPNDY QITSYFSFYH YCMLGLMEK VSSKNIPLVE SLKNEANDAQ 960
 SYSAKTLEY FDLIEKNRTY CKDFLKALNA PFSYNLPRFK NLSIEALFDK NIVYEQADLK 1020
 KE 1022

(SEQ ID NO: 112).

[0258] An exemplary direct repeat sequence of CasRX/Cas13d proteins may comprise or consist of the sequence

[0259] CasRX/Cas13d 160582958 _gene49834 (SEQ ID NO: 112) comprises or consists of the nucleic acid sequence:

CasRX/Cas13d DR:

gaactacacc cctctgttct tgtaggggtc taacac 36

(SEQ ID NO: 113).

[0260] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d 250twins_35838_GL0110300:

MGNKQRVSAQ KRRENAKLCN QQKARQAESQ RDKIKNMNVE KMKNTNTNDI KHTKTTAKKL 60

GLKSTIIADK	KIILTSFINE	QSSKTANIEK	VAGFKGDTID	TISYTFRMFR	SEINPGEIVI	120
SKGDDLSEFA	NPANFPIGRD	YVKIRSALEK	QYFGKEFPED	NLHVQIAYNV	ADIKKILSVY	180
INNIIYMFYN	LARSEEYDIF	YNSQSENSGR	DCDVIGSLY	QASYRNQDAN	RFEKDGKKKA	240
IDSLLDDTRA	YYTYFDGLFS	VPKREDDGKI	KESEKEKAKD	QNFVDLRLLS	VGRQLTFHSD	300
KSNNEAYLFD	LSKLTRAAQD	ENRRQDIQSL	LNILNSTCRS	NLEGVNGDFV	KHAKNNLYVL	360
NQLYPSLKAN	DLIGEYYNFI	VKKENRNIGI	RLITVRELII	EHNYTNLKDS	KYDTYRNKIY	420
TVLNFILFRE	IQENSIAIKN	FREKLIRSTEK	AEQPALYQAF	ANKIYPMVQA	KFAKAIDLFE	480
EQYKTKFKSE	FKGGISIENM	QQQNILLQTE	NIDYFSKYVL	FLTKFLDGKE	INELLCALIN	540
KFDNIADLLD	ISKQIGTPVV	FCADYESLND	AAKIAENIRL	IKNIAHLRPA	IQEAQSSKDN	600
ADAAGTPATL	LIDAYNMLNT	DIQLVYGEAA	YEELRKDLFE	RKNGTKYNKK	GKKVDVYDHK	660
ERNFLINNVI	KSKWFFYIAK	YVKPADCAKM	MSNKKMIEFA	LRDLPETQIK	RYYYYTITGNE	720
ALGDAESLKG	VIIEQLHAFS	IKNTLLSIKN	MGEGEYKIQQ	IGSSKEKLKA	IVNLYLTVAY	780
LLTKSLVKVN	IRFSIAFGCL	ERDLVLQKKS	EKKFDAIINE	ILLEDDKIRK	ECDKERAQAK	840
TLPRELAQER	FAQIKRRESG	CYFKSYHVDY	YLSKNSNEFK	QNHIDFAVTS	YRNNVEHLNV	900
VHCKTKYFSE	VKDVKSYYGV	YCYIMQRMCL	DELIKNQDK	PDVRQTFEY	NRLKDHGTY	960
SKNLMWLLNF	PFAYNLARYK	NLSNEDLFNA	KNNDQKSK			998

(SEQ ID NO: 114).

[0261] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence:

CasRX/Cas13d 250twins_36050_GL0158985:

MKKKHQSAAE	KRQVKKLKNQ	EKAQKYASEP	SPLQSDTAGV	ECSQKKT VVS	HIASSKTLAK	60
AMGLKSTLVM	GDKLVITSFA	ASKAVGGAGY	KSANIEKITD	LQGRVIEEHE	RMFSADVGEK	120
NIELSKNDCH	TNVNPNVVTN	IGKDYIGLKS	RLEQEFFGKT	FENDNLHVQL	AYNILDIKKI	180
LGTYVNNIIY	IFYNLNRAFT	GRDERMYDDL	IGTLYAYKPM	EAQQTYLLKG	DKDMRRFEV	240
KQLLQNTSAY	YVYGTLFKEK	VKAKSKKEQR	AKAEIDACT	AHNYDVLRL	SLMRQLCMHS	300
VAGTAFKLAE	SALENIEDVL	SADLKEILDE	AFSGAVNKL	DGFVQHSNN	LYVLQQLYPN	360
ETIERIAEKY	YRLTVRKEDL	NMGVNIKKLR	ELIVGQYFPE	VLDKEYDLSK	NGDSVVTYRS	420
KIYTMNYIL	LYLEDHDS	RESMVEALRQ	NREGDEGKEE	IYRQFAKKVW	NGVSGLFGVC	480
LNLFKTEKRN	KFRSKVALPD	VSGAAYMLSS	ENIDYFVKML	FFVCKFLDGK	EINELLCALI	540
NKFDNIADIL	DAAAQCGSSV	WFVDSYRFFE	RSRRISAQIR	IVKNIASKDF	KSKKDSDES	600
YPEQLYLDAL	ALLGDVISKY	KQNRDGSVVI	DDQGNVLT	QYKRFRYEFF	EEIKRDESGG	660
IKYKSGKPE	YNHQRRNFIL	NNVLKSKWFF	YVVKYNRPSS	CRELMKNKEI	LRFVLRDIPD	720
SQVRRYFKAV	QGEAYASAE	AMRTRLVDAL	SQFSVTACLD	EVGGMTDKEF	ASQRAVDSKE	780
KLRAIIRLYL	TVAYLITKSM	VKVNTREFSIA	FSVLDERDYYL	LIDGKKKSSD	YTGEDMLALT	840
RKFVGEDAGL	YREWKEKNAE	AKDKYFDKAE	RKKVLRQNDK	MIRKMHFTPH	SLNYVQKNLE	900
SVQSNGLAAV	IKEYRNAVAH	LNIINRLDEY	IGSARADSY	SLYCYCLQMY	LSKNFSVGYL	960
INVQQLLEH	HTYMKDLMWL	LNIPFAYNLA	RYKNLSNEKL	FYDEEAAAEK	ADKAENERGE	1020

(SEQ ID NO: 115).

[0262] Yan et al. (2018) *Mol Cell*. 70(2):327-339 (doi: 10.1016/j.molcel.2018.02.2018) and Konermann et al. (2018) *Cell* 173(3):665-676 (doi: 10.1016/j.cell/2018.02.033) have described CasRX/Cas13d proteins and both of which are incorporated by reference herein in their

entireties. Also see WO Publication Nos. WO2018/183703 (CasM) and WO2019/006471 (Cas13d), which are incorporated herein by reference in their entirety.

[0263] Exemplary wild type Cas13d proteins of the disclosure may comprise or consist of the amino acid sequence:

Cas13d (*Ruminococcus flavefaciens* XPD3002) sequence:

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1 IEKKKSFAKG MGVKSTLVSG SKVYMTTFAE GSDARLEKIV EGDSIRSVNE GEAFSAEMAD
61 KNAGYKIGNA KFSHPKGYAV VANNPLYTGP VQQDMLGLKE TLEKRYFGES ADGNDNICIQ
121 VIHNIIDIEK ILAEYITNAA YAVNNISGLD KDIIGFGKFS TVYTYDEFKD PEHHRAAFNN
181 NDKLINAIKA QYDEFDNFLD NPRLGYFGQA FFSKEGRNYI INYGNECYDI LALLSGLAHW
241 VVANNEEESR ISRTWLYNLD KNLDNEYIST LNYLYDRITN ELTNSFSKNS AANVNYIAET
301 LGINPAEFAE QYFRFSIMKE QKNLGFNITK LREVMLEDRKD MSEIRKNHKV FDSIRTKVYT
361 MMDFVIYRY IEDAKVAAA NKSLPDNEKS LSEKDIFVIN LRGSFNDDQK DALYYDEANR
421 IWRKLENIMH NIKEFRGNKT REYKKKDAPR LPRILPAGRD VSAFSKLMYA LTMFLDGKEI
481 NDLLTTLINK FDNIQSFLKV MPLIGVNAKF VEEYAFFKDS AKIADELRLI KSFARMGEPI
541 ADARRAMYID AIRILGTNLS YDELKALADT FSLDENGKSL KKGKHGMRNF IINNVISNKR
601 FHYLIRYGDP AHLHEIAKNE AVVKFVLGRI ADIQKKQGQN GKNQIDRYE TCIGKDKGKS
661 VSEKVDALTK IITGMNYDQF DKKRSVIEDT GRENAEREKF KKIISLYLTV IYHILKNIVN
721 INARYVIGFH CVERDAQLYK EKGYNINLKK LEEKGFSSVT KLCAGIDETA PDKRKDEVEK
781 MAERAKESID SLESANPKLY ANYIKYSDEK KAEETFQIN REKAKTALNA YLRNTKWNVI
841 IREDLLRIDN KTCTLFANKA VALEVARYVH AYINDIAEVN SYFQLYHYIM QRIIMNERYE
901 KSSGKVSEYF DAVNDEKKYN DRLLKLLCVP FGYCIPRFKN LSIEALFDRN EAAKFDKEKK
961 KVSNGS (SEQ ID NO: 45).
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[0264] Exemplary wild type Cas13d proteins of the disclosure may comprise or consist of the amino acid sequence:

[0265] Cas13d (contig e-k87_11092736):

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MKRQKTFAKRIGIKSTVAYGQGYAITTFGKGSKAEIAVRSADPPEETLPTESDATLSIHAKFA
KAGRDGREFKCGDVDETRIHTSRSEYESLISNPAESPREDYLGLKGTLEKFFGDEYPKDNLRI
QIIYSILDIQKILGLYVEDILHFVDGLQDEPEDLVGLGLGDEKMQKLLSKALPYMGFFGSTDVF
KVTKKREERAAADEHNAKVFRALGAIRQKLAHFVKESLAI FGANANMPIRFFQGATGGRQLWN
DVIAPLWKKRIERVRKSFLSNSAKNLWVLYQVFKDDTDEKKKARARQYYHFSVLKEGKNLGFNL
TKTREYFLDKFFPIFHSSAPDVKRKVDTFRSKFYAILDFIIYEASVSVANSQGQMGKVAPWKGAI
DNALVKLREAPDEEAKEKIYNVLAASIRNDSLFLRLKSACDKFGAEQNRPVFPNELRNNRDIRN
VRSEWLEATQDVDAAAFVQLIAFLCNFLEGKEINELVTALIKKFEGIQALIDLLRNLEGVDSIR
FENEFALFNDDKGNMAGRIARQLRLLASVGKMKPDMTDAKRVLYKSALEILGAPPDEVSEWLA
ENILLDKSNNDYQKAKKTVNPFERNYIAKNVITSRSFYLVRYAKPTAVRKLMSNPKIVRYVLKR
LPEKQVASYYSAIWTQSESNSNEMVKLIEMIDRLTTEIAGFSFAVLKDKKDSIVSASRESRAVN
LEVERLKKLTTLYMSIAYIAVKSLVKVNARYFIAYSALERDLYFFNEKYGEEFRLHFIPYELNG
KTCQFEYLAAILKYYLARDEETLKRKCEICEEIKVGCEKHKKNANPPYEYDQEWIDKKKALNSER
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KACERRLHFSTHWAQYATKRDENMAKHPQKWYDILASHYDELLALQATGWLATQARNDAEHLNP
VNEFDVYIEDLRRYPEGTPKNKDYHIGSYFEIYHYIRQRAYLEEVLA KRKEYRDSGSFTDEQLD
KLQKILDDIRARGSYDKNLLKLEYLPFAYNLPYKNTTTEALFDDDSVSGKKRVAEWREREREKTR
EAEREQRRQR (SEQ ID NO: 46) .

[0266] An exemplary direct repeat sequence of Cas13d (contig e-k87_11092736) (SEQ ID NO: 46) comprises or consists of the nucleic acid sequence:

[0267] Cas13d (contig e-k87_11092736) Direct Repeat Sequence):

GTGAGAAGTCTCCTTATGGGGAGATGCTAC (SEQ ID NO: 47) .

[0268] Exemplary wild type Cas13d proteins of the disclosure may comprise or consist of the amino acid sequence:

[0269] Cas13d (160582958_gene49834):

MKNSVTFKLIQAQENKEAARKKAKDIAEQARIAKRNGVVKKEENRINRIQIEIQTQKKSNTQNA
YHLKSLAKAAGVKS VFAIGNDLLMTGFGPGNDATIEKRVFQNRAIETLSSPEQYSAEFQNKQFK
IKGNIKVLNHSTQKMEEIQTELQDNYNRPHFDLLGCKNVLEQKYFGRTFSDNIHVQIAYNIMDI
EKLLTPYINNIIYTLNELMRDNSKDDFFGCDSHFSVAYLYDELKAGYSDRLKTKPNLSKNIDRI
WNNFCNYMNSDSGNTEARLAYFGELFYKPKETGDAKSDYKTHLSNNQKEEWELKSDKEVYNI FA
ILCDLRHFCTHGESITPSGKPFYPNLEKNLFPEAKQVLNSLFEEKAESLGAEAFGKTAGKTDVS
ILLKVFEKEQASQKEQQALLKEYYDFKVQKTYKNMGFSIKKLREAIM EIPDAAKFKDDLYSSLR
HKLYGLFDFILVKHFLDTSSEN LQNNDI FRQLRACRCEEEKDQVYRSIAVKVWEKVKKELNM
FKQVVVIPSLSKDELKQMEMTKNTELLSSIETISTQASLFSEMI FMMTYLLDGKEINLLCTSLI
EKFENIASFNEVLKSPQIGYETKYTEGYAFFKNADKTAKELRQVNNMARM TKPLGGVNTKCVMY
NEAAKILGAKPMSKAELESVFNLDNHDYTYSPSGKKIPNKNFRNFI INNVITSRRFLYLIRYGN
PEKIRKIAINPSIIISFVLKQIPDEQIKRYPPCIGKRTDDVTLMRDELGKMLQSVNFEQFSRVN
NKQNAKQNPNGEKARLQACVRLYLTVPYLFIKNMVNINARYVLAFHCLERDHALCFNSRKLND
SYNEMANKFQMRKAKKEQYEKEYCKKQETGTAHTKKIEKLNQQIAYIDKDIKNMHSYTCRNY
RNLVAHLNVVSKLQNYVSELPNDYQITSYFSFYHYCMQLGLMEKVSSKNIPLVESLKNEANDAQ
SYSAKKTLEYFDLIEKNRTYCKDFL KALNAPFSYNLPRFKNLSIEALFDKNIVYEQADLKKE
(SEQ ID NO: 48) .

[0270] An exemplary direct repeat sequence of Cas13d (160582958_gene49834) (SEQ ID NO: 48) comprises or consists of the nucleic acid sequence:

[0271] Cas13d (160582958_gene49834) Direct Repeat Sequence:

GAACTACACCCCTCTGTTCTTGTAGGGGTCTAACAC (SEQ ID NO: 49) .

[0272] Exemplary wild type Cas13d proteins of the disclosure may comprise or consist of the amino acid sequence:

[0273] Cas13d (contig tpg|DJXD01000002.1| ; uncultivated *Ruminococcus* assembly, UBA7013, from sheep gut metagenome):

MKKQKSKKTVSKTSGLKEALSVQGTVMITSFGKGNMANLSYKIPSSQKPQNLNSSAGLKNVEVS
GKKIKFQGRHPKIAATTDNPLFKPQPGMDLLCLKDKLEMHYFGKTFDDNIHIQLIYQILDIEKIL
AVHVNNIVFTLDNVLHPQKEELTEDFIGAGGWRINLDYQTLRGQTNKYDRFKNYIKRKELLYFG
EAFYHENERRYEDIFAILTLLSALRQFCFHSDLSSDES DHVNSFWLYQLEDQLSDEFKETLSI
LWEEVTERIDSEFLKTNTVNLHILCHVFPKESKETIVRAYYEFLIKKSFKNMGFSIKKLREIML
EQSDLKSFKEDKYNVRAKLYKLDFDIITYYYDHHA FEKEALVSSLRSSLTEENKEEIYIKTAR
TLASALGADFKKAAADVNAKNIRDYQKKANDYRISFEDIKIGNTGIGYFSELIYMLTLLLDGKE
INDLLTTLINKFDNIISFIDILKKLNLEFKFKPEYADFFNMTNCRYTLEELRVINSIARMQKPS
ADARKIMYRDALRILGMDNRPDEEIDRELERTMPVGADGKFIKKGQGRNFIFASNVIESSRFHY
LVRYNNPHKTRTLVKNPNVVKFVLEGIPETQIKRYFDVCKGQEIPPTSDKSAQIDVLARIISSV
DYKIFEDVPQSAKINKDDPSRNFSDALKKQRYQAIVSLYLTVMYLITKNLVYVNSRYVIAFHCL
ERDAFLHGVTLPKMNKKIVYSQLTTHLLTDKNYTTYGHLKNQKGHRKQWYVLVKNNLQNSDITAV
SSFRNIVAHISVVRNSNEYISGIGELHSYFELYHYLVQSMIAKNNWYDTSHQPKTAEYLNNLKK
HHTYCKDFVKAYCIPFGYVVPYKNLTINELFDRNNPNPEPKKEEV (SEQ ID NO: 50) .

[0274] An exemplary direct repeat sequence of Cas13d (contig tpg|DJXD01000002.1| ; uncultivated *Ruminococcus* assembly, UBA7013, from sheep gut metagenome) (SEQ ID NO: 50) comprises or consists of the nucleic acid sequence:

[0275] Cas13d (contig tpg|DJXD01000002.1| ; uncultivated *Ruminococcus* assembly, UBA7013, from sheep gut metagenome): CAACTACAACCCCGTAAAAATACGGGGTTCTGAAAC (SEQ ID NO: 51) .

[0276]

[0277] In some embodiments of the disclosure, a CjeCas9-endonuclease fusions and gRNA molecule may comprise or consist of the nucleic acid sequence of:

E43-CjeCas9 and sgRNA plasmid (U6: N's=sgRNA spacer, E43, CjeCas9)

gtttattacagggacagcagagatccagtttggttaattaaggtaccgagggcctatttcccatgattccttcattttgcatatacgatacaagg
ctgttagagagataattagaattaatttgactgtaaacacaaagatatttagtacaaaatacgtgacgtagaaagtaataatttctgggtagttg
cagttttaaattatgttttaaattggactatcatatgcttaccgtaacttgaaagtatttcgatttctggccttatatatcttGTGGAAAGG
ACGAAACACCNNNNNNNNNNNNNNNNNNNNGTTTTAGTCCCTGAAGGGACTAAAAT
AAAGAGTTTGCGGGACTCTGCGGGGTACAATCCCCTAAAACCGCTTTTTTTCCTGC
AGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAGCTT
TTGTTCCCTTTAGTGAGGGTTAATTGCGCGAATTTCGCTAGCTAGGTCTTGAAAGGAG
TGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCC
GAGAAGTTGGGGGGAGGGGTGCGCAATTGATCCGGTGCCTAGAGAAGGTGGCGCG
GGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGG
GAGAACCGTATATAAGTGCAAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGGGTTTG
CCGCCAGAACACAGGACCGGTTCTAGAGCGCTATTTAGAACCatgTGTTCTCCCCAA
GAATCTGGCATGACCGCTCTTTCAGCGAGGATGTTGACGCGAAGCAGATCCCT
GGGACCTGGGGCCGGGGCCACGAGGGTGTGCGGAAGAACCAGGACCGTTGCGA
CGGAGGGAAGCAGCAGCGGAAGCTCGGAAATCCCATTCTCCGGTTAAACGACC
CCGCAAGGCACAACGGCTCAGGGTTGCTTACGAGGGGAGCGATTCCGAAAAGG
GTGAAGGAGCAGAGCCCTTGAAGGTTCCAGTATGGGAACCCCAGGATTGGCAG
CAGCAGCTTGTAACATCCGAGCAATGAGGAACAAAAAAGATGCACCTGTTGA
TCACCTCGGAACCGAACATTGTTATGATTCTAGTGCGCCGCCAAAAGTCCGCC
GGTATCAGGTTCTGTTGAGTTTGATGCTGAGTAGTCAGACTAAGGACCAGGTT
ACGGCCGGAGCAATGCAACGGCTTCGGGCACGGGGACTCACGGTCGATAGCAT
TTTGACAGACCGATGACGCAACATTGGGTAACTCATATATCCAGTTGGCTTCTG
GCGGAGCAAAGTGAAGTACATCAAGCAGACCTCAGCCATTCTCCAACAACATT
ACGGAGGTGATATACCCGCAAGCGTAGCTGAACTGGTAGCACTGCCGGGCGTC
GGTCCCAAAATGGCACATCTGGCTATGGCGGTTGCTTGGGGAACGGTGTCTGG
TATCGCAGTTGATACGCATGTCCACCGCATCGCCAATCGGCTGAGGTGGACTA
AAAAAGCCACTAAGTCTCCTGAAGAAACACGGGCTGCTCTGGAAGAGTGGCTT
CCACGAGAGCTGTGGCATGAAATCAATGGATTGCTGGTTGGTTTCGGGCAGCA
GACATGCTTGCCCGTGCACCCCCGGTGTCTATGCTTGCTTGAACCAGGCTTTGT
GCCCAGCTGCCCAGGGCCTGAGTGGAAGTGAGACACCGGGAACATCTGAGTCTGC
GACCCCGGAGAGCacaacGCGCGAATCCTGGCCTTCGcgATTGGCATTAGCAGCAT
CGGCTGGGCATTCTCTGAAAACGACGAACTGAAGGATTGCGGCGTGCGAATTT
TCACTAAGGTCGAAAATCCCCAAAACCTGGTGAATCACTCGCTCTCCCTAGACGAC
TGGCACGCTCCGCACGAAAGAGGGCTTGCCCGCCGCAAGGCACGCTTGAACCAT
CTTAAACACCTTATTGCAAATGAGTTTAAACTGAATTATGAGGACTACCAATCC
TTTGACGAGTCTCTTGCTAAAGCCTACAAAGGGAGCCTTATATCCCCGTATGAG
CTCCGGTTCAGAGCACTCAACGAACTGCTGTCCAACAGGATTTTGCTCGCGT
GATTCTCCACATAGCGAAGAGGCGAGGATACGATGACATTAACAAACAGTGATG
ATAAGGAAAAAGGGGCCATACTCAAAGCGATTAAAGCAAAATGAAGAGAAGCTC
GCTAACTATCAATCAGTAGGGGAGTATCTCTATAAAGAGTACTTCCAGAAGTTC
AAAGAAAATAGCAAGGAATTTACTAATGTCCGGAATAAAAAGGAGTCTTACGA
AAGATGTATTGCGCAATCTTTCCTCAAGGACGAGCTCAAATTGATTTTCAAGAA
ACAAAGGGAATTTGGGTTTCACTTCTCAAAAAAATTTGAGGAAGAGGTTCTGA
GCGTTGCCTTTTACAAACGCGCCCTTAAGGACTTCTCACATCTCGTAGGGAATT
GTAGTTTCTTACCGATGAAAAACGGGCGCCAAAAAATAGCCCTTTGGCTTTTA
TGTTTGTGCTCTGACTCGCATCATTAATCTGCTCAACAACCTTAAAAACACGG

AAGGGATTCTGTACACAAAGGATGATCTGAACGCTCTGCTTAACGAAGTTTTGA
AGAACGGGACTTTGACCTACAAACAAACCAAAAAGCTTCTTGGTCTCAGTGATG
ACTACGAATTCAAGGGAGAAAAAGGGACATATTTTCATCGAATTCAAGAAGTATA
AGGAGTTCATCAAAGCCTTGGGCGAGCACAACTTGTCTCAAGATGATCTCAAC
GAAATTGCTAAGGATATCACTCTGATTAAAGACGAGATCAAGCTCAAAAAGGC
GTTGGCGAAGTATGACCTTAACCAAAAACCAATAGATAGCCTCAGCAAGTTGG
AATTTAAAGATCACTTGAATATAAGTTTCAAGGCCCTTAAGTTGGTCACCCCCT
TGATGCTTGAAGGAAAGAAATATGATGAGGCATGTAATGAGCTGAATCTCAAG
GTTGCTATTAACGAAGACAAAAAAGATTTTCTCCAGCTTTCAATGAGACTTAC
TATAAGGACGAGGTTACCAATCCTGTGGTGCTCCGAGCCATCAAAGAGTATCG
AAAGGTCCTGAATGCTTTGCTCAAAAAATACGGTAAGGTACACAAAAATAAATAT
TGAGCTCGCAAGGGAGGTCGGTAAGAACCACTCCCAGCGCGCCAAAAATAGAAA
AGGAACAGAATGAAAATTACAAAGCGAAAAAGGACGCCGAGCTCGAGTGCGAA
AAGCTGGGCCTGAAAATAAACAGCAAGAACATTCTCAAACCTCCGCCTCTTCAA
GAACAAAAAGAATTTTGTGCTTATAGTGGTGAGAAAAATAAAAAATCTCCGATCTT
CAAGACGAGAAGATGCTCGAAATAGACgcgATATATCCATATAGCAGGTCTTTTG
ACGATTCTTACATGAATAAAGTGCTTGTTTTCACTAAGCAGAATCAGGAAAAGT
TGAATCAGACCCCCCTTTGAGGCCTTTGGCAACGACTCAGCAAAGTGGCAGAAG
ATCGAGGTCTTGGCTAAGAATCTTCCTACTAAGAAACAGAAAAGGATATTGGAT
AAGAACTATAAAGACAAAGAACAAAGAAGAACTTTAAAGACCGCAACCTCAATGA
CACCAGATACATAGCAAGATTGGTTCTGAACTACACAAAAGATTATTTGGACTT
CTTGCCGCTGTCTGATGATGAGAACACGAACTCAACGACACGCAAAAGGGGT
CTAAAGTCCACGTCGAAGCTAAATCTGGGATGCTCACCTCAGCATTGAGGCAT
ACGTGGGGATTCTCAGCAAAGGACCGAAACAATCACCTGCACCATGCCATTGA
CGCAGTTATCATAGCGTATGCCAATAATTCAATAGTAAAAGCGTTTAGCGACTT
CAAGAAGGAACAAGAGTCCAACAGCGCCGAGCTCTACGCAAAAAAGATTAGTG
AACTCGACTACAAAAACAAAAGAAAATTCTTTGAGCCGTTACAGCGGATTTTCGAC
AGAAGGTATTGGATAAAATAGATGAAATTTTCGTGAGCAAACCCGAAAGGAAA
AAGCCCTCAGGCGCCTTGCACGAAGAGACTTTCAGGAAGGAAGAGGAATTCTA
CCAAAGCTACGGCGGAAAGAGGGAGTTTTGAAGGCTCTCGAACTTGGAAGA
TTAGGAAGGTGAACGGCAAGATAGTGAAAAACGGCGATATGTTCCGGGTTGAT
ATCTTCAAACATAAAAAAACGAATAAATTTTATGCTGTGCCTATATACACTATG
GACTTCGCACTTAAGGTCCTGCCGAATAAGGCGGTAGCCCGATCTAAAAAAGG
CGAAATTAAGGACTGGATTTTGATGGATGAAAATTACGAGTTCTGCTTTTCTCT
CTACAAGGATTCCCTTATATTGATACAGACGAAAGATATGCAGGAACCGGAATT
CGTGTATTACAACGCTTTTACTTCCTCTACGGTATCTTTGATTGTCTCCAAACAT
GACAACAAATTCGAAACACTCAGTAAAAACCAAAAGATTCTCTTTAAAAATGCG
AACGAGAAAGAAGTAATTGCAAAATCAATTGGCATCCAAAATTTGAAAGTTTTT
GAAAAATATATAGTATCTGCCCTCGGAGAGGTTACTAAAGCGGAATTTAGACA
GCGAGAGGACTTCAAAAATCAGGTCCACCCAAGAAAAAACGCAAGGTGGAAGA
TCCGAAGAAAAAGCGAAAAGTGGATGTGtaaCGTTTTCCGGGACGCCGGCTGGATGA
TCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCACCCCAACTTGTTTATTGC
AGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCAT
TTTTTTCCTGCAATTCTAGTTGTGGTTTGTCCAACTCATCAATGTATCTTATCATGTC
TGTATACCG (SEQ ID NO: 202).

[0278] In some embodiments of the disclosure, a CjeCas9-endonuclease fusions and gRNA molecule may comprise or consist of the nucleic acid sequence of:

E67-CjeCas9 and sgRNA plasmid (U6: N's=sgRNA spacer, E67, CjeCas9)

gtttattacagggacagcagagatccagtttggttaattaaggtaccgagggcctatttcccatgattcctcatatttgcataacgatacaagg
ctgttagagagataattagaattaatttgactgtaaacacaaagatattagtacaaaatacgtgacgtagaaagtaataatttctgggtagtttg
cagttttaaattatgttttaaattggactatcatatgcttaccgtaactgaaagtatttcgatttctggccttatatatcttGTGGAAAGG
ACGAAACACCNNNNNNNNNNNNNNNNNNNNGTTTTAGTCCCTGAAGGGACTAAAAT
AAAGAGTTTGCGGGACTCTGCGGGGTACAATCCCCTAAAACCGCTTTTTTTCCTGC
AGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAGCTT
TTGTTCCCTTTAGTGAGGGTTAATTGCGCGAATTTCGCTAGCTAGGTCTTGAAAGGAG
TGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCC
GAGAAGTTGGGGGGAGGGGTCTGGCAATTGATCCGGTGCCTAGAGAAGGTGGCGCG
GGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGG
GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGGGTTTG
CCGCCAGAACACAGGACCGGTTCTAGAGCGCTATTTAGAACCatgCAGGAGGTAATA
GCGGGGCTTGAGCGATTACCTTTGCCTTCGAAAAAGACGTAGAGATGCAGAA
GGGAACCGGCCTGCTCCCATTTCAAGGTATGGACAAATCAGCATCTGCCGTGT
GCAATTTTTTACCAAGGGTCTGTGTGAAAAGGGGAAGCTCTGTCCATTTCCGCC
ATGATCGCGGAGAGAAGATGGTGGTGTGTAAGCACTGGCTGAGAGGGCTTTGC
AAAAAAGGCGACCACTGCAAATTTCTTCACCAATATGACCTGACTCGAATGCCT
GAGTGTTATTTTTACAGTAAGTTCGGTGACTGTAGCAACAAAGAATGCAGCTTC
TTGCATGTCAAACCAGCATTCAAGTCACAGGATTGCCCGTGGTACGATCAGGG
TTTTTGCAAGGACGGTCCCCCTCTGCAAATATCGACACGTACCCAGAATTATGTG
CCTTAATTACCTGGTTCGGCTTCTGTCTGAAGGGCCAAAATGTCAGTTTGCTCA
AAAAATTCGCGAGTTCAAATTGCTCCCTGGGTCTAAAATTTGGGAACCCCAGGA
TTGGCAGCAGCAGCTTGTAACATCCGAGCAATGAGGAACAAAAAAGATGCAC
CTGTTGATCACCTCGGAACCGAACATTGTTATGATTCTAGTGCGCCGCCAAAAG
TCCGCCGGTATCAGGTTCTGTTGAGTTTGATGCTGAGTAGTCAGACTAAGGAC
CAGGTTACGGCCGGAGCAATGCAACGGCTTCGGGCACGGGGACTCACGGTCG
ATAGCATTTTGCAGACCGATGACGCAACATTGGGTAAACTCATATATCCAGTTG
GCTTCTGGCGGAGCAAAGTGAAGTACATCAAGCAGACCTCAGCCATTCTCCAA
CAACATTACGGAGGTGATATACCCGCAAGCGTAGCTGAACTGGTAGCACTGCC
GGGCGTTCGGTCCCAAAATGGCACATCTGGCTATGGCGGTTGCTTGGGGAACGG
TGTCTGGTATCGCAGTTGATACGCATGTCCACCGCATCGCCAATCGGCTGAGG
TGGACTAAAAAAGCCACTAAGTCTCCTGAAGAAACACGGGCTGCTCTGGAAGA
GTGGCTTCCACGAGAGCTGTGGCATGAAATCAATGGATTGCTGGTTGGTTTCG
GGCAGCAGACATGCTTGCCCGTGACCCCCGGTGTTCATGCTTGCTTGAACCAG
GCTTTGTGCCAGCTGCCCAGGGCCTGAGTGGAAGTGAGACACCGGGAACATCT
GAGTCTGCGACCCCGGAGAGCacaaacGCGCGAATCCTGGCCTTCGcgATTGGCATT
AGCAGCATCGGCTGGGCATTCTCTGAAAACGACGAAGTGAAGGATTGCGGCGT
GCGAATTTTCACTAAGGTCGAAAATCCCAAACTGGTGAATCACTCGCTCTCCC
TAGACGACTGGCACGCTCCGCACGAAAGAGGCTTGCCCGCCGCAAGGCACGCT
TGAACCATCTTAAACACCTTATTGCAAATGAGTTTAAACTGAATTATGAGGACT
ACCAATCCTTTGACGAGTCTCTTGCTAAAGCCTACAAAGGGAGCCTTATATCCC
CGTATGAGCTCCGGTTCAGAGCACTCAACGAAGTCTGTCCAAACAGGATTTT

GCTCGCGTGATTCTCCACATAGCGAAGAGGCGAGGATACGATGACATTAAAAA
CAGTGATGATAAGGAAAAAGGGGCCATACTCAAAGCGATTAAGCAAAATGAAG
AGAAGCTCGCTAACTATCAATCAGTAGGGGAGTATCTCTATAAAGAGTACTTCC
AGAAGTTCAAAGAAAAATAGCAAGGAATTTACTAATGTCCGGAATAAAAAAGGAG
TCTTACGAAAGATGTATTGCGCAATCTTTCCTCAAGGACGAGCTCAAATTGATT
TTCAAGAAACAAAGGGAATTTGGGTTTCAGCTTCTCAAAAAAATTTGAGGAAGA
GGTTCTGAGCGTTGCCTTTTACAAACGCGCCCTTAAGGACTTCTCACATCTCGT
AGGGAATTGTAGTTTCTTCACCGATGAAAAACGGGCGCCAAAAAATAGCCCTTT
GGCTTTTATGTTTGTGCTCTGACTCGCATCATTAATCTGCTCAACAACCTTAA
AAACACGGAAGGGATTCTGTACACAAAGGATGATCTGAACGCTCTGCTTAACG
AAGTTTTGAAGAACGGGACTTTGACCTACAAACAAACCAAAAAGCTTCTTGGTCT
TCAGTGATGACTACGAATTCAAGGGAGAAAAAGGGACATATTTTCATCGAATTCA
AGAAGTATAAGGAGTTCATCAAAGCCTTGGGCGAGCACAACTTGTCTCAAGAT
GATCTCAACGAAATTGCTAAGGATATCACTCTGATTAAAGACGAGATCAAGCTC
AAAAAGGCGTTGGCGAAGTATGACCTTAACCAAAACCAAAATAGATAGCCTCAG
CAAGTTGGAATTTAAAGATCACTTGAATATAAGTTTCAAGGCCCTTAAGTTGGT
CACCCCTTGATGCTTGAAGGAAAGAAATATGATGAGGCATGTAATGAGCTGA
ATCTCAAGGTTGCTATTAACGAAGACAAAAAAGATTTCTCCAGCTTTCAATG
AGACTTACTATAAGGACGAGGTTACCAATCCTGTGGTGCTCCGAGCCATCAAA
GAGTATCGAAAGGTCCTGAATGCTTTGCTCAAAAAATACGGTAAGGTACACAA
AATAAATATTGAGCTCGCAAGGGAGGTCGGTAAGAACCCTCCAGCGCGCCA
AAATAGAAAAGGAACAGAATGAAAATTACAAAGCGAAAAAGGACGCCGAGCTC
GAGTGCGAAAAGCTGGGCCTGAAAATAAACAGCAAGAACATTCTCAAACCTCCG
CCTCTTCAAAGAACAAAAAGAATTTTGTGCTTATAGTGGTGAGAAAAATAAAAT
CTCCGATCTTCAAGACGAGAAGATGCTCGAAATAGACgagATATATCCATATAGC
AGGTCTTTTGACGATTCTTACATGAATAAAGTGCTTGTTTTCACTAAGCAGAAT
CAGGAAAAGTTGAATCAGACCCCTTTGAGGCCTTTGGCAACGACTCAGCAAA
GTGGCAGAAGATCGAGGTCTTGGCTAAGAATCTTCCTACTAAGAAACAGAAAA
GGATATTGGATAAGAACTATAAAGACAAAAGAACAAAAGAACTTTAAAGACCGC
AACCTCAATGACACCAGATACATAGCAAGATTGGTTCTGAACTACACAAAAGAT
TATTTGGACTTCTTGCCGCTGTCTGATGATGAGAACACGAAACTCAACGACACG
CAAAAGGGGTCTAAAGTCCACGTCGAAGCTAAATCTGGGATGCTCACCTCAGC
ATTGAGGCATACGTGGGGATTCTCAGCAAAGGACCGAAACAATCACCTGCACC
ATGCCATTGACGCAGTTATCATAGCGTATGCCAATAATTCAATAGTAAAAGCGT
TTAGCGACTTCAAGAAGGAACAAGAGTCCAACAGCGCCGAGCTCTACGCAAAA
AAGATTAGTGAAGTCTGACTACAAAAACAAAAGAAAATTCTTTGAGCCGTTCAGC
GGATTTGACAGAAAGGTATTGGATAAAATAGATGAAATTTTCGTGAGCAAACCC
GAAAGGAAAAAGCCCTCAGGCGCCTTGACAGAAAGAGACTTTCAGGAAGGAAGA
GGAATTCTACCAAAGCTACGGCGGAAAAGAGGGAGTTTTGAAGGCTCTCGAAC
TTGGAAGATTAGGAAGGTGAACGGCAAGATAGTGAAAAACGGCGATATGTTT
CGGGTTGATATCTTCAAACATAAAAAAACGAATAAATTTTATGCTGTGCCTATA
TACACTATGGACTTCGCACTTAAGGTCCTGCCGAATAAGGCGGTAGCCCGATC
TAAAAAAGGCGAAATTAAGGACTGGATTTTGTGATGGATGAAAATTACGAGTTCTG
CTTTTCTCTCTACAAGGATTCCCTTATATTGATACAGACGAAAGATATGCAGGA
ACCGGAATTCGTGTATTACAACGCTTTTACTTCTCTACGGTATCTTTGATTGT
CTCCAAACATGACAACAAATTCGAAACACTCAGTAAAAACCAAAAGATTCTCTT

TAAAAATGCGAACGAGAAAGAAGTAATTGCAAAATCAATTGGCATCCAAAATTT
GAAAGTTTTTTGAAAAATATATAGTATCTGCCCTCGGAGAGGTTACTAAAGCGGA
ATTAGACAGCGAGAGGACTTCAAAAAATCAGGTCCACCCAAGAAAAAACGCAA
 GGTGGAAGATCCGAAGAAAAAGCGAAAAGTGGATGTGtaaCGTTTTCCGGGACGCCG
 GCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCCACCCCAACT
 TGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAA
 ATAAAGCATTTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATC
 TTATCATGTCTGTATACCG (SEQ ID NO: 203).

gRNA Target Sequences

[0279] In some embodiments of the compositions of the disclosure, a target sequence of an RNA molecule comprises a sequence motif corresponding to the first RNA binding protein and/or the second RNA binding protein.

[0280] In some embodiments of the compositions and methods of the disclosure, the sequence motif is a signature of a disease or disorder.

[0281] A sequence motif of the disclosure may be isolated or derived from a sequence of foreign or exogenous sequence found in a genomic sequence, and therefore translated into an mRNA molecule of the disclosure or a sequence of foreign or exogenous sequence found in an RNA sequence of the disclosure.

[0282] A sequence motif of the disclosure may comprise or consist of a mutation in an endogenous sequence that causes a disease or disorder. The mutation may comprise or consist of a sequence substitution, inversion, deletion, insertion, transposition, or any combination thereof.

[0283] A sequence motif of the disclosure may comprise or consist of a repeated sequence. In some embodiments, the repeated sequence may be associated with a microsatellite instability (MSI). MSI at one or more loci results from impaired DNA mismatch repair mechanisms of a cell of the disclosure. A hypervariable sequence of DNA may be transcribed into an mRNA of the disclosure comprising a target sequence comprising or consisting of the hypervariable sequence.

[0284] A sequence motif of the disclosure may comprise or consist of a biomarker. The biomarker may indicate a risk of developing a disease or disorder. The biomarker may indicate a healthy gene (low or no determinable risk of developing a disease or disorder). The biomarker may indicate an edited gene. Exemplary biomarkers include, but are not limited to, single

nucleotide polymorphisms (SNPs), sequence variations or mutations, epigenetic marks, splice acceptor sites, exogenous sequences, heterologous sequences, and any combination thereof.

[0285] A sequence motif of the disclosure may comprise or consist of a secondary, tertiary or quaternary structure. The secondary, tertiary or quaternary structure may be endogenous or naturally occurring. The secondary, tertiary or quaternary structure may be induced or non-naturally occurring. The secondary, tertiary or quaternary structure may be encoded by an endogenous, exogenous, or heterologous sequence.

[0286] In some embodiments of the compositions and methods of the disclosure, a target sequence of an RNA molecule comprises or consists of between 2 and 100 nucleotides or nucleic acid bases, inclusive of the endpoints. In some embodiments, the target sequence of an RNA molecule comprises or consists of between 2 and 50 nucleotides or nucleic acid bases, inclusive of the endpoints. In some embodiments, the target sequence of an RNA molecule comprises or consists of between 2 and 20 nucleotides or nucleic acid bases, inclusive of the endpoints.

[0287] In some embodiments of the compositions and methods of the disclosure, a target sequence of an RNA molecule is continuous. In some embodiments, the target sequence of an RNA molecule is discontinuous. For example, the target sequence of an RNA molecule may comprise or consist of one or more nucleotides or nucleic acid bases that are not contiguous because one or more intermittent nucleotides are positioned in between the nucleotides of the target sequence.

[0288] In some embodiments of the compositions and methods of the disclosure, a target sequence of an RNA molecule is naturally occurring. In some embodiments, the target sequence of an RNA molecule is non-naturally occurring. Exemplary non-naturally occurring target sequences may comprise or consist of sequence variations or mutations, chimeric sequences, exogenous sequences, heterologous sequences, chimeric sequences, recombinant sequences, sequences comprising a modified or synthetic nucleotide or any combination thereof.

[0289] In some embodiments of the compositions and methods of the disclosure, a target sequence of an RNA molecule binds to a guide RNA of the disclosure.

[0290] In some embodiments of the compositions and methods of the disclosure, a target sequence of an RNA molecule binds to a first RNA binding protein of the disclosure.

[0291] In some embodiments of the compositions and methods of the disclosure, a target sequence of an RNA molecule binds to a second RNA binding protein of the disclosure.

RNA Molecules

[0292] In some embodiments of the compositions and methods of the disclosure, an RNA molecule of the disclosure comprises a target sequence. In some embodiments, the RNA molecule of the disclosure comprises at least one target sequence. In some embodiments, the RNA molecule of the disclosure comprises one or more target sequence(s). In some embodiments, the RNA molecule of the disclosure comprises two or more target sequences.

[0293] In some embodiments of the compositions and methods of the disclosure, an RNA molecule of the disclosure is a naturally occurring RNA molecule. In some embodiments, the RNA molecule of the disclosure is a non-naturally occurring molecule. Exemplary non-naturally occurring RNA molecules may comprise or consist of sequence variations or mutations, chimeric sequences, exogenous sequences, heterologous sequences, chimeric sequences, recombinant sequences, sequences comprising a modified or synthetic nucleotide or any combination thereof.

[0294] In some embodiments of the compositions and methods of the disclosure, an RNA molecule of the disclosure comprises or consists of a sequence isolated or derived from a virus.

[0295] In some embodiments of the compositions and methods of the disclosure, an RNA molecule of the disclosure comprises or consists of a sequence isolated or derived from a prokaryotic organism. In some embodiments, an RNA molecule of the disclosure comprises or consists of a sequence isolated or derived from a species or strain of archaea or a species or strain of bacteria.

[0296] In some embodiments of the compositions and methods of the disclosure, the RNA molecule of the disclosure comprises or consists of a sequence isolated or derived from a eukaryotic organism. In some embodiments, an RNA molecule of the disclosure comprises or consists of a sequence isolated or derived from a species of protozoa, parasite, protist, algae, fungi, yeast, amoeba, worm, microorganism, invertebrate, vertebrate, insect, rodent, mouse, rat, mammal, or a primate. In some embodiments, an RNA molecule of the disclosure comprises or consists of a sequence isolated or derived from a human.

[0297] In some embodiments of the compositions and methods of the disclosure, the RNA molecule of the disclosure comprises or consists of a sequence derived from a coding sequence from a genome of an organism or a virus. In some embodiments, the RNA molecule of the disclosure comprises or consists of a primary RNA transcript, a precursor messenger RNA (pre-

mRNA) or messenger RNA (mRNA). In some embodiments, the RNA molecule of the disclosure comprises or consists of a gene product that has not been processed (e.g. a transcript). In some embodiments, the RNA molecule of the disclosure comprises or consists of a gene product that has been subject to post-transcriptional processing (e.g. a transcript comprising a 5' cap and a 3' polyadenylation signal). In some embodiments, the RNA molecule of the disclosure comprises or consists of a gene product that has been subject to alternative splicing (e.g. a splice variant). In some embodiments, the RNA molecule of the disclosure comprises or consists of a gene product that has been subject to removal of non-coding and/or intronic sequences (e.g. a messenger RNA (mRNA)).

[0298] In some embodiments of the compositions and methods of the disclosure, the RNA molecule of the disclosure comprises or consists of a sequence derived from a non-coding sequence (e.g. a non-coding RNA (ncRNA)). In some embodiments, the RNA molecule of the disclosure comprises or consists of a ribosomal RNA. In some embodiments, the RNA molecule of the disclosure comprises or consists of a small ncRNA molecule. Exemplary small RNA molecules of the disclosure include, but are not limited to, microRNAs (miRNAs), small interfering (siRNAs), piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), extracellular or exosomal RNAs (exRNAs), and small Cajal body-specific RNAs (scaRNAs). In some embodiments, the RNA molecule of the disclosure comprises or consists of a long ncRNA molecule. Exemplary long RNA molecules of the disclosure include, but are not limited to, X-inactive specific transcript (Xist) and HOX transcript antisense RNA (HOTAIR).

[0299] In some embodiments of the compositions and methods of the disclosure, the RNA molecule of the disclosure contacted by a composition of the disclosure in an intracellular space. In some embodiments, the RNA molecule of the disclosure contacted by a composition of the disclosure in a cytosolic space. In some embodiments, the RNA molecule of the disclosure contacted by a composition of the disclosure in a nucleus. In some embodiments, the RNA molecule of the disclosure contacted by a composition of the disclosure in a vesicle, membrane-bound compartment of a cell, or an organelle.

[0300] In some embodiments of the compositions and methods of the disclosure, the RNA molecule of the disclosure contacted by a composition of the disclosure in an extracellular space. In some embodiments, the RNA molecule of the disclosure contacted by a composition of the

disclosure in an exosome. In some embodiments, the RNA molecule of the disclosure contacted by a composition of the disclosure in a liposome, a polymersome, a micelle or a nanoparticle. In some embodiments, the RNA molecule of the disclosure contacted by a composition of the disclosure in an extracellular matrix. In some embodiments, the RNA molecule of the disclosure contacted by a composition of the disclosure in a droplet. In some embodiments, the RNA molecule of the disclosure contacted by a composition of the disclosure in a microfluidic droplet.

[0301] In some embodiments of the compositions and methods of the disclosure, a RNA molecule of the disclosure comprises or consists of a single-stranded sequence. In some embodiments, the RNA molecule of the disclosure comprises or consists of a double-stranded sequence. In some embodiments, the double-stranded sequence comprises two RNA molecules. In some embodiments, the double-stranded sequence comprises one RNA molecule and one DNA molecule. In some embodiments, including those wherein the double-stranded sequence comprises one RNA molecule and one DNA molecule, compositions of the disclosure selectively bind and, optionally, selectively cut the RNA molecule.

Fusion Proteins

[0302] In some embodiments of the compositions and methods of the disclosure, the composition comprises a sequence encoding a target RNA-binding fusion protein comprising (a) a sequence encoding a first RNA-binding polypeptide or portion thereof; and (b) a sequence encoding a second RNA-binding polypeptide, wherein the first RNA-binding polypeptide binds a target RNA, and wherein the second RNA-binding polypeptide comprises RNA-nuclease activity.

[0303] In some embodiments, a target RNA-binding fusion protein is an RNA-guided target RNA-binding fusion protein. RNA-guided target RNA-binding fusion proteins comprise at least one RNA-binding polypeptide which corresponds to a gRNA which guides the RNA-binding polypeptide to target RNA. RNA-guided target RNA-binding fusion proteins include without limitation, RNA-binding polypeptides which are CRISPR/Cas-based RNA-binding polypeptides or portions thereof.

[0304] In some embodiments, a target RNA-binding fusion protein is not an RNA-guided target RNA-binding fusion protein and as such comprises at least one RNA-binding polypeptide which is capable of binding a target RNA without a corresponding gRNA sequence. Such non-guided RNA-binding polypeptides include, without limitation, at least one RNA-binding protein

or RNA-binding portion thereof which is a PUF (Pumilio and FBF homology family). This type RNA-binding polypeptide can be used in place of a gRNA-guided RNA binding protein such as CRISPR/Cas. The unique RNA recognition mode of PUF proteins (named for *Drosophila* Pumilio and *C. elegans* fem-3 binding factor) that are involved in mediating mRNA stability and translation are well known in the art. The PUF domain of human Pumilio1, also known in the art, binds tightly to cognate RNA sequences and its specificity can be modified. It contains eight PUF repeats that recognize eight consecutive RNA bases with each repeat recognizing a single base. Since two amino acid side chains in each repeat recognize the Watson-Crick edge of the corresponding base and determine the specificity of that repeat, a PUF domain can be designed to specifically bind most 8-nt RNA. *Wang et al., Nat Methods. 2009; 6(11): 825-830*. See also WO2012/068627 which is incorporated by reference herein in its entirety.

[0305] In some embodiments of the non-guided RNA-binding fusion proteins of the disclosure, the fusion protein comprises at least one RNA-binding protein or RNA-binding portion thereof which is a PUMBY (Pumilio-based assembly) protein. RNA-binding protein PumHD (Pumilio homology domain, a member of the PUF family), which has been widely used in native and modified form for targeting RNA, has been engineered to yield a set of four canonical protein modules, each of which targets one RNA base. These modules (*i.e.*, Pumby, for Pumilio-based assembly) can be concatenated in chains of varying composition and length, to bind desired target RNAs. The specificity of such Pumby–RNA interactions is high, with undetectable binding of a Pumby chain to RNA sequences that bear three or more mismatches from the target sequence. *Katarzyna et al., PNAS, 2016; 113(19): E2579-E2588*. See also US 2016/0238593 which is incorporated by reference herein in its entirety.

[0306] In some embodiments of the compositions of the disclosure, at least one of the RNA-binding proteins or RNA-binding portions thereof is a PPR protein. PPR proteins (proteins with pentatricopeptide repeat (PPR) motifs derived from plants) are nuclear-encoded and exclusively controlled at the RNA level organelles (chloroplasts and mitochondria), cutting, translation, splicing, RNA editing, genes specifically acting on RNA stability. PPR proteins are typically a motif of 35 amino acids and have a structure in which a PPR motif is about 10 contiguous amino acids. The combination of PPR motifs can be used for sequence-selective binding to RNA. PPR proteins are often comprised of PPR motifs of about 10 repeat domains. PPR domains or RNA-

binding domains may be configured to be catalytically inactive. WO 2013/058404 incorporated herein by reference in its entirety.

[0307] In some embodiments, the fusion protein disclosed herein comprises a linker between the at least two RNA-binding polypeptides. In some embodiments, the linker is a peptide linker. In some embodiments, the peptide linker comprises one or more repeats of the tri-peptide GGS. In other embodiments, the linker is a non-peptide linker. In some embodiments, the non-peptide linker comprises polyethylene glycol (PEG), polypropylene glycol (PPG), co-poly(ethylene/propylene) glycol, polyoxyethylene (POE), polyurethane, polyphosphazene, polysaccharides, dextran, polyvinyl alcohol, polyvinylpyrrolidones, polyvinyl ethyl ether, polyacryl amide, polyacrylate, polycyanoacrylates, lipid polymers, chitins, hyaluronic acid, heparin, or an alkyl linker.

[0308] In some embodiments, the at least one RNA-binding protein does not require multimerization for RNA-binding activity. In some embodiments, the at least one RNA-binding protein is not a monomer of a multimer complex. In some embodiments, a multimer protein complex does not comprise the RNA binding protein. In some embodiments, the at least one of RNA-binding protein selectively binds to a target sequence within the RNA molecule. In some embodiments, the at least one RNA-binding protein does not comprise an affinity for a second sequence within the RNA molecule. In some embodiments, the at least one RNA-binding protein does not comprise a high affinity for or selectively bind a second sequence within the RNA molecule. In some embodiments, the at least one RNA-binding protein comprises between 2 and 1300 amino acids, inclusive of the endpoints.

[0309] In some embodiments, the sequence encoding the at least one RNA-binding protein of the fusion proteins disclosed herein further comprises a sequence encoding a nuclear localization signal (NLS). In some embodiments, the sequence encoding a nuclear localization signal (NLS) is positioned 3' to the sequence encoding the RNA binding protein. In some embodiments, the at least one RNA-binding protein comprises an NLS at a C-terminus of the protein. In some embodiments, the sequence encoding the at least one RNA-binding protein further comprises a first sequence encoding a first NLS and a second sequence encoding a second NLS. In some embodiments, the sequence encoding the first NLS or the second NLS is positioned 3' to the sequence encoding the RNA-binding protein. In some embodiments, the at least one RNA-binding protein comprises the first NLS or the second NLS at a C-terminus of the protein. In

some embodiments, the at least one RNA-binding protein further comprises an NES (nuclear export signal) or other peptide tag or secretory signal.

[0310] In some embodiments, a fusion protein disclosed herein comprises the at least one RNA-binding protein as a first RNA-binding protein together with a second RNA-binding protein comprising or consisting of a nuclease domain. In some embodiments, the second RNA binding protein binds RNA in a manner in which it associates with RNA. In some embodiments, the second RNA binding protein associates with RNA in a manner in which it cleaves RNA.

[0311] In some embodiments, the second RNA-binding polypeptide is operably configured to the first RNA-binding polypeptide at the C-terminus of the first RNA-binding polypeptide. In some embodiments, the second RNA-binding polypeptide is operably configured to the first RNA-binding polypeptide at the N-terminus of the first RNA-binding polypeptide.

Vectors

[0312] In some embodiments of the compositions and methods of the disclosure, a vector comprises a guide RNA of the disclosure. In some embodiments, the vector comprises at least one guide RNA of the disclosure. In some embodiments, the vector comprises one or more guide RNA(s) of the disclosure. In some embodiments, the vector comprises two or more guide RNAs of the disclosure. In some embodiments, the vector further comprises a fusion protein of the disclosure. In some embodiments, the fusion protein comprises a first RNA binding protein and a second RNA binding protein.

[0313] In some embodiments of the compositions and methods of the disclosure, a first vector comprises a guide RNA of the disclosure and a second vector comprises a fusion protein of the disclosure. In some embodiments, the first vector comprises at least one guide RNA of the disclosure. In some embodiments, the first vector comprises one or more guide RNA(s) of the disclosure. In some embodiments, the first vector comprises two or more guide RNA(s) of the disclosure. In some embodiments, the fusion protein comprises a first RNA binding protein and a second RNA binding protein. In some embodiments, the first vector and the second vector are identical. In some embodiments, the first vector and the second vector are not identical.

[0314] In some embodiments of the compositions and methods of the disclosure, the vector is or comprises a component of a “2-component RNA targeting system” comprising (a) nucleic acid sequence encoding a RNA-targeted fusion protein of the disclosure; and (b) a single guide

RNA (sgRNA) sequence comprising: on its 5' end, an RNA sequence (e.g., spacer sequence) that hybridizes to or specifically binds to a target RNA sequence; and on its 3' end, an RNA sequence (e.g., scaffold sequence) capable of specifically binding to or associating with the CRISPR/Cas protein of the fusion protein; and wherein the 2-component RNA targeting system recognizes and alters the target RNA in a cell in the absence of a PAMmer. In some embodiments, the sequences of the 2-component system are comprised within a single (e.g., unitary) vector. In some embodiments, the spacer sequence of the 2-component system targets a repeat sequence selected from the group consisting of CUG, CCUG, CAG, and GGGGCC. In some embodiments, the spacer sequence of the 2-component system targets an RNA sequence involved in an adaptive immune response. In some embodiments, a spacer sequence of the 2-component system comprises a portion of a nucleic acid sequence encoding a protein component of an adaptive immune response, and wherein the protein component is selected from the group consisting of Beta-2-microglobulin (β 2M), Human Leukocyte Antigen A (HLA-A), Human Leukocyte Antigen B (HLA-B), Human Leukocyte Antigen C (HLA-C), Cluster of Differentiation 28 (CD28), Cluster of Differentiation 80 (CD80), Cluster of Differentiation 86 (CD86), Inducible T-cell Costimulator (ICOS), ICOS Ligand (ICOSLG), OX40L, Interleukin 12 (IL12), and CC Chemokine Receptor 7 (CCR7). In some embodiments, the 2-component system comprises a spacer which is a portion of a nucleic acid sequence encoding a protein component of an adaptive immune response and which is about 20 or 21 nucleotides in length. In some embodiments, the 2-component system comprises a first and second spacer comprised within a singular gRNA. In some embodiments, the 2-component system comprises a first and second spacer sequence comprised within first and second gRNA sequences. In some embodiments, the first spacer targets a repeat sequence and the second spacer targets RNA involved in an adaptive immune response.

[0315] In some embodiments of the compositions and methods of the disclosure, a vector of the disclosure is a viral vector. In some embodiments, the viral vector comprises a sequence isolated or derived from a retrovirus. In some embodiments, the viral vector comprises a sequence isolated or derived from a lentivirus. In some embodiments, the viral vector comprises a sequence isolated or derived from an adenovirus. In some embodiments, the viral vector comprises a sequence isolated or derived from an adeno-associated virus (AAV). In some

embodiments, the viral vector is replication incompetent. In some embodiments, the viral vector is isolated or recombinant. In some embodiments, the viral vector is self-complementary.

[0316] In some embodiments of the compositions and methods of the disclosure, the viral vector comprises a sequence isolated or derived from an adeno-associated virus (AAV). In some embodiments, the viral vector comprises an inverted terminal repeat sequence or a capsid sequence that is isolated or derived from an AAV of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 or AAV12. In some embodiments, the viral vector is replication incompetent. In some embodiments, the viral vector is isolated or recombinant (rAAV). In some embodiments, the viral vector is self-complementary (scAAV).

[0317] In some embodiments of the compositions and methods of the disclosure, a vector of the disclosure is a non-viral vector. In some embodiments, the vector comprises or consists of a nanoparticle, a micelle, a liposome or lipoplex, a polymersome, a polyplex or a dendrimer. In some embodiments, the vector is an expression vector or recombinant expression system. As used herein, the term “recombinant expression system” refers to a genetic construct for the expression of certain genetic material formed by recombination.

[0318] In some embodiments of the compositions and methods of the disclosure, an expression vector, viral vector or non-viral vector provided herein, includes without limitation, an expression control element. An “expression control element” as used herein refers to any sequence that regulates the expression of a coding sequence, such as a gene. Exemplary expression control elements include but are not limited to promoters, enhancers, microRNAs, post-transcriptional regulatory elements, polyadenylation signal sequences, and introns. Expression control elements may be constitutive, inducible, repressible, or tissue-specific, for example. A “promoter” is a control sequence that is a region of a polynucleotide sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. In some embodiments, expression control by a promoter is tissue-specific. Non-limiting exemplary promoters include CMV, CBA, CAG, Cbh, EF-1a, PGK, UBC, GUSB, UCOE, hAAT, TBG, Desmin, MCK, C5-12, NSE, Synapsin, PDGF, MecP2, CaMKII, mGluR2, NFL, NFH, nβ2, PPE, ENK, EAAT2, GFAP, MBP, and U6 promoters. An “enhancer” is a region of DNA that can be bound by activating proteins to increase the likelihood or frequency

of transcription. Non-limiting exemplary enhancers and posttranscriptional regulatory elements include the CMV enhancer and WPRE.

[0319] In some embodiments of the compositions and methods of the disclosure, an expression vector, viral vector or non-viral vector provided herein, includes without limitation, vector elements such as an IRES or 2A peptide sites for configuration of “multicistronic” or “polycistronic” or “bicistronic” or tricistronic” constructs, i.e., having double or triple or multiple coding areas or exons, and as such will have the capability to express from mRNA two or more proteins from a single construct. Multicistronic vectors simultaneously express two or more separate proteins from the same mRNA. The two strategies most widely used for constructing multicistronic configurations are through the use of an IRES or a 2A self-cleaving site. An “IRES” refers to an internal ribosome entry site or portion thereof of viral, prokaryotic, or eukaryotic origin which are used within polycistronic vector constructs. In some embodiments, an IRES is an RNA element that allows for translation initiation in a cap-independent manner. The term “self-cleaving peptides” or “sequences encoding self-cleaving peptides” or “2A self-cleaving site” refer to linking sequences which are used within vector constructs to incorporate sites to promote ribosomal skipping and thus to generate two polypeptides from a single promoter, such self-cleaving peptides include without limitation, T2A, and P2A peptides or sequences encoding the self-cleaving peptides.

[0320] In some embodiments, the vector is a viral vector. In some embodiments, the vector is an adenoviral vector, an adeno-associated viral (AAV) vector, or a lentiviral vector. In some embodiments, the vector is a retroviral vector, an adenoviral/retroviral chimera vector, a herpes simplex viral I or II vector, a parvoviral vector, a reticuloendotheliosis viral vector, a polioviral vector, a papillomaviral vector, a vaccinia viral vector, or any hybrid or chimeric vector incorporating favorable aspects of two or more viral vectors. In some embodiments, the vector further comprises one or more expression control elements operably linked to the polynucleotide. In some embodiments, the vector further comprises one or more selectable markers. In some embodiments, the AAV vector has low toxicity. In some embodiments, the AAV vector does not incorporate into the host genome, thereby having a low probability of causing insertional mutagenesis. In some embodiments, the AAV vector can encode a range total of polynucleotides from 4.5 kb to 4.75 kb. In some embodiments, exemplary AAV vectors that may be used in any of the herein described compositions, systems, methods, and kits can

include an AAV1 vector, a modified AAV1 vector, an AAV2 vector, a modified AAV2 vector, an AAV3 vector, a modified AAV3 vector, an AAV4 vector, a modified AAV4 vector, an AAV5 vector, a modified AAV5 vector, an AAV6 vector, a modified AAV6 vector, an AAV7 vector, a modified AAV7 vector, an AAV8 vector, an AAV9 vector, an AAV.rh10 vector, a modified AAV.rh10 vector, an AAV.rh32/33 vector, a modified AAV.rh32/33 vector, an AAV.rh43 vector, a modified AAV.rh43 vector, an AAV.rh64R1 vector, and a modified AAV.rh64R1 vector and any combinations or equivalents thereof. In some embodiments, the lentiviral vector is an integrase-competent lentiviral vector (ICLV). In some embodiments, the lentiviral vector can refer to the transgene plasmid vector as well as the transgene plasmid vector in conjunction with related plasmids (e.g., a packaging plasmid, a rev expressing plasmid, an envelope plasmid) as well as a lentiviral-based particle capable of introducing exogenous nucleic acid into a cell through a viral or viral-like entry mechanism. Lentiviral vectors are well-known in the art (see, e.g., Trono D. (2002) *Lentiviral vectors*, New York: Springer-Verlag Berlin Heidelberg and Durand et al. (2011) *Viruses* 3(2):132-159 doi: 10.3390/v3020132). In some embodiments, exemplary lentiviral vectors that may be used in any of the herein described compositions, systems, methods, and kits can include a human immunodeficiency virus (HIV) 1 vector, a modified human immunodeficiency virus (HIV) 1 vector, a human immunodeficiency virus (HIV) 2 vector, a modified human immunodeficiency virus (HIV) 2 vector, a sooty mangabey simian immunodeficiency virus (SIV_{SM}) vector, a modified sooty mangabey simian immunodeficiency virus (SIV_{SM}) vector, a African green monkey simian immunodeficiency virus (SIV_{AGM}) vector, a modified African green monkey simian immunodeficiency virus (SIV_{AGM}) vector, an equine infectious anemia virus (EIAV) vector, a modified equine infectious anemia virus (EIAV) vector, a feline immunodeficiency virus (FIV) vector, a modified feline immunodeficiency virus (FIV) vector, a Visna/maedi virus (VNV/VMV) vector, a modified Visna/maedi virus (VNV/VMV) vector, a caprine arthritis-encephalitis virus (CAEV) vector, a modified caprine arthritis-encephalitis virus (CAEV) vector, a bovine immunodeficiency virus (BIV), or a modified bovine immunodeficiency virus (BIV).

Nucleic Acids

[0321] Provided herein are the nucleic acid sequences encoding the fusion proteins disclosed herein for use in gene transfer and expression techniques described herein. It should be understood, although not always explicitly stated that the sequences provided herein can be used

to provide the expression product as well as substantially identical sequences that produce a protein that has the same biological properties. These “biologically equivalent” or “biologically active” or “equivalent” polypeptides are encoded by equivalent polynucleotides as described herein. They may possess at least 60%, or alternatively, at least 65%, or alternatively, at least 70%, or alternatively, at least 75%, or alternatively, at least 80%, or alternatively at least 85%, or alternatively at least 90%, or alternatively at least 95% or alternatively at least 98%, identical primary amino acid sequence to the reference polypeptide when compared using sequence identity methods run under default conditions. Specific polypeptide sequences are provided as examples of particular embodiments. Modifications to the sequences to amino acids with alternate amino acids that have similar charge. Additionally, an equivalent polynucleotide is one that hybridizes under stringent conditions to the reference polynucleotide or its complement or in reference to a polypeptide, a polypeptide encoded by a polynucleotide that hybridizes to the reference encoding polynucleotide under stringent conditions or its complementary strand. Alternatively, an equivalent polypeptide or protein is one that is expressed from an equivalent polynucleotide.

[0322] The nucleic acid sequences (e.g., polynucleotide sequences) disclosed herein may be codon-optimized which is a technique well known in the art. In some embodiments disclosed herein, exemplary Cas sequences, such as e.g., SEQ ID NO: 46 (Cas13d), are codon optimized for expression in human cells. Codon optimization refers to the fact that different cells differ in their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. It is also possible to decrease expression by deliberately choosing codons for which the corresponding tRNAs are known to be rare in a particular cell type. Codon usage tables are known in the art for mammalian cells, as well as for a variety of other organisms. Based on the genetic code, nucleic acid sequences coding for, e.g., a Cas protein, can be generated. In some embodiments, such a sequence is optimized for expression in a host or target cell, such as a host cell used to express the Cas protein or a cell in which the disclosed methods are practiced (such as in a mammalian cell, e.g., a human cell). Codon preferences and codon usage tables for a particular species can be used to engineer isolated nucleic acid molecules encoding a Cas protein (such as one encoding a protein having at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to its corresponding wild-type protein) that takes advantage of the codon usage preferences of that particular species. For example, the Cas proteins disclosed herein can be designed to have codons that are preferentially used by a particular organism of interest. In one example, a Cas nucleic acid sequence is optimized for expression in human cells, such as one having at least 70%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, or at least 99% sequence identity to its corresponding wild-type or originating nucleic acid sequence. In some embodiments, an isolated nucleic acid molecule encoding at least one Cas protein (which can be part of a vector) includes at least one Cas protein coding sequence that is codon optimized for expression in a eukaryotic cell, or at least one Cas protein coding sequence codon optimized for expression in a human cell. In one embodiment, such a codon optimized Cas coding sequence has at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to its corresponding wild-type or originating sequence. In another embodiment, a eukaryotic cell codon optimized nucleic acid sequence encodes a Cas protein having at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to its corresponding wild-type or originating protein. In another embodiment, a variety of clones containing functionally equivalent nucleic acids may be routinely generated, such as nucleic acids which differ in sequence but which encode the same Cas protein sequence. Silent mutations in the coding sequence result from the degeneracy (i.e., redundancy) of the genetic code, whereby more than one codon can encode the same amino acid residue. Thus, for example, leucine can be encoded by CTT, CTC, CTA, CTG, TTA, or TTG; serine can be encoded by TCT, TCC, TCA, TCG, AGT, or AGC; asparagine can be encoded by AAT or AAC; aspartic acid can be encoded by GAT or GAC; cysteine can be encoded by TGT or TGC; alanine can be encoded by GCT, GCC, GCA, or GCG; glutamine can be encoded by CAA or CAG; tyrosine can be encoded by TAT or TAC; and isoleucine can be encoded by ATT, ATC, or ATA. Tables showing the standard genetic code can be found in various sources (see, for example, Stryer, 1988, Biochemistry, 3rd Edition, W.H. Freeman and Co., NY).

[0323] “Hybridization” refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any

other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PC reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[0324] Examples of stringent hybridization conditions include: incubation temperatures of about 25°C to about 37°C; hybridization buffer concentrations of about 6x SSC to about 10x SSC; formamide concentrations of about 0% to about 25%; and wash solutions from about 4x SSC to about 8x SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40°C to about 50°C; buffer concentrations of about 9x SSC to about 2x SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5x SSC to about 2x SSC. Examples of high stringency conditions include: incubation temperatures of about 55°C to about 68°C; buffer concentrations of about 1x SSC to about 0.1x SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1x SSC, 0.1x SSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed.

“Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the present invention.

Cells

[0325] In some embodiments of the compositions and methods of the disclosure, a cell of the disclosure is a prokaryotic cell.

[0326] In some embodiments of the compositions and methods of the disclosure, a cell of the disclosure is a eukaryotic cell. In some embodiments, the cell is a mammalian cell. In some

embodiments, the cell is a bovine, murine, feline, equine, porcine, canine, simian, or human cell. In some embodiments, the cell is a non-human mammalian cell such as a non-human primate cell.

[0327] In some embodiments, a cell of the disclosure is a somatic cell. In some embodiments, a cell of the disclosure is a germline cell. In some embodiments, a germline cell of the disclosure is not a human cell.

[0328] In some embodiments of the compositions and methods of the disclosure, a cell of the disclosure is a stem cell. In some embodiments, a cell of the disclosure is an embryonic stem cell. In some embodiments, an embryonic stem cell of the disclosure is not a human cell. In some embodiments, a cell of the disclosure is a multipotent stem cell or a pluripotent stem cell. In some embodiments, a cell of the disclosure is an adult stem cell. In some embodiments, a cell of the disclosure is an induced pluripotent stem cell (iPSC). In some embodiments, a cell of the disclosure is a hematopoietic stem cell (HSC).

[0329] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is an immune cell. In some embodiments, an immune cell of the disclosure is a lymphocyte. In some embodiments, an immune cell of the disclosure is a T lymphocyte (also referred to herein as a T-cell). Exemplary T-cells of the disclosure include, but are not limited to, naïve T cells, effector T cells, helper T cells, memory T cells, regulatory T cells (Tregs) and Gamma delta T cells. In some embodiments, an immune cell of the disclosure is a B lymphocyte. In some embodiments, an immune cell of the disclosure is a natural killer cell. In some embodiments, an immune cell of the disclosure is an antigen-presenting cell.

[0330] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is a muscle cell. In some embodiments, a muscle cell of the disclosure is a myoblast or a myocyte. In some embodiments, a muscle cell of the disclosure is a cardiac muscle cell, skeletal muscle cell or smooth muscle cell. In some embodiments, a muscle cell of the disclosure is a striated cell.

[0331] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is an epithelial cell. In some embodiments, an epithelial cell of the disclosure forms a squamous cell epithelium, a cuboidal cell epithelium, a columnar cell epithelium, a stratified cell epithelium, a pseudostratified columnar cell epithelium or a transitional cell epithelium. In some embodiments, an epithelial cell of the disclosure forms a

gland including, but not limited to, a pineal gland, a thymus gland, a pituitary gland, a thyroid gland, an adrenal gland, an apocrine gland, a holocrine gland, a merocrine gland, a serous gland, a mucous gland and a sebaceous gland. In some embodiments, an epithelial cell of the disclosure contacts an outer surface of an organ including, but not limited to, a lung, a spleen, a stomach, a pancreas, a bladder, an intestine, a kidney, a gallbladder, a liver, a larynx or a pharynx. In some embodiments, an epithelial cell of the disclosure contacts an outer surface of a blood vessel or a vein.

[0332] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is a neuronal cell. In some embodiments, a neuron cell of the disclosure is a neuron of the central nervous system. In some embodiments, a neuron cell of the disclosure is a neuron of the brain or the spinal cord. In some embodiments, a neuron cell of the disclosure is a neuron of the retina. In some embodiments, a neuron cell of the disclosure is a neuron of a cranial nerve or an optic nerve. In some embodiments, a neuron cell of the disclosure is a neuron of the peripheral nervous system. In some embodiments, a neuron cell of the disclosure is a neuroglial or a glial cell. In some embodiments, a glial of the disclosure is a glial cell of the central nervous system including, but not limited to, oligodendrocytes, astrocytes, ependymal cells, and microglia. In some embodiments, a glial of the disclosure is a glial cell of the peripheral nervous system including, but not limited to, Schwann cells and satellite cells.

[0333] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is a primary cell.

[0334] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is a cultured cell.

[0335] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is in vivo, in vitro, ex vivo or in situ.

[0336] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is autologous or allogeneic.

Masking Modified Cells of the Disclosure

[0337] Compositions of the disclosure simultaneously deliver a gene therapy and prevent expression of antigens derived from the gene therapy construct or associated delivery vector from display on the surface of a modified cell of the disclosure.

[0338] By inhibiting or reducing expression of a component of an adaptive immune response in the modified cell, the modified cell is invisible to a host immune system. For example, compositions of the disclosure may simultaneously target an RNA molecule associated with a genetic disease or disorder and an RNA molecule that encodes the β 2M subunit of the MHC I. By selectively targeting an RNA molecule that encodes the β 2M subunit of the MHC I, the composition prevents the modified cell from displaying one or more antigen peptides derived from an RNA targeting construct, vector, or combination thereof on the surface of the modified cell. Consequently, a subject's immune system does not identify the modified cell as containing foreign sequences and does not attempt to mount an immune response directed at the modified cell. This method increases the therapeutic efficacy of the treatment of the genetic disease or disorder while avoiding a common side effect of gene therapy.

[0339] In some embodiments of the compositions and methods of the disclosure, the component of an adaptive immune response comprises or consists of a component of a type I major histocompatibility complex (MHC I), a type II major histocompatibility complex (MHC II), a T-cell receptor (TCR), a costimulatory molecule or a combination thereof. In some embodiments, the MHC I component comprises an α 1 chain, an α 2 chain, an α 3 chain, or a β 2M protein. In some embodiments, the component of an adaptive immune response comprises or consists of an MHC I β 2M protein. In some embodiments, the MHC II component comprises an α 1 chain, an α 2 chain, a β 1 chain, or a β 2 chain. In some embodiments, the TCR component comprises an α -chain and a β -chain. In some embodiments, the costimulatory molecule comprises a Cluster of Differentiation 28 (CD28), a Cluster of Differentiation 80 (CD80), a Cluster of Differentiation 86 (CD86), an Inducible T-cell COStimulator (ICOS), or an ICOS Ligand (ICOSLG) protein.

[0340] An α -chain of an MHC I may be encoded by an HLA gene, including but not limited to, HLA-A, HLA-B and HLA-C.

[0341] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding an α -chain derived from an HLA-A gene comprising or consisting of 20 nucleotides of the sequence of

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1  atggcgcgtca tggcgccccc aaccctcgtc ctgctactct cgggggctct ggccctgacc
61  cagacctggg cgggctctca ctccatgagg tatttcttca catccgtgtc ccggcccggc
121 cgcggggagc cccgcttcat cgcagtgggc tacgtggacg acacgcagtt cgtgcgggtc
181 gacagcgacg ccgcgagcca gaggatggag ccgcgggcgc cgtggataga gcaggagggt
241 ccggagtatt gggacgggga gacacggaaa gtgaaggccc actcacagac tcaccgagtg

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301 gacctgggga ccctgcgcgg ctactacaac cagagcgagg ccggttctca caccgtccag
361 aggatgtgtg gctgcgacgt ggggtcggac tggcgcttcc tccgcgggta ccaccagtac
421 gcctacgacg gcaaggatta catcgccctg aaagaggacc tgcgctcttg gaccgcggcg
481 gacatggcag ctcagaccac caagcacaag tgggaggcgg cccatgtggc ggagcagttg
541 agagcctacc tggagggcac gtgctgtggag tggctccgca gatacctgga gaacgggaag
601 gagacgctgc agcgcacgga cgccccaaa acgcatatga ctcaccacgc tgtctctgac
661 catgaagcca ccctgaggtg ctgggccttg agcttctacc ctgcgagat cactactgacc
721 tggcagcggg atggggagga ccagaccag gacacggagc tcgtggagac caggcctgca
781 ggggatggaa ccttcagaa gtgggcggct gtggtggtgc cttctggaca ggagcagaga
841 taaacctgcc atgtgcagca tgagggttg cccaagcccc tcacctgag atgggagccg
901 tcttcccagc ccaccatccc catcgtgggc atcattgctg gcctgggtct ctttgagct
961 gtgatcactg gagctgtggt cgctgctgtg atgtggagga ggaagagctc agatagaaaa
1021 ggagggagct actctcaggc tgcaagcagt gacagtgcc agggctctga tgtgtctctc
1081 acagcttgta aagtgtga (SEQ ID NO: 216).

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[0342] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding an α -chain derived from an HLA-B gene comprising or consisting of 20 nucleotides of the sequence of

```

1  tgggttagga gaagagggat caggacgaag tcccaggccc cgggcggggc tctcagggtc
61  tcaggctccg agggccgcgt ctgcaatggg gaggcgcagc gttggggatt cccactccc
121 acgagtttca cttcttctcc caacctatgt cgggtccttc ttccaggata ctctgacgc
181 gtccccattt cccactccca ttgggtgtcg ggtgtctaga gaagccaatc agcgtcgccg
241 tggtdccagt tctaaagtcc ccacgcacc acccgactc agaatctcct cagacgccga
301 gatgcgggtc acggcacccc gaaccgtcct cctgctgctc tcggcggccc tggccctgac
361 cgagacctgg gccggtgagt gcgggtcggc agggaaatgg cctctgtggg gaggagcgag
421 gggaccgcag gcgggggcgc aggaccggg gagccgcgcc gggagagggg tcgggcgggt
481 ctacgcccct cctcgcccc aggtcccac tccatgaggt atttcacac cgccatgtcc
541 cggcccggcc gcggggagcc ccgcttcac accgtgggct acgtggacga cagctgttc
601 gtgaggttcg acagcgacgc cagagtccg aggaaggagc cgcgggcgcc atggatagag
661 caggaggggc cggagtattg ggaccgggag acacagatct ccaagaccaa cacacagact
721 taccgagaga gcctgcggaa cctgcgcggc tactacaacc agagcgaggc cggtagtgta
781 ccccgcccg gggcgaggt cagactccc catccccac gtacggcccg ggtcgccccg
841 agtctccggg tccgagatcc gccccctga ggccgcggga cccgccaga ccctcgaccg
901 gcgagagccc caggcgcgtt taccgggtt cattttcagt tgaggccaaa atcccccgcg
961 gttggtcggg gcggggcggg gcggggctcg ggggacgggg ctgaccgcgg ggcctgggce
1021 aggtctcac acttggcaga ggatgtatgg ctgcgacctg gggccgcagc ggcgcctcct
1081 ccgcggttat aaccagttag cctacgacgg caaggattac atcgccctga acgaggacct
1141 gagctcctgg accgcggcgg acaccgcggc tcagatcacc cagcgcaagt gggaggcggc
1201 ccgtgtggcg gagcaggaca gagcctacct ggagggcctg tgcgtggagt cgctccgcag
1261 atacctggag aacgggaagg agacgtgca gcgcgcgggt accaggggca gtggggagcc
1321 ttccccatct cctataggtc gccggggatg gctcccacg agaagaggag gaaaatggga
1381 tcagcgctag aatgtcgccc tcccttgaat ggagaatggc atgagttttc ctgagtttcc
1441 tctgagggcc ccctcttctc tctaggacaa taaggaatga cgtctctgag gaaatggagg
1501 ggaagacagt ccctagaata ctgatcagg gtcccctttg acccctgcag cagccttggg
1561 aaccgtgact ttctctcag gccttgttct ctgcctcaca ctcagtgtgt ttggggctct
1621 gattccagca cttctgagtc actttacct cactcagatc gggagcagaa gtccctgttc
1681 cccgtcaga gactcgaact ttccaatgaa taggagatta tcccagggtc ctgcgtccag
1741 gctggtgtct gggttctgtg ccccttcccc accccagggt tctgttccat tctcaggctg
1801 gtcacatggg tggctcctag gtgtcccatg agagatgcaa agcgcctgaa ttttctgact
1861 cttcccatca gacccccaa agacacatgt gaccaccac cccatctctg accatgaggc
1921 caccctgagg tgctggggcc tgggcttcta ccctgcggag atcacactga cctggcagcg
1981 ggatggcgag gacaaactc aggacaccga gcttgtggag accagaccag caggagatag
2041 aaccttcag aagtgggcag ctgtgtgtgt gccttctgga gaagagcaga gatacacatg
2101 ccatgtacag catgaggggc tgccgaagcc cctcacctg agatggggta aggaggggga

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2161 tgaggggtca tatctgttct cagggaaagc aggagccctt ctggagccct tcagcagggg
2221 cagggcccct catcttcccc tcctttccca gagccatctt cccagtcacac catccccatc
2281 gtgggcattg ttgctggcct ggctgtccta gcagttgtgg tcatcggagc tgtggtcgct
2341 actgtgatgt gtaggaggaa gagctcaggt aggggaaggg tgaggggtgg ggtctgggtt
2401 ttcttgtccc actgggggtt tcaagcccca ggtagaagtg ttccctgcct cattactggg
2461 aagcagcatc cacacagggg ctaacgcagc ctgggaccct gtgtgccagc acttactctt
2521 ttgtgcagca catgtgacaa tgaaggacgg atgtatcgcc ttgatgggtg tgggtgtggg
2581 gtcctgattc cagcattcat gagtcagggg aaggtccctg ctaaggacag accttaggag
2641 ggcagttggg ccaggaccca cacttgcttt cctcgtgttt cctgatcctg ccttgggtct
2701 gtagtcatac ttctggaaat tccttttggt tccaagacga ggaggttcct ctaagatctc
2761 atggccctgc ttccctcccag tcccctcaca ggacattttc ttcccacagg tggaaaagga
2821 gggagctact ctcaggctgc gtgtaagtgg tgggggtggg agtgtggagg agctcaccca
2881 ccccataatt cctcctgtcc cacgtctcct gagggctctg accaggctct gtttttgttc
2941 tactccagcc agcgacagtg cccagggtct tgatgtgtct ctcacagctt gaaaagggtg
3001 gattcttggg gtctagagtg ggtgggggtg cgggtctggg ggtgggtggg gcagtgggga
3061 aaggcctggg taatggagat tctttgattg ggatgtttcg cgtgtgtggg gggctgttca
3121 gagtgtcatc acttaccatg actaaccaga atttgttcat gactgttgtt ttctgtagcc
3181 tgagacagct gtcttgtgag ggactgagat gcaggatttc ttcacgcctc cctttgtga
3241 cttcaagagc ctctggcatc tctttctgca aaggcacctg aatgtgtctg cgtccctgtt
3301 agcataatgt gaggaggtgg agagacagcc cacccttgtg tccactgtga cccctgttcg
3361 catgctgacc tgtgtttcct cccca (SEQ ID NO: 217).

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[0343] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding an α -chain derived from an HLA-C gene comprising or consisting of 20 nucleotides of the sequence of

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1 tccgcagtc cggttctaaa gtccccagtc acccaccgga actcacattc tccccagagg
61 ccgagatgc ggtcatggcg ccccagagccc tcctcctgct gctctcggga ggccctggccc
121 tgaccgagac ctgggcctgc tcccactcca tgaggtatth cgacaccgccc gtgtcccggc
181 ccggccgcgg agagccccgc ttcattctcag tgggctacgt ggacgacacg cagttcgtgc
241 ggttcgacag cgacgccgcg agtccgagag gggagccgcg ggcgccgtgg gtggagcagg
301 aggggccgga gtattgggac cgggagacac agaagtacaa gcgccaggca caggctgacc
361 gagtgagcct gcggaacctg cgcggtactt acaaccagag cgaggacggg tctcacaccc
421 tccagaggat gtctggctgc gacctggggc ccgacggggc cctcctccgc gggatgacc
481 agtccgccta cgacggcaag gattacatcg cctgaacga ggacctgcgc tccctggacc
541 ccgcgacac cgcggtcag atcaccagc gcaagttgga ggcggccggt cggcgaggc
601 agctgagagc ctacctggag ggcacgtgcg tggagtggct ccgagatac ctggagaacg
661 ggaaggagac gctgcagcgc gcagaacccc caaagacaca cgtgaccac caccctctt
721 ctgacctga ggccacctg aggtgctggg cctgggctt ctacctgcg gagatcacac
781 tgacctggca gcgggatggg gaggaccaga cccaggacac cgagcttgtg gagaccaggc
841 cagcaggaga tggaacctc cagaagtggg cagctgtggg ggtgccttct ggacaagagc
901 agagatacac gtgccatatg cagcacgagg ggctgcaaga gcccctcacc ctgagctggg
961 agccatcttc ccagcccacc atccccatca tgggcatcgt tgctggcctg gctgtcctgg
1021 ttgtcctagc tgtccttgga gctgtgggtc ccgctatgat gtgtaggagg aagagctcag
1081 gtggaaaagg agggagctgc tctcaggctg cgtgcagcaa cagtgccag ggctctgatg
1141 agtctctcat cacttgtaaa gcctgagaca gctgcctgtg tgggactgag atgcaggatt
1201 tcttcacacc tctcctttgt gacttcaaga gcctctggca tctctttctg caaaggcacc
1261 tgaatgtgtc tgcgttcctg ttagcataat gtgaggaggt ggagagacag cccacccccg
1321 tgtccaccgt gacccctgtc cccacactga cctgtgttcc ctcccgcac atctttcctg
1381 ttccagagag gtggggctgg atgtctccat ctctgtctca aattcatggg gcactgagct
1441 gcaacttctt acttccttaa tgaagttaag aacctgaata taaatttgtg ttctcaaata
1501 tttgctatga agcgttgatg gattaattaa ataagtcaat tccatagaag tgagagagca
1561 aataaagacc tgagaacctt ccagaa (SEQ ID NO: 218).

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[0344] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding an α -chain derived from an HLA-C gene comprising or consisting of 20 nucleotides of the sequence of

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1  tccgcagtc cggttctaaa gtccccagtc acccaccgga actcacattc tccccagagg
61  ccgagatgcg ggtcatggcg ccccgagccc tcctcctgct gctctcggga ggccctggccc
121  tgaccgagac ctgggcctgc tcccactcca tgaggtattt cgacaccgcc gtgtcccggc
181  ccggccgcgg agagccccgc ttcactctcag tgggctacgt ggacgacacg cagttcgtgc
241  ggttcgacag cgacgcccg agtccgagag gggagccgcg ggccgctggg gtggagcagg
301  aggggccgga gtattgggac cgggagacac agaactacaa gcgccaggca caggctgacc
361  gagtgcgact gcggaacctg cgcggctact acaaccagag cgaggacggg tctcacaccc
421  tccagaggat gtatggctgc gacctggggc ccgacgggcg cctcctccgc gggatgacc
481  agtccgccta cgacggcaag gattacatcg ccctgaacga ggacctgcgc tcctggaccg
541  ccgcgacac cgcggtcag atcaccacgc gcaagtggga ggccggccgt gcggcggagc
601  agctgagagc ctacctggag ggcagtgcg tggagtggct ccgcagatac ctggagaacg
661  ggaaggagac gctgcagcgc gcagaacccc caaagacaca cgtgacccac caccctctct
721  ctgaccatga ggccacctg aggtgctggg cctgggctt ctacctgctg gagatcacac
781  tgacctggca gcgggatggg gaggacaga ccaggacac cgagcttggt gagaccaggc
841  cagcaggaga tggaaacctt cagaagtggg cagctgtggt ggtgccttct ggacaagagc
901  agagatacac gtgccatatg cagcacgagg ggctgcaaga gccctcacc ctgagctggg
961  agccatcttc ccagcccacc atccccatca tgggcatcgt tgcctggctg gctgtcctgg
1021  ttgtcctagc tgccttgga gctgtggtca ccgctatgat gtgtaggagg aagagctcag
1081  gtggaaaagg agggagctgc tctcaggctg cgtgcagcaa cagtgccag ggctctgatg
1141  agtctctcat cacttgtaaa gcctgagaca gctgcctgtg tgggactgag atgcaggatt
1201  tcttcacacc tctcctttgt gacttcaaga gcctctggca tctctttctg caaaggcgctc
1261  tgaatgtgtc tgcgttcctg ttagcataat gtgaggaggt ggagagacag cccacccccg
1321  tgtccaccgt gaccctgtc cccacactga cctgtgttcc ctcccgatc atctttcctg
1381  ttccagagag gtggggctgg atgtctccat ctctgtctca aattcatggt gactgagct
1441  gcaacttctt acttcctaa tgaagttaag aacctgaata taaatttggt ttctcaaata
1501  tttgctatga agcgttgatg gattaattaa ataagtcaat tcctagaagt tgagagagca
1561  aataaagacc tgagaacctt ccagaa (SEQ ID NO: 219).

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[0345] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding an β 2M protein comprising or consisting of 20 nucleotides of the sequence of

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1  attcctgaag ctgacagcat tcgggccgag atgtctcgct ccgtggcctt agctgtgctc
61  gcgctactct ctctttctgg cctggaggct atccagcgta ctccaaagat tcagggtttac
121  tcacgtcatc cagcagagaa tggaaagtca aatttcctga attgctatgt gtctgggttt
181  catccatccg acattgaagt tgacttactg aagaatggag agagaattga aaaagtggag
241  cattcagact tgtctttcag caaggactgg tctttctatc tcttgacta cactgaattc
301  accccactg aaaaagatga gtatgcctgc cgtgtgaacc atgtgacttt gtcacagccc
361  aagatagtta agtgggatcg agacatgtaa gcagcatcat ggaggtttga agatgccgca
421  tttggattgg atgaattcca aattctgctt gcttgctttt taatattgat atgcttatac
481  acttacactt tatgcacaaa atgtagggtt ataataatgt taacatggac atgatcttct
541  ttataattct actttgagtg ctgtctccat gtttgatgta tctgagcagg ttgctccaca
601  ggtagctcta ggagggctgg caacttagag gtggggagca gagaattctc ttatccaaca
661  tcaacatctt ggtcagattt gaactcttca atctcttgca ctcaaagctt gttaagatag
721  ttaagcgtgc ataagttaac ttccaattta catactctgc ttagaatttg ggggaaaatt
781  tagaaatata attgacagga ttattggaaa tttgttataa tgaatgaaac attttgtcat
841  ataagattca tatttacttc ttatacattt gataaagtaa ggcatgggtt tgggttaatct
901  ggtttatatt tgttcacaa gttaaataaa tcataaaact tgatgtgtta tctcttatat
961  ctactccca ctattacccc tttattttca aacagggaag cagtcttcaa gttccacttg

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1021 gtaaaaaatg tgaacccctt gtatatagag tttggctcac agtgtaaagg gcctcagtga
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1141 acacagctgt ctagtgggag gccagccttc tatatttttag ccagcgttct ttcctgctggg
1201 ccaggtcatg aggagtatgc agactctaag agggagcaaa agtatctgaa ggattttaata
1261 ttttagcaag gaatagatat acaatcatcc cttgggtctcc ctgggggatt ggtttcagga
1321 ccccttcttg gacaccaaat ctatggatat ttaagtccct tctataaaat ggtatagtat
1381 ttgcatataa cctatccaca tcctcctgta tacttttaaat catttctaga ttacttgtaa
1441 tacctaatac aatgtaaagt ctatgcaaat agttgttatt gtttaaggaa taatgacaag
1501 aaaaaaaagt ctgtacatgc tcagtaaaga cacaaccatc cctttttttc cccagtgttt
1561 ttgatccatg gtttgctgaa tccacagatg tggagcccct ggatacggaa ggcccgtctg
1621 actttgaatg acaaataaca gatttaaaat tttcaaggca tagttttata cctga (SEQ

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ID NO: 220) .

[0346] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CD28 protein comprising or consisting of 20 nucleotides of the sequence of

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[0347] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CD28 protein comprising or consisting of 20 nucleotides of the sequence of

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222) .

[0348] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CD28 protein comprising or consisting of 20 nucleotides of the sequence of

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3901 ccagccattt gcactgccag ctgggaacta taccagacct ggatactgat cccaaagtgt
3961 taaattcaac tacatgctgg agattagaga tggtgccaat aaaggacca gaaccaggat
4021 cttgattgct atagacttat taataatcca ggtcaaagag agtgacacac actctctcaa
4081 gacctgggtt gagggagtct gtgttatctg caaggccatt tgaggctcag aaagtctctc
4141 tttcctatag atatatgcat actttctgac atataggaat gtatcaggaa tactcaacca
4201 tcacagggcat gttcctacct cagggccttt acatgtcctg tttactctgt ctagaatgtc
4261 cttctgtaga tgacctggct tgccctgtca cccttcaggt ccttgctcaa gtgtcatctt
4321 ctcccctagt taaactaccc cacacctgtt ctgctttcct tgcttatttt tctccatagc
4381 attttacat ctcttacatt agacatttct cttattttat tgtagtttat aagcttcatg
4441 aggcaagtaa ctttgctttg tttcttgctg tatctccagt gccagagca gtgcctggta
4501 tataataaat atttattgac tgagtgtaaa aaaaaaaaaa aa (SEQ ID NO: 223).

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[0349] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CD80 protein comprising or consisting of 20 nucleotides of the sequence of

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1 gacaagtact gagtgaactc aaaccctctg taaagtaaca gaagttagaa ggggaaatgt
61 cgctctctctg aagattaccc aaagaaaaag tgatttgtca ttgctttata gactgtaaga
121 agagaacatc tcagaagtgg agtcttacct tgaaatcaaa ggattttaaag aaaaagtgga

```

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181 attttttcttc agcaagctgt gaaactaaat ccacaacctt tggagaccca ggaacaccct
241 ccaatctctg tgtgttttgt aaacatcact ggagggtctt ctacgtgagc aattggattg
301 tcatcagccc tgcctgtttt gcacctggga agtgccctgg tcttacttgg gtccaaattg
361 ttggctttca cttttgaccc taagcatctg aagccatggg ccacacacgg aggcagggaa
421 catcaccatc caagtgtcca tacctcaatt tctttcagct cttggtgctg gctggtcttt
481 ctcaattctg ttcaggtgtt atccacgtga ccaaggaagt gaaagaagtg gcaacgctgt
541 cctgtggtca caatgtttct gttgaagagc tggcacaacac tcgcatctac tggcaaaagg
601 agaagaaaat ggtgctgact atgatgtctg gggacatgaa tatatggccc gagtacaaga
661 accggaccat ctttgatata actaataacc tctccattgt gatcctggct ctgcgcccac
721 ctgacgaggg cacatacgag tgtgttgttc tgaagtatga aaaagacgct ttcaagcggg
781 aacacctggc tgaagtgcg ttatcagtc aagctgactt ccctacacct agtatatctg
841 actttgaaat tccaacttct aatattagaa ggataatttg ctcaacctct ggagggtttc
901 cagagcctca cctctcctgg ttggaaaatg gagaagaatt aaatgccatc aacacaacag
961 tttcccaaga tcctgaaact gagctctatg ctgttagcag caaactggat ttcaatatga
1021 caaccaacca cagcttcatg tgtctcatca agtatggaca tttaagagtg aatcagacct
1081 tcaactggaa tacaaccaag caagagcatt ttctgataa cctgctccca tcctgggcca
1141 ttaccttaat ctcagtaaat ggaatttttg tgatatgctg cctgacctac tgctttgccc
1201 caagatgcag agagagaagg aggaatgaga gattgagaag ggaaagtgtg cgccctgtat
1261 aacagtgtcc gcagaagcaa ggggctgaaa agatctgaag gtcccacctc catttgcaat
1321 tgacctcttc tgggaacttc ctcagatgga caagattacc ccaccttgcc ctttacgtat
1381 ctgctcttag gtgcttcttc acttcagttg ctttgagga agtgtctaga ggaatatggt
1441 gggcacagaa gtagctctgg tgacctgat caaggtgttt tgaaatgcag aattcttgag
1501 ttctggaagg gacttttagag aataccagtg ttattaatga caaaggcact gaggcccagg
1561 gaggtgaccc gaattataaa ggccagcgcc agaaccacga tttcctaact ctggtgctct
1621 ttccctttat cagtttgact gtggcctgtt aactggtata tacatatata tgtcaggcaa
1681 agtgctgctg gaagtagaat ttgtccaata acaggtcaac ttcagagact atctgatttc
1741 ctaatgtcag agtagaagat tttatgctgc tgtttacaaa agcccaatgt aatgcatagg
1801 aagtatggca tgaacatctt taggagacta atggaaatat tattggtgtt taccagatg
1861 tccatttttt tcattgtgtt ctctattgct gctctctcac tccccatga ggtacagcag
1921 aaaggagaac tatccaaaac taatttcctc tgacatgtaa gacgaatgat ttaggtacgt
1981 caaagcagta gtcaaggagg aaagggatag tccaaagact taactggttc atattggact
2041 gataatctct ttaaatggct ttatgctagt ttgacctcat ttgtaaaata tttatgagaa
2101 agttctcatt taaaatgaga tcgttgttta cagtgtatgt actaagcagt aagctatctt
2161 caaatgtcta aggtagtaac tttccatagg gcctccttag atccctaaga tggctttttc
2221 tccttggtat ttctgggtct ttctgacatc agcagagaac tggaaagaca tagccaactg
2281 ctgttcatgt tactcatgac tcctttctct aaaactgcct tccacaattc actagaccag
2341 aagtggacgc aacttaagct gggataatca cattatcatc tgaaaatctg gagttgaaca
2401 gcaaaagaag acaacatttc tcaaatgcac atctcatggc agctaagcca catggctggg
2461 atttaaagcc tttagagcca gcccatggct ttagctacct cactatgctg cttcacaac
2521 cttgctcctg tgtaaaacta tattctcagt gtagggcaga gaggtctaac accaacataa
2581 ggtactagca gtgtttcccg tattgacagg aatacttaac tcaataattc ttttcttttc
2641 catttagtaa cagttgtgat gactatgttt ctattctaag taattcctgt attctacagc
2701 agatactttg tcagcaatac taagggaaga aacaaagttg aaccgtttct ttaataa

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(SEQ ID NO: 224)

[0350] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a CD80 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of comprising SEQ ID NO: 330 to SEQ ID NO: 3067.

[0351] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CD86 protein comprising or consisting of 20 nucleotides of the sequence of:

```

1  agtcattgcc gaggaaggct tgcacaggggt gaaagctttg cttctctgct gctgtaacag
61 ggactagcac agacacacgg atgagtgggg tcatttccag atattaggtc acagcagaag
121 cagccaaaat ggatccccag tgcactatgg gactgagtaa cattctcttt gtgatggcct
181 tcctgctctc tgggtgctgct cctctgaaga ttcaagctta tttcaatgag actgcagacc
241 tgccatgcc aattgcaaac tctcaaaacc aaagcctgag tgagctagta gtattttggc
301 aggaccagga aaacttggtt ctgaatgagg tatacttagg caaagagaaa tttgacagtg
361 ttcatctcaa gtatatgggc cgcacaagtt ttgattcgga cagttggacc ctgagacttc
421 acaatcttca gatcaaggac aagggttgt atcaatgtat catccatcac aaaaagccca
481 caggaatgat tcgcatccac cagatgaatt ctgaactgtc agtgcttgct aacttcagtc
541 aacctgaaat agtaccaatt tctaataata cagaaaatgt gtacataaat ttgacctgct
601 catctataca cggttacca gaacctaaga agatgagtgt tttgctaaga accaagaatt
661 caactatcga gtatgatggt attatgcaga aatctcaaga taatgtcaca gaactgtacg
721 acgtttccat cagcttgctt gtttcattcc ctgatgttac gagcaatatg accatcttct
781 gtattctgga aactgacaag acgcggtttt tatcttcacc tttctctata gagcttgagg
841 accctcagcc tccccagac cacattcctt ggattacagc tgtacttcca acagttatta
901 tatgtgtgat ggttttctgt ctaattctat ggaaatggaa gaagaagaag cggcctcgca
961 actcttataa atgtggaacc aacacaatgg agaggggaaga gagtgaacag accaagaaaa
1021 gagaaaaaat ccatatacct gaaagatctg atgaagccca gcgtgttttt aaaagttcga
1081 agacatcttc atgcgacaaa agtgatacat gtttttaatt aaagagtaaa gcccatacaa
1141 gtattcattt tttctaccct ttcttttgta agttcctggg caaccttttt gatttcttcc
1201 agaaggcaaa aagacattac catgagtaat aagggggctc caggactccc tctaagtggg
1261 atagcctccc tgtaactcca gctctgctcc gtatgccaa agggagacttt aattctctta
1321 ctgcttcttt tcacttcaga gcacacttat gggccaagcc cagcttaatg gctcatgacc
1381 tggaaataaa atttaggacc aatacctcct ccagatcaga ttcttctctt aatttcatag
1441 attgtgtttt ttttttaaat agacctctca atttctggaa aactgccttt tatctgccc
1501 gaattctaag ctggtgcccc actgaatttt gtgtacctgt gactaaacaa ctacctctc
1561 agtctgggtg ggacttatgt atttatgacc ttatagtgtt aatatcttga aacatagaga
1621 tctatgtact gtaatagtgt gattactatg ctctagagaa aagtctacct ctgctaagga
1681 gttctcatcc ctctgtcagg gtcagtaagg aaaacggtgg cctagggtag aggcaacaat
1741 gagcagacca acctaaattt ggggaaatta ggagaggcag agatagaacc tggagccact
1801 tctatctggg ctgttgctaa tattgaggag gcttgcccca cccaacaagc catagtggag
1861 agaactgaat aaacaggaaa atgccagagc ttgtgaacct tgtttctctt gaagaactga
1921 ctagttagat ggcctgggga agctgtgaaa gaacaaaaag agatcacaat actcaaaaga
1981 gagagagaga gaaaaagag agatcttgat ccacagaaat acatgaaatg tctggtctgt
2041 ccaccccatc aacaagtctt gaaacaagca acagatggat agtctgtcca aatggacata
2101 agacagacag cagtttccct ggtggtcagg gaggggtttt ggtgataccc aagttattgg
2161 gatgtcatct tcctggaagc agagctgggg agggagagcc atcaccttga taatgggatg
2221 aatggaagga ggcttaggac tttccactcc tggctgagag aggaagagct gcaacggaat
2281 taggaagacc aagacacaga tcaccggggg cttaacttagc ctacagatgt cctacgggaa
2341 cgtgggctgg ccagcatag ggctagcaaa tttgagttgg atgattgttt ttgctcaagg
2401 caaccagagg aaacttgcat acagagacag atatactggg agaaatgact ttgaaaacct
2461 ggctctaagg tgggatcact aagggatggg gcagtctctg cccaaacata aagagaactc
2521 tggggagcct gagccacaaa aatgttcctt tattttatgt aaacctcaa gggttataga
2581 ctgccatgct agacaagctt gtccatgtaa tattcccatg tttttaccct gccctgctt
2641 tgattagact ctagcacct ggctagtttc taacatgttt tgtgcagcac agtttttaat
2701 aaatgcttgt tacattcatt taaaaaaaaa aaaaa. (SEQ ID NO: 226)

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[0352] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a CD86 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of SEQ ID NO: 3068 to SEQ ID NO: 5783.

[0353] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CD86 protein comprising or consisting of 20 nucleotides of the sequence of

```

1  ccctttctgt  atttgagttc  taccgtcagt  cctggcatta  tttctctctc  tacaaggagc
61  cttaggaggt  acggggagct  cgaaataact  ccttttggtt  tattcttacc  accttgcttc
121  tgtgttcctt  ggaatgctg  ctgtgcttat  gcatctggtc  tctttttgga  gctacagtgg
181  acaggcattt  gtgacagcac  tatgggactg  agtaacattc  tctttgtgat  ggccttcctg
241  ctctctggtg  ctgctcctct  gaagattcaa  gcttatttca  atgagactgc  agacctgcca
301  tgccaatttg  caaactctca  aaaccaaagc  ctgagtgagc  tagtagtatt  ttggcaggac
361  caggaaaact  tggttctgaa  tgaggtatac  ttaggcaaag  agaaatttga  cagtgttcat
421  tccaagtata  tgggccgcac  aagttttgat  tcggacagtt  ggaccctgag  acttcacaat
481  cttcagatca  aggacaaggg  cttgtatcaa  tgtatcatcc  atcacaaaaa  gccacagga
541  atgattcgca  tccaccagat  gaattctgaa  ctgtcagtgc  ttgctaactt  cagtcaacct
601  gaaatagtac  caatttctaa  tataacagaa  aatgtgtaca  taaatttgac  ctgctcatct
661  atacacggtt  acccagaacc  taagaagatg  agtgttttgc  taagaaccaa  gaattcaact
721  atcgagtatg  atggtattat  gcagaaatct  caagataatg  tcacagaact  gtacgacgtt
781  tccatcagct  tgtctgtttc  attccctgat  gttacgagca  atatgaccat  cttctgtatt
841  ctggaaactg  acaagacgcg  gcttttatct  tcacctttct  ctatagagct  tgaggacctt
901  cagcctcccc  cagaccacat  tccttggtat  acagctgtac  ttccaacagt  tattatatgt
961  gtgatggttt  tctgtctaat  tctatggaaa  tggaagaaga  agaagcggcc  tcgcaactct
1021  tataaatgtg  gaaccaacac  aatggagagg  gaagagagtg  aacagaccaa  gaaaagagaa
1081  aaaatccata  tacctgaaag  atctgatgaa  gccagcgtg  tttttaaaag  ttccaagaca
1141  tcttcatgcg  acaaaagtga  tacatgtttt  taattaaaga  gtaaagccca  tacaagtatt
1201  cattttttct  accctttcct  ttgtaagttc  ctgggcaacc  tttttgattt  cttccagaag
1261  gcaaaaagac  attaccatga  gtaataaggg  ggctccagga  ctccctctaa  gtggaatagc
1321  ctccctgtaa  ctccagctct  gctccgtatg  ccaagaggag  actttaattc  tcttactgct
1381  tcttttcact  tcagagcaca  cttatgggcc  aagcccagct  taatggtcca  tgacctggaa
1441  ataaaattta  ggaccaatac  ctccctccaga  tcagattctt  ctcttaattt  catagattgt
1501  gttttttttt  taaatagacc  tctcaatttc  tgaaaaactg  ccttttatct  gcccagaatt
1561  ctaagctggg  gcccactga  attttgtgta  cctgtgacta  aacaactacc  tcctcagtct
1621  ggggtgggact  tatgtattta  tgacctata  gtgttaatat  cttgaaacat  agagatctat
1681  gtactgtaat  agtgtgatta  ctatgctcta  gagaaaagtc  taccctgct  aaggagtctt
1741  catccctctg  tcagggtcag  taaggaaaac  ggtggcctag  ggtacaggca  acaatgagca
1801  gaccaaccta  aatttgggga  aattaggaga  ggcagagata  gaacctggag  ccacttctat
1861  ctgggctggt  gctaattatt  aggaggcttg  cccacccaa  caagccatag  tggagagaac
1921  tgaataaaca  ggaaaatgcc  agagcttgtg  aaccctgttt  ctcttgaaga  actgactagt
1981  gagatggcct  ggggaagctg  tgaaagaacc  aaaagagatc  acaatactca  aaagagagag
2041  agagagaaaa  aagagagatc  ttgatccaca  gaaatacatg  aaatgtctgg  tctgtccacc
2101  ccatcaacaa  gtcttgaaac  aagcaacaga  tggatagtct  gtccaaatgg  acataagaca
2161  gacagcagtt  tccctgggtg  tcaggagggg  gttttgggtg  tacccaagtt  attgggatgt
2221  catcttcctg  gaagcagagc  tggggaggga  gagccatcac  cttgataatg  ggatgaatgg
2281  aaggaggctt  aggactttcc  actcctggct  gagagaggaa  gagctgcaac  ggaattagga
2341  agaccaagac  acagatcacc  cggggcttac  ttagcctaca  gatgtcctac  gggaacgtgg
2401  gctggcccag  catagggcta  gcaaatttga  gttggatgat  tgtttttgct  caaggcaacc
2461  agaggaaact  tgcatacaga  gacagatata  ctgggagaaa  tgactttgaa  aacctggctc
2521  taaggtggga  tcactaaggg  atggggcagt  ctctgcccaa  acataaagag  aactctgggg

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2581 agcctgagcc acaaaaatgt tcctttatatt tatgtaaacc ctcaaggggt atagactgcc
 2641 atgctagaca agcttgtcca tgtaatatcc ccatgttttt accctgcccc tgccttgatt
 2701 agactcctag cacctggcta gtttctaaca tgttttgtgc agcacagttt ttaataaatg
 2761 cttgttacat tcattttaaaa aaaaaaaaaa. (SEQ ID NO: 227)

[0354] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CD86 protein comprising or consisting of 20 nucleotides of the sequence of

1 ccctttctgt atttgagttc taccgtcagt cctggcatta tttctctctc tacaaggagc
 61 cttaggaggt acggggagct cgcaaatact cctttttggt tattcttacc accttgcttc
 121 tgtgttcctt gggaatgctg ctgtgcttat gcatctggtc tcttttttga gctacagtgg
 181 acaggcattt gtgacagcac tatgggactg agtaacattc tctttgtgat ggccttcctg
 241 ctctctgggtg ctgctcctct gaagattcaa gcttatttca atgagactgc agacctgcc
 301 tgccaatttg caaactctca aaaccaaagc ctgagtgaac tagtagtatt ttggcaggac
 361 caggaaaact tggttctgaa tgaggtatag ttaggcaaag agaaatttga cagtgttcat
 421 tccaagtata tgggcccgcac aagttttgat tcggacagtt ggaccctgag acttcacaat
 481 cttcagatca aggacaaggg cttgtatcaa tgtatcatcc atcacaaaaa gccacagga
 541 atgattcgca tccaccagat gaattctgaa ctgtcagtgc ttgctaactt cagtcaacct
 601 gaaatagtac caatttctaa tataacagaa aatgtgtaca taaatttgac ctgctcatct
 661 atacacggtt acccagaacc taagaagatg agtgttttgc taagaaccaa gaattcaact
 721 atcgagtatg atggtattat gcagaaatct caagataatg tcacagaact gtacgacgtt
 781 tccatcagct tgtctgtttc attcctgatg gttacgagca atatgacctt cttctgtatt
 841 ctggaaaactg acaagacgcg gcttttatct tcacctttct ctataggaac caacacaatg
 901 gagaggggaag agagtgaaca gaccaagaaa agagaaaaaa tccatatacc tgaagatct
 961 gatgaagccc agcgtgtttt taaaagtctg aagacatctt catgcgacaa aagtgatata
 1021 tgtttttaat taaagagtaa agcccatata agtattcatt ttttctacct tttcctttgt
 1081 aagttcctgg gcaacctttt tgatttcttc cagaaggcaa aaagacatta ccatgagtaa
 1141 taaggggggt ccaggactcc ctctaagtgg aatagcctcc ctgtaactcc agctctgctc
 1201 cgtatgccaa gaggagactt taattctctt actgcttctt ttcacttcag agcacactta
 1261 tgggccaagc ccagcttaat ggctcatgac ctggaaataa aatttaggac caatacctcc
 1321 tccagatcag attcttctct taatttcata gattgtgttt tttttttaa tagacctctc
 1381 aatttctgga aaactgcctt ttatctgccc agaattctaa gctggtgccc cactgaattt
 1441 tgtgtacctg tgactaaaca actacctctc cagtctgggt gggacttatg tatttatgac
 1501 cttatagtgt taatatcttg aaacataag atctatgtac tgtaattgag tgattactat
 1561 gctctagaga aaagtctacc cctgctaagg agttctcatc cctctgtcag ggtcagtaag
 1621 gaaaacggtg gcctagggta caggcaacaa tgagcagacc aacctaaatt tggggaaatt
 1681 aggagaggca gagatagaac ctggagccac ttctatctgg gctgttgcta atattgagga
 1741 ggcttgcccc acccaacaag ccatagtgga gagaactgaa taaacaggaa aatgccagag
 1801 cttgtgaacc ctgtttctct tgaagaactg actagtgaga tggcctgggg aagctgtgaa
 1861 agaaccacaaa gagatcacia tactcaaaag agagagagag agaaaaaaga gagatcttga
 1921 tccacagaaa tacatgaaat gtctggtctg tccaccccat caacaagtct tgaacaagc
 1981 aacagatgga tagtctgtcc aaatggacat aagacagaca gcagtttccc tgggtggtcag
 2041 ggagggggtt tgggtgatacc caagttattg ggatgtcatc ttcctggaag cagagctggg
 2101 gagggagagc catcaccttg ataattgggat gaatggaagg aggcttagga ctttccactc
 2161 ctggctgaga gaggaagagc tgcaacggaa ttaggaagac caagacacag atcacccggg
 2221 gcttacttag cctacagatg tcctacggga acgtgggctg gccagcaca gggctagcaa
 2281 atttgagttg gatgattgtt tttgctcaag gcaaccagag gaaacttgca tacagagaca
 2341 gatatactgg gagaaatgac tttgaaaacc tggctctaag gtgggatcac taagggatgg
 2401 ggaggtctct gcccaaacat aaagagaact ctggggagcc tgagccacaa aaatgttctc
 2461 ttatttttatg taaacctca agggttatag actgccatgc tagacaagct tgtccatgta
 2521 atattcccat gtttttacct tggccctgcc ttgattagac tcctagcacc tggctagttt
 2581 ctaacatggt ttgtgcagca cagtttttaa taaatgcttg ttacattcat taaaaaaaaa
 2641 aaaaaa. (SEQ ID NO: 228)

[0355] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CD86 protein comprising or consisting of 20 nucleotides of the sequence of

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1  agtcattgcc gaggaaggct tgcacagggt gaaagctttg cttctctgct gctgtaacag
61 ggactagcac agacacacgg atgagtgggg tcatttccag atattaggtc acagcagaag
121 cagccaaaat ggatccccag tgcactatgg gactgagtaa cattctcttt gtgatggcct
181 tcctgctctc tgctaacttc agtcaacctg aaatagtacc aatttctaata ataacagaaa
241 atgtgtacat aaatttgacc tgctcatcta tacacggtta ccagaacct aagaagatga
301 gtgttttgct aagaaccaag aattcaacta tcgagtatga tggattatg cagaaatctc
361 aagataatgt cacagaactg tacgacgttt ccatcagctt gtctgtttca ttccctgatg
421 ttacgagcaa tatgaccatc ttctgtattc tggaaactga caagacgcgg cttttatctt
481 cacctttctc tatagagctt gaggacctc agcctcccc agaccacatt cttggatta
541 cagctgtact tccaacagtt attatatgtg tgatggtttt ctgtctaatt ctatggaaat
601 ggaagaagaa gaagcggcct cgcaactctt ataatgtgg aaccaacaca atggagaggg
661 aagagagtga acagaccaag aaaagagaaa aaatccatat acctgaaaga tctgatgaag
721 ccagcgtgt ttttaaaagt tcgaagacat cttcatgcga caaaagtgat acatgttttt
781 aattaaagag taaagcccat acaagtattc attttttcta ccctttcctt tgtaagttcc
841 tgggcaacct ttttgatttc ttccagaagg caaaaagaca ttaccatgag taataagggg
901 gctccaggac tccctctaag tggaaatagc tccctgtaac tccagctctg ctccgtatgc
961 caagaggaga ctttaattct cttactgctt cttttcactt cagagcacac ttatgggcca
1021 agcccagctt aatggctcat gacctgaaa taaaatttag gaccaatacc tctccagat
1081 cagattcttc tcttaatttc atagattgtg tttttttttt aaatagacct ctcaatttct
1141 ggaaaactgc cttttatctg ccagaattc taagctgggt cccactgaa ttttgtgtac
1201 ctgtgactaa acaactacct cctcagtctg ggtgggactt atgtatttat gaccttatag
1261 tgtaaatatc ttgaaacata gagatctatg tactgtaata gtgtgattac tatgctctag
1321 agaaaagtct acccctgcta aggagttctc atccctctgt cagggtcagt aaggaaaacg
1381 gtggcctagg gtacaggcaa caatgagcag accaacctaa atttggggaa attaggagag
1441 gcagagatag aacctggagc cacttctatc tgggctgttg ctaatatgga ggaggcttgc
1501 cccacccaac aagccatagt ggagagaact gaataaacag gaaaatgcca gagcttgtga
1561 accctgtttc tcttgaagaa ctgactagtg agatggcctg gggaagctgt gaaagaacca
1621 aaagagatca caatactcaa aagagagaga gagagaaaaa agagagatct tgatccacag
1681 aaatacatga aatgtctggt ctgtccacc catcaacaag tcttgaaaca agcaacagat
1741 ggatagtctg tccaaatgga cataagacag acagcagttt cctggtggt caggaggagg
1801 tttgtgtgat acccaagtta ttgggatgtc atcttcctg aagcagagct ggggaggagg
1861 agccatcacc ttgataatgg gatgaatgga aggaggctta ggactttcca ctccctggctg
1921 agagaggaag agctgcaacg gaattaggaa gaccaagaca cagatcccc ggggcttact
1981 tagcctacag atgtcctacg ggaacgtggg ctggcccagc atagggctag caaatttgag
2041 ttggatgatt gtttttgctc aaggcaacca gaggaactt gcatacagag acagatatatc
2101 tgggagaaat gactttgaaa acctggctct aaggtgggat cactaaggga tggggcagtc
2161 tctgcccata cataaagaga actctgggga gcctgagcca caaaaatgtt cttttatttt
2221 atgtaaaccc tcaagggtta tagactgcca tgctagacaa gcttggtccat gtaatatcc
2281 catgttttta ccctgcccct gccttgatta gactcctagc acctggctag tttctaaca
2341 gttttgtgca gcacagtttt taataaatgc ttgttacatt catttaaaaa aaaaaaaaa.

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(SEQ ID NO: 229)

[0356] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CD86 protein comprising or consisting of 20 nucleotides of the sequence of

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1  agtcattgcc gaggaaggct tgcacagggt gaaagctttg cttctctgct gctgtaacag

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61 ggactagcac agacacacgg atgagtgggg tcatttccag atattaggtc acagcagaag
121 cagccaaaat ggatccccag tgggtgctgct cctctgaaga ttcaagctta tttcaatgag
181 actgcagacc tgccatgcca atttgcaaac tctcaaaacc aaagcctgag tgagctagta
241 gtatttttggc aggaccagga aaacttggtt ctgaatgagg tatacttagg caaagagaaa
301 tttgacagtg ttcattccaa gtatatgggc cgcacaagtt ttgattcgga cagttggacc
361 ctgagacttc acaatcttca gatcaaggac aagggcttgt atcaatgtat catccatcac
421 aaaaagccca caggaatgat tcgcatccac cagatgaatt ctgaactgtc agtgcttgct
481 aacttcagtc aacctgaaat agtaccaatt tctaataata cagaaaatgt gtacataaat
541 ttgacctgct catctataca cggttaccca gaacctaaga agatgagtgt tttgctaaga
601 accaagaatt caactatcga gtatgatggt attatgcaga aatctcaaga taatgtcaca
661 gaactgtacg acgtttccat cagcttgtct gtttcattcc ctgatgttac gagcaatatg
721 accatcttct gtattctgga aactgacaag acgcggcttt tatcttcacc tttctctata
781 gagcttgagg accctcagcc tccccagac cacattcctt ggattacagc tgtacttcca
841 acagttatta tatgtgtgat ggttttctgt ctaattctat ggaaatggaa gaagaagaag
901 cggcctcgca actcttataa atgtggaacc aacacaatgg agagggaaga gagtgaacag
961 accaagaaaa gagaaaaaat ccatatacct gaaagatctg atgaagccca gcgtgttttt
1021 aaaagttcga agacatcttc atgcgacaaa agtgatacat gtttttaatt aaagagtaaa
1081 gccatacaa gtattcattt tttctaccct ttcctttgta agttcctggg caaccttttt
1141 gatttcttcc agaaggcaaa aagacattac catgagtaat aagggggctc caggactccc
1201 tctaagtgga atagcctccc tgtaactcca gctctgctcc gtatgccaag aggagacttt
1261 aattctctta ctgcttcttt tcacttcaga gcacacttat gggccaagcc cagcttaatg
1321 gctcatgacc tggaaataaa atttaggacc aatacctcct ccagatcaga ttcttctctt
1381 aatttcatag attgtgtttt ttttttaaat agacctctca atttctggaa aactgccttt
1441 tatctgcccc gaattctaag ctgggtgcccc actgaatttt gtgtacctgt gactaaacaa
1501 ctacctctc agtctgggtg ggacttatgt atttatgacc ttatagtgtt aatatcttga
1561 aacatagaga tctatgtact gtaatagtgt gattactatg ctctagagaa aagtctaccc
1621 ctgctaagga gttctcatcc ctctgtcagg gtcagtaagg aaaacgggtg cctagggtac
1681 aggcaacaat gagcagacca acctaaattt ggggaaatta ggagaggcag agatagaacc
1741 tggagccact tctatctggg ctgttgctaa tattgaggag gcttgccca cccaacaagc
1801 catagtggag agaactgaat aaacaggaaa atgccagagc ttgtgaaccc tgtttctctt
1861 gaagaactga ctagtगतat ggctgggga agctgtgaaa gaaccaaag agatcacaat
1921 actcaaaaga gagagagaga gaaaaaagag agatcttgat ccacagaaat acatgaaatg
1981 tctggtctgt ccaccccatc aacaagtctt gaaacaagca acagatggat agtctgtcca
2041 aatggacata agacagacag cagtttccct ggtggtcagg gaggggtttt ggtgataccc
2101 aagttatttg gatgtcatct tcctggaagc agagctgggg agggagagcc atcaccttga
2161 taatgggatg aatggaagga ggcttaggac tttccactcc tggctgagag aggaagagct
2221 gcaacggaat taggaagacc aagacacaga tcaccgggg cttacttagc ctacagatgt
2281 cctacgggaa cgtgggctgg cccagcatag ggctagcaaa tttgagttgg atgattgttt
2341 ttgctcaagg caaccagagg aaacttgcac acagagacag atatactggg agaaatgact
2401 ttgaaaacct ggctctaagg tgggatcact aagggatggg gcagtctctg cccaaacata
2461 aagagaactc tggggagcct gagccacaaa aatgttcctt tattttatgt aaacctcaa
2521 gggttataga ctgccatgct agacaagctt gtccatgtaa tattcccatg tttttaccct
2581 gccctgctt tgattagact cctagcacct ggctaagttt taacatgttt tgtgcagcac
2641 agtttttaat aaatgcttgt tacattcatt taaaaaaaa aaaaa.

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(SEQ ID NO: 230)

[0357] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding ICOSLG protein comprising or consisting of 20 nucleotides of the sequence of

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1 AGTTAGAGCC GATCTCCCGC GCGCCGAGGT TGCTCCTCTC CGAGGTCTCC CGCGGCCCAA
61 GTTCTCCGCG CCGCGAGGTC TCCGCGCCCC GAGGTCTCCG CGGCCGAGG TCTCCGCCCG
121 CACCATGCGG CTGGGCAGTC CTGGACTGCT CTTCTTGCTC TTCAGCAGCC TTCGAGCTGA
181 TACTCAGGAG AAGGAAGTCA GAGCGATGGT AGGCAGCGAC GTGGAGCTCA GCTGCGCTTG

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241 CCCTGAAGGA AGCCGTTTTG ATTTAAATGA TGTTTACGTA TATTGGCAAA CCAGTGAGTC
301 GAAAACCGTG GTGACCTACC ACATCCCACA GAACAGCTCC TTGGAAAACG TGGACAGCCG
361 CTACCGGAAC CGAGCCCTGA TGTCACCGGC CGGCATGCTG CGGGGCGACT TCTCCCTGCG
421 CTTGTTCAAC GTCACCCCCC AGGACGAGCA GAAGTTTCAC TGCCTGGTGT TGAGCCAATC
481 CCTGGGATTC CAGGAGGTTT TGAGCGTTGA GGTTACACTG CATGTGGCAG CAAACTTCAG
541 CGTGCCCGTC GTCAGCGCCC CCCACAGCCC CTCCCAGGAT GAGCTCACCT TCACGTGTAC
601 ATCCATAAAC GGCTACCCCA GGCCCAACGT GTACTGGATC AATAAGACGG ACAACAGCCT
661 GCTGGACCAG GCTCTGCAGA ATGACACCGT CTTCTTGAAC ATGCGGGGCT TGTATGACGT
721 GGTCAGCGTG CTGAGGATCG CACGGACCCC CAGCGTGAAC ATTGGCTGCT GCATAGAGAA
781 CGTGCTTCTG CAGCAGAACC TGACTGTCGG CAGCCAGACA GGAAATGACA TCGGAGAGAG
841 AGACAAGATC ACAGAGAATC CAGTCAGTAC CGGCGAGAAA AACGCGGCCA CGTGGAGCAT
901 CCTGGCTGTC CTGTGCCTGC TTGTGGTCGT GGCGGTGGCC ATAGGCTGGG TGTGCAGGGA
961 CCGATGCCTC CAACACAGCT ATGCAGGTGC CTGGGCTGTG AGTCCGGAGA CAGAGCTCAC
1021 TGGTGAGTTT GCCGTGGGAA GCAGCAGGTT CTGGGGGGCC CAGGGGAGGC TTGGCTGCCA
1081 GCTGTCTTTC AGAGTTTCAA AAAACTTTCA AAAGGCAAAA GTCCCTTGCC TTGAACAAC
1141 GTTGTTCCTG GAGACGCAGC GAAGCCCTCG ATGGTGCGCA TGGCATTTC TGCAGCCTCC
1201 CCTTGGCATG GGATGGCATC CTGGTGTGCA CTTTGTACA CTGCGATGGG ATTTTCCCAA
1261 CATGCACAGA AGCAGAGAGA CGAGTGCTAG ACCCCCGCGC TCCCCAGTGC CCAGCCCCGA
1321 CCAGGTTGTC CAGGGCGGGT CCAGGCACCG GCGCCAGCC CCCATGGGGT GTCCGGAGTG
1381 GGTCCAGGCA CCGGCGCCCA GCCCCGTGG GGTGTCCAGG GCGGGTCCAG GCACCGGCGC
1441 CCAGCCCTG TGGGGTGTCC GGAGTGGGTC CGGGCACCGC CAGCTTCTCT CTGTGGCAGC
1501 CACTCCTGCA GCTCTCGTTT GCCCCCTCAGT TCCAGGAGCA ACATAGATGT GGATTCTCTG
1561 CCAATTTGGG AAAAATGTCC ACACACGGTC ACCCACCTGG CAGGTGCCTC TGGCTGCAAG
1621 GGGCGTGGG CTTCGCAGGC AGGCCAGCCG GGCTCCCCGC CATGGGCCAG GATCCCCTCC
1681 GAGCCCTGTT TGCCGCCAG GAGAAGGGGT TCCCCGGGGA CAGTGGGCTC AGGGTGTGCG
1741 CAGCCACCAT GCTGTGGTGT CACCTGTGGA CCCAGGCGAG CTGATGGCCG ACCGAGAAA
1801 CGCACTTCCA AGGCCAGGTC GGCCATCCA GATGATGCAG GAACACAGCT TGCTAAAAAC
1861 ACGGCGGGCC TGTCCCCGTC GGAGCCAGTC GAAGTTCCTT GAACAGGCCG CTGTTTCCGA
1921 AGCTTTAAAC CCTGTGTTTC CACCAAGCTG AGTCCTGAGA AAACCGACGT CTGCCTGCAG
1981 AAGGGAAAGG GGTGCTTCAT GTTCCTCTCT CTCCTTCATC TCCCT
(SEQ ID NO: 231).

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[0358] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a IOSLG protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 5784 to SEQ ID NO: 7789.

[0359] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding OX40L protein comprising or consisting of 20 nucleotides of the sequence of

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1  GGCCCTGGGA CCTTTGCCTA TTTTCTGATT GATAGGCTTT GTTTTGTCTT TACCTCCTTC
61  TTTCTGGGGA AAACCTCAGT TTTATCGCAC GTTCCCCTTT TCCATATCTT CATCTTCCCT
121 CTACCCAGAT TGTGAAGATG GAAAGGGTCC AACCCTGGA AGAGAATGTG GGAAATGCAG
181 CCAGGCCAAG ATTCGAGAGG AACAACTAT TGCTGGTGGC CTCTGTAATT CAGGACTGG
241 GGCTGCTCCT GTGCTTCACC TACATCTGCC TGCCTTCTC TGCTCTTCAG GTATCACATC
301 GGTATCCTCG AATTCAAAGT ATCAAAGTAC AATTTACCGA ATATAAGAAG GAGAAAGGTT
361 TCATCCTCAC TTCCCAAAAG GAGGATGAAA TCATGAAGGT GCAGAACAAC TCAGTCATCA
421 TCAACTGTGA TGGGTTTTAT CTCATCTCCC TGAAGGGCTA CTTCTCCCAG GAAGTCAACA
481 TTAGCCTTCA TTACCAGAAG GATGAGGAGC CCCTCTTCCA ACTGAAGAAG GTCAGGTCTG
541 TCAACTCCTT GATGGTGGCC TCTCTGACTT ACAAAGACAA AGTCTACTTG AATGTGACCA
601 CTGACAATAC CTCCCTGGAT GACTTCCATG TGAATGGCGG AGAACTGATT CTTATCCATC

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661 AAAATCCTGG TGAATTCCTGT GTCCTTTGAG GGGCTGATGG CAATATCTAA AACCAGGCAC
721 CAGCATGAAC ACCAAGCTGG GGGTGGACAG GGCATGGATT CTTCATTGCA AGTGAAGGAG
781 CCTCCCAGCT CAGCCACGTG GGATGTGACA AGAAGCAGAT CCTGGCCCTC CCGCCCCCAC
841 CCCTCAGGGA TATTTAAAC TATTTTATA TACCAGTTAA TCTTATTTAT CCTTATATTT
901 TCTAAATTGC CTAGCCGTCA CACCCCAAGA TTGCCTTGAG CCTACTAGGC ACCTTTGTGA
961 GAAAGAAAAA ATAGATGCCT CTTCTTCAAG ATGCATTGTT TCTATTGGTC AGGCAATTGT
1021 CATAATAAAC TTATGTCATT GAAAACGGTA CCTGACTACC ATTTGCTGGA AATTTGACAT
1081 GTGTGTGGCA TTATCAAAAT GAAGAGGAGC AAGGAGTGAA GGAGTGGGGT TATGAATCTG
1141 CCAAAGGTGG TATGAACCAA CCCCTGGAAG CCAAAGCGGC CTCTCCAAGG TTAAATTGAT
1201 TGCAGTTTGC ATATTGCCA AATTTAAACT TTCTCATTTG GTGGGGGTTT AAAAGAAGAA
1261 TCAGCTTTGTG AAAAATCAGG ACTTGAAGAG AGCCGTCTAA GAAATACCAC GTGCTTTTTT
1321 TCTTTACCAT TTTGCTTTCC CAGCCTCCAA ACATAGTTAA TAGAAATTTT CCTTCAAAGA
1381 ACTGTCTGGG GATGTGATGC TTTGAAAAAT CTAATCAGTG ACTTAAGAGA GATTTTCTTG
1441 TATACAGGGA GAGTGAGATA ACTTATTGTG AAGGGTTAGC TTTACTGTAC AGGATAGCAG
1501 GGAACCTGGAC ATCTCAGGGT AAAAGTCAGT ACGGATTTTA ATAGCCTGGG GAGGAAAACA
1561 CATTCTTTGC CACAGACAGG CAAAGCAACA CATGCTCATC CTCCTGCCTA TGCTGAGATA
1621 CGCACTCAGC TCCATGTCTT GTACACACAG AAACATTGCT GGTTCAGA AATGAGGTGA
1681 TCCTATTATC AAATTCAATC TGATGTCAAA TAGCACTAAG AAGTTATTGT GCCTTATGAA
1741 AAATAATGAT CTCTGTCTAG AAATACCATA GACCATATAT AGTCTCACAT TGATAATTGA
1801 AACTAGAAGG GTCTATAATC AGCCTATGCC AGGGCTTCAA TGGAATAGTA TCCCCTTATG
1861 TTTAGTTGAA ATGTCCCCTT AACTTGATAT AATGTGTTAT GCTTATGGCG CTGTGGACAA
1921 TCTGATTTTT CATGTCAACT TTCCAGATGA TTTGTAACCT CTCTGTGCCA AACCTTTTAT
1981 AAACATAAAT TTTTGAGATA TGTATTTTAA AATTGTAGCA CATGTTTCCC TGACATTTTC
2041 AATAGAGGAT ACAACATCAC AGAATCTTTC TGGATGATTC TGTGTTATCA AGGAATTGTA
2101 CTGTGCTACA ATTATCTCTA GAATCTCCAG AAAGGTGGAG GGCTGTTGCG CCTTACACTA
2161 AATGGTCTCA GTTGGATTTT TTTTTCCTGT TTTCTATTTT CTCTTAAGTA CACCTTCAAC
2221 TATATTCCCA TCCCTCTATT TTAATCTGTT ATGAAGGAAG GTAAATAAAA ATGCTAAATA
2281 GAAGAAATTG TAGGTAAGGT AAGAGGAATC AAGTTCTGAG TGGCTGCCAA GGCCTACACA
2341 GAATCATAAT CATGGCTAAA TATTTATGGA GGGCCTACTG TGGACCAGGC ACTGGGCTAA
2401 ATACTTACAT TTACAAGAAT CATTCTGAGA CAGATATTCA ATGATATCTG GCTTCACTAC
2461 TCAGAAGATT GTGTGTGTGT TTGTGTGTGT GTGTGTGTGT GTATTTCACT TTTTGTATT
2521 GACCATGTTT TGCAAAATTG CAGTTACTCA GTGAGTGATA TCCGAAAAAG TAAACGTTTA
2581 TGACTATAGG TAATATTTAA GAAAATGCAT GGTTCATTTT TAAGTTTGGA ATTTTTATCT
2641 ATATTTCTCA CAGATGTGCA GTGCACATGC AGGCCTAAGT ATATGTTGTG TGTGTTGTTT
2701 GTCTTTGATG TCATGGTCCC CTCTCTTAGG TGCTCACTCG CTTTGGGTGC ACCTGGCCTG
2761 CTCTTCCCAT GTTGGCCTCT GCAACCACAC AGGGATATTT CTGCTATGCA CCAGCCTCAC
2821 TCCACCTTCC TTCCATCAAA AATATGTGTG TGTGTCTCAG TCCCTGTAAG TCATGTCCTT
2881 CACAGGGAGA ATTAACCCTT CGATATACAT GGCAGAGTTT TGTGGGAAAA GAATTGAATG
2941 AAAAGTCAGG AGATCAGAAT TTTAAATTTG ACTTAGCCAC TAACTAGCCA TGTAACCTTG
3001 GGAAAGTCAT TTCCCATTTC TGGGTCTTGC TTTTCTTTCT GTTAAATGAG AGGAATGTTA
3061 AATATCTAAC AGTTTAGAAT CTTATGCTTA CAGTGTATAT TGTGAATGCA CATATTAAAT
3121 GTCTATGTTT TTGTTGCTAT GAGTCAAGGA GTGTAACCTT CTCCTTTACT ATGTTGAATG
3181 TATTTTTTTT TGGACAAGCT TACATCTTCC TCAGCCATCT TTGTGAGTCC TTCAAGAGCA
3241 GTTATCAATT GTTAGTTAGT TATTTTCTAT TTAGAGAATG CTTAAGGGAT TCCAATCCCG
3301 ATCCAAATCA TAATTTGTTT TTAAGTATAC TGGGCAGGTC CCCTATTTTA AGTCATAATT
3361 TTGTATTTAG TGCTTTCCTG GCTCTCAGAG AGTATTAATA TTGATATTAA TAATATAGTT
3421 AATAGTAATA TTGCTATTTA CATGGAAACA AATAAAAGAT CTCAGAATTC ACTAAAAAAA
3481 AAAA (SEQ ID NO: 232).

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[0360] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a OX40L protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 7790 to SEQ ID NO: 11254.

[0361] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding IL12 protein comprising or consisting of 20 nucleotides of the sequence of

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1  TTTTCGCTTTC  ATTTTGGGCC  GAGCTGGAGG  CGGCGGGGCC  GTCCCGGAAC  GGCTGCGGCC
61  GGGCACCCCG  GGAGTTAATC  CGAAAGCGCC  GCAAGCCCCG  CGGGCCGGCC  GCACCGCACG
121  TGTCAACGAG  AAGCTGATGT  AGAGAGAGAC  ACAGAAGGAG  ACAGAAAGCA  AGAGACCAGA
181  GTCCCGGGAA  AGTCCTGCCG  CGCCTCGGGA  CAATTATAAA  AATGTGGCCC  CCTGGGTCAG
241  CCTCCCAGCC  ACCGCCCTCA  CCTGCCGCGG  CCACAGGTCT  GCATCCAGCG  GCTCGCCCTG
301  TGTCCCTGCA  GTGCCGGCTC  AGCATGTGTC  CAGCGCGCAG  CCTCCTCCTT  GTGGCTACCC
361  TGGTCCTCCT  GGACCACCTC  AGTTTGGCCA  GAAACCTCCC  CGTGGCCACT  CCAGACCCAG
421  GAATGTTCCC  ATGCCCTTCA  CACTCCCAA  ACCTGCTGAG  GGCCGTGAGC  AACATGCTCC
481  AGAAGGCCAG  ACAAACCTA  GAATTTTACC  CTTGCACTTC  TGAAGAGATT  GATCATGAAG
541  ATATCACAAA  AGATAAAACC  AGCACAGTGG  AGGCCTGTTT  ACCATTGGAA  TTAACCAAGA
601  ATGAGAGTTG  CCTAAATTCC  AGAGAGACCT  CTTTCATAAC  TAATGGGAGT  TGCCTGGCCT
661  CCAGAAAGAC  CTCTTTTATG  ATGGCCCTGT  GCCTTAGTAG  TATTATGAA  GACTTGAAGA
721  TGTACCAGGT  GGAGTTCAG  ACCATGAATG  CAAAGCTTCT  GATGGATCCT  AAGAGCCAGA
781  TCTTTCTAGA  TCAAAACATG  CTGGCAGTTA  TTGATGAGCT  GATGCAGGCC  CTGAATTTCA
841  ACAGTGAGAC  TGTGCCACAA  AAATCCTCCC  TTGAAGAACC  GGATTTTTAT  AAAACTAAAA
901  TCAAGCTCTG  CATACTTCTT  CATGCTTTC  GAATTCGGGC  AGTGACTATT  GATAGAGTGA
961  TGAGCTATCT  GAATGCTTCC  TAAAAAGCGA  GGTCCCTCCA  AACCCTTGTC  ATTTTATATA
1021  AACTTTGAAA  TGAGGAAACT  TTGATAGGAT  GTGGATTAAG  AACTAGGGAG  GGGGAAAGAA
1081  GGATGGGACT  ATTACATCCA  CATGATACCT  CTGATCAAGT  ATTTTGTACA  TTTACTGTGG
1141  ATAAATTGTT  TTTAAGTTTT  CATGAATGAA  TTGCTAAGAA  GGGAAAATAT  CCATCCTGAA
1201  GGTGTTTTTC  ATTCACTTTA  ATAGAAGGGC  AAATATTTAT  AAGCTATTTT  TGTACCAAAG
1261  TGTTTGTGGA  AACAAACATG  TAAGCATAAC  TTATTTTAAA  ATATTTATTT  ATATAACTTG
1321  GTAATCATGA  AAGCATCTGA  GCTAACTTAT  ATTTATTTAT  GTTATATTTA  TTAAATTATT
1381  TATCAAGTGT  ATTTGAAAAA  TATTTTAAAG  TGTTCTAAAA  ATAAAAGTAT  TGAATTAAAG
1441  TGAATAAAAA  (SEQ ID NO: 233).

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[0362] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding an IL12 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 11255 to SEQ ID NO: 12685.

[0363] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CCR7 protein comprising or consisting of 20 nucleotides of the sequence of

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1  CACTTCCTCC  CCAGACAGGG  GTAGTGCGAG  GCCGGGCACA  GCCTTCCTGT  GTGGTTTTAC
61  CGCCAGAGA  GCGTCATGGA  CCTGGGGAAA  CCAATGAAAA  GCGTGCTGGT  GGTGGCTCTC
121  CTTGTCATTT  TCCAGGTATG  CCTGTGTCAA  GATGAGGTCA  CGGACGATTA  CATCGGAGAC
181  AACACCACAG  TGGACTACAC  TTTGTTGCGAG  TCTTTGTGCT  CCAAGAAGGA  CGTGCGGAAC
241  TTTAAAGCCT  GGTTCCTCCC  TATCATGTAC  TCCATCATTT  GTTTCGTGGG  CCTACTGGGC
301  AATGGGCTGG  TCGTGTTGAC  CTATATCTAT  TTCAAGAGGC  TCAAGACCAT  GACCGATACC
361  TACCTGCTCA  ACCTGGCGGT  GGCAGACATC  CTCTTCCTCC  TGACCCTTCC  CTTCTGGGCC
421  TACAGCGCGG  CCAAGTCCTG  GGTCTTCGGT  GTCCACTTTT  GCAAGCTCAT  CTTTGCCATC
481  TACAAGATGA  GCTTCTTCAG  TGGCATGCTC  CTACTTCTTT  GCATCAGCAT  TGACCGCTAC
541  GTGGCCATCG  TCCAGGCTGT  CTCAGCTCAC  CGCCACCGTG  CCCGCGTCCT  TCTCATCAGC
601  AAGCTGTCTT  GTGTGGGCAT  CTGGATACTA  GCCACAGTGC  TCTCCATCCC  AGAGCTCCTG
661  TACAGTGACC  TCCAGAGGAG  CAGCAGTGAG  CAAGCGATGC  GATGCTCTCT  CATCACAGAG

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721 CATGTGGAGG CCTTTATCAC CATCCAGGTG GCCCAGATGG TGATCGGCTT TCTGGTCCCC
781 CTGCTGGCCA TGAGCTTCTG TTACCTTGTC ATCATCCGCA CCCTGCTCCA GGCACGCAAC
841 TTTGAGCGCA ACAAGGCCAT CAAGGTGATC ATCGCTGTGG TCGTGGTCTT CATAGTCTTC
901 CAGCTGCCCT ACAATGGGGT GGTCTGGGCC CAGACGGTGG CCAACTTCAA CATCACCAGT
961 AGCACCTGTG AGCTCAGTAA GCAACTCAAC ATCGCCTACG ACGTCACCTA CAGCCTGGCC
1021 TCGTCCGCT GCTGCGTCAA CCCTTTCTTG TACGCCTTCA TCGGCGTCAA GTTCCGCAAC
1081 GATCTCTTCA AGCTCTTCAA GGACCTGGGC TGCCTCAGCC AGGAGCAGCT CCGGCAGTGG
1141 TCTTCCTGTC GGCACATCCG GCGCTCCTCC ATGAGTGTGG AGGCCGAGAC CACCACCACC
1201 TTCTCCCAT AGGCGACTCT TCTGCCTGGA CTAGAGGGAC CTCTCCAGG GTCCCTGGGG
1261 TGGGGATAGG GAGCAGATGC AATGACTCAG GACATCCCC CGCCAAAAGC TGCTCAGGGA
1321 AAAGCAGCTC TCCCTCAGA GTGCAAGCCC CTGCTCCAGA AGATAGCTT ACCCCAATCC
1381 CAGCTACCTC AACCAATGCC AAAAAAAGAC AGGGCTGATA AGCTAACACC AGACAGACAA
1441 CACTGGGAAA CAGAGGCTAT TGTCCCCTAA ACCAAAAACT GAAAGTGAAA GTCCAGAAAC
1501 TGTTCACACC TGCTGGAGTG AAGGGGCCAA GGAGGGTGAG TGCAAGGGGC GTGGGAGTGG
1561 CCTGAAGAGT CCTCTGAATG AACCTTCTGG CCTCCACAG ACTCAAATGC TCAGACCAGC
1621 TCTTCGAAA ACCAGGCCTT ATCTCCAAGA CCAGAGATAG TGGGGAGACT TCTTGGCTTG
1681 GTGAGGAAAA GCGGACATCA GCTGGTCAA CAACTCTCT GAACCCCTCC CTCCATCGTT
1741 TTCTTCACTG TCCTCCAAGC CAGCGGGAAT GGCAGCTGCC ACGCCGCCCT AAAAGCACAC
1801 TCATCCCTC ACTTGCCGCG TCGCCCTCCC AGGCTCTCAA CAGGGGAGAG TGTGGTGTTC
1861 CCTGCAGGCC AGGCCAGCTG CCTCCGCGTG ATCAAAGCCA CACTCTGGGC TCCAGAGTGG
1921 GGATGACATG CACTCAGCTC TTGGCTCCAC TGGGATGGGA GGAGAGGACA AGGGAAATGT
1981 CAGGGGCGGG GAGGGTGACA GTGGCCGCCC AAGGCCACG AGCTTGTTC TTGTTCTTTG
2041 TCACAGGGAC TGAAAACCTC TCCTCATGTT CTGCTTTCGA TTCGTTAAGA GAGCAACATT
2101 TTACCCACAC ACAGATAAAG TTTTCCCTTG AGGAAACAAC AGCTTTAAAA GAAAAAGAAA
2161 AAAAAAGTCT TTGGTAAATG GCAAAAAAAA AAAAAAAA AAAAAA
(SEQ ID NO: 234).

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[0364] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a CCR7 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 12686 to SEQ ID NO: 14872.

[0365] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule, wherein the spacer sequence and the target sequence are reverse complements of one another. In some embodiments, compositions of the disclosure may comprise a single (i.e., singular) gRNA comprising a) a first spacer sequence that specifically binds to a first target RNA sequence and b) a second spacer sequence that specifically binds to a second target RNA sequence, wherein the first and second spacer sequences each bind different target RNA sequences. In some embodiments, first and second spacer sequences which bind different target RNA sequences are not comprised within a single (i.e., singular) gRNA but rather a first spacer sequence is comprised within a first gRNA and a second spacer sequence is comprised within a second gRNA sequence. In some embodiments, a spacer sequence disclosed herein comprises a portion of a nucleic acid sequence encoding a protein component of the adaptive immune response, wherein the protein component

is selected from the group consisting of Beta-2-microglobulin (β 2M), Human Leukocyte Antigen A (HLA-A), Human Leukocyte Antigen B (HLA-B), Human Leukocyte Antigen C (HLA-C), Cluster of Differentiation 28 (CD28), Cluster of Differentiation 80 (CD80), Cluster of Differentiation 86 (CD86), Inducible T-cell Costimulator (ICOS), ICOS Ligand (ICOSLG), OX40L, Interleukin 12 (IL12), and CC Chemokine Receptor 7 (CCR7). In some embodiments, a spacer which is a portion of a nucleic acid sequence encoding a protein component of an adaptive immune response is about 20 or 21 nucleotides in length.

[0366] All nucleotide sequences of the disclosure may include a uracil (U) or a thymine (T) interchangeably.

[0367] Exemplary, non-limiting Zika NS5 targeting spacer sequences of sgRNAs include, but are not limited to: gcaatgatcttcattgttgggagc (SEQ ID NO: 196), gaacctgttgatgaactcttc (SEQ ID NO: 197), gttggtgattagagcttcattc (SEQ ID NO: 198), and gaggatcctcgttcaagaatcc (SEQ ID NO: 199).

[0368] Exemplary, non-limiting lambda NS5 targeting spacer sequences of sgRNAs include, but are not limited to: GTGATAAGTGGGAATGCCATG (SEQ ID NO: 200) and GNNNNNNNNNNNNNNNNNNNNNGUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 201).

Methods of Simultaneous Treatment of Disease and Prevention of Immune Response

[0369] The disclosure provides compositions and methods for the simultaneous treatment of a disease or disorder in a subject by delivering a gene therapy to a cell and prevention of an immune response to the cell receiving the gene therapy. For example, the composition shown in Figure 4 may be administered to a subject wherein gRNA 1 binds to a target sequence within an RNA molecule that encodes a component of an adaptive immune response and gRNA2 binds to a target sequence within an RNA molecule associated with a disease or disorder. By targeting an RNA molecule that encodes a component of an adaptive immune response gRNA1 prevents the display of an antigen associated with the composition or a vector comprising the composition on the surface of the cell, thereby masking the cell from the subject's immune system. gRNA2 simultaneously targets a second RNA molecule to treat a disease or disorder of the disclosure.

[0370] In alternative embodiments, gRNA1 and gRNA2 of the composition shown in Figure 4, for example, can each target a distinct RNA molecule encoding a component of the adaptive

immune response. For example, while gRNA1 targets an RNA molecule encoding a β 2M polypeptide, gRNA2 targets a costimulatory molecule (ICOSLG, CD80, CD86, OX40L, IL12 or CCR7).

[0371] In some embodiments, compositions of the disclosure may comprise or consist of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 gRNAs.

[0372] In some embodiments, compositions of the disclosure may comprise or consist of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 gRNAs, the expression of which is under the control of a constitutive promoter (e.g. U6) and a fusion protein comprising a first RNA binding protein and a second RNA binding protein, the expression of which fusion is under the control of a viral promoter, which may be optionally constitutive (e.g. EFS).

[0373] In some embodiments, compositions of the disclosure may comprise or consist of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 gRNAs, the expression of which is under the control of a first promoter and a fusion protein comprising a first RNA binding protein and a second RNA binding protein, the expression of which fusion is under the control of a second promoter, wherein the first promoter drives stronger expression of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 gRNAs than the second promoter drives expression of the fusion protein. In some embodiments, compositions of the disclosure may comprise or consist of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 gRNAs, the expression of which is under the control of a first promoter and a fusion protein comprising a first RNA binding protein and a second RNA binding protein, the expression of which fusion is under the control of a second promoter, wherein the first promoter drives weaker expression of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 gRNAs than the second promoter drives expression of the fusion protein. By varying the relative strength of the promoters driving expression of the gRNA versus fusion protein components of the compositions of the disclosure, the compositions may be provided in ratiometric doses while expressing the gRNA and fusion protein from the same vector. Thus, the compositions of the disclosure may comprise gRNAs that bind RNA molecules associated with two or more diseases as well as two or more components of an adaptive immune response. In some embodiments, the compositions of the disclosure may comprise fusion proteins disclosed herein, wherein at least one of the fusion partner proteins is an endonuclease such as, without limitation, RNase1, RNase4, RNase6, RNase7, RNase8, RNase2, RNase6PL, RNaseL, RNaseT2, RNase11, RNaseT2-like, NOB1, ENDOV, ENDOG, ENDOD1, hFEN1, hSLFN14, hLACTB2, APEX2, ANG, HRSP12,

ZC3H12A, RIDA, PDL6, NTHL, KIAA0391, APEX1, AGO2, EXOG, ZC3H12D, ERN2, PELO, YBEY, CPSF4L, hCG_2002731, ERCC1, RAC1, RAA1, RAB1, DNA2, FLJ35220, FLJ13173, ERCC4, RNase1(K41R), RNase1(K41R, D121E), RNase1(K41R, D121E, H119N), RNase1(H119N), RNase1(R39D, N67D, N88A, G89D, R91D, H119N), RNase1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E), RNase1(R39D, N67D, N88A, G89D, R91D), TENM1, TENM2, RNaseK, TALEN, ZNF638, or PIN of hSMG6.

Methods of Use

[0374] The disclosure provides a method of modifying level of expression of an RNA molecule of the disclosure or a protein encoded by the RNA molecule comprising contacting the composition and the RNA molecule under conditions suitable for binding of one or more of the guide RNA or the fusion protein (or a portion thereof) to the RNA molecule.

[0375] The disclosure provides a method of modifying an activity of a protein encoded by an RNA molecule comprising contacting the composition and the RNA molecule under conditions suitable for binding of one or more of the guide RNA or the fusion protein (or a portion thereof) to the RNA molecule.

[0376] The disclosure provides a method of modifying level of expression of an RNA molecule of the disclosure or a protein encoded by the RNA molecule comprising contacting the composition and a cell comprising the RNA molecule under conditions suitable for binding of one or more of the guide RNA or the fusion protein (or a portion thereof) to the RNA molecule. In some embodiments, the cell is in vivo, in vitro, ex vivo or in situ. In some embodiments, the composition comprises a vector comprising composition comprising a guide RNA of the disclosure and a fusion protein of the disclosure. In some embodiments, the vector is an AAV.

[0377] The disclosure provides a method of modifying an activity of a protein encoded by an RNA molecule comprising contacting the composition and a cell comprising the RNA molecule under conditions suitable for binding of one or more of the guide RNA or the fusion protein (or a portion thereof) to the RNA molecule. In some embodiments, the cell is in vivo, in vitro, ex vivo or in situ. In some embodiments, the composition comprises a vector comprising composition comprising a guide RNA of the disclosure and a fusion protein of the disclosure. In some embodiments, the vector is an AAV.

[0378] The disclosure provides a method of modifying level of expression of an RNA molecule of the disclosure or a protein encoded by the RNA molecule comprising contacting the

composition and the RNA molecule under conditions suitable for RNA nuclease activity wherein the fusion protein induces a break in the RNA molecule.

[0379] The disclosure provides a method of modifying an activity of a protein encoded by an RNA molecule comprising contacting the composition and the RNA molecule under conditions suitable for RNA nuclease activity wherein the fusion protein induces a break in the RNA molecule.

[0380] The disclosure provides a method of modifying a level of expression of an RNA molecule of the disclosure or a protein encoded by the RNA molecule comprising contacting the composition and a cell comprising the RNA molecule under conditions suitable for RNA nuclease activity wherein the fusion protein induces a break in the RNA molecule. In some embodiments, the cell is in vivo, in vitro, ex vivo or in situ. In some embodiments, the composition comprises a vector comprising composition comprising a guide RNA of the disclosure and a fusion protein of the disclosure. In some embodiments, the vector is an AAV.

[0381] The disclosure provides a method of modifying an activity of a protein encoded by an RNA molecule comprising contacting the composition and a cell comprising the RNA molecule under conditions suitable for RNA nuclease activity wherein the fusion protein induces a break in the RNA molecule. In some embodiments, the cell is in vivo, in vitro, ex vivo or in situ. In some embodiments, the composition comprises a vector comprising composition comprising a guide RNA of the disclosure and a fusion protein of the disclosure. In some embodiments, the vector is an AAV.

[0382] The disclosure provides a method of treating a disease or disorder comprising administering to a subject a therapeutically effective amount of a composition of the disclosure.

[0383] The disclosure provides a method of treating a disease or disorder comprising administering to a subject a therapeutically effective amount of a composition of the disclosure, wherein the composition comprises a vector comprising composition comprising a guide RNA of the disclosure and a fusion protein of the disclosure and wherein the composition modifies a level of expression of an RNA molecule of the disclosure or a protein encoded by the RNA molecule.

[0384] The disclosure provides a method of treating a disease or disorder comprising administering to a subject a therapeutically effective amount of a composition of the disclosure, wherein the composition comprises a vector comprising composition comprising a guide RNA of

the disclosure and a fusion protein of the disclosure and wherein the composition modifies an activity of a protein encoded by an RNA molecule.

[0385] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, a genetic disease or disorder. In some embodiments, the genetic disease or disorder is a single-gene disease or disorder. In some embodiments, the single-gene disease or disorder is an autosomal dominant disease or disorder, an autosomal recessive disease or disorder, an X-chromosome linked (X-linked) disease or disorder, an X-linked dominant disease or disorder, an X-linked recessive disease or disorder, a Y-linked disease or disorder or a mitochondrial disease or disorder. In some embodiments, the genetic disease or disorder is a multiple-gene disease or disorder. In some embodiments, the genetic disease or disorder is a multiple-gene disease or disorder. In some embodiments, the single-gene disease or disorder is an autosomal dominant disease or disorder including, but not limited to, Huntington's disease, neurofibromatosis type 1, neurofibromatosis type 2, Marfan syndrome, hereditary nonpolyposis colorectal cancer, hereditary multiple exostoses, Von Willebrand disease, and acute intermittent porphyria. In some embodiments, the single-gene disease or disorder is an autosomal recessive disease or disorder including, but not limited to, Albinism, Medium-chain acyl-CoA dehydrogenase deficiency, cystic fibrosis, sickle-cell disease, Tay-Sachs disease, Niemann-Pick disease, spinal muscular atrophy, and Roberts syndrome. In some embodiments, the single-gene disease or disorder is X-linked disease or disorder including, but not limited to, muscular dystrophy, Duchenne muscular dystrophy, Hemophilia, Adrenoleukodystrophy (ALD), Rett syndrome, and Hemophilia A. In some embodiments, the single-gene disease or disorder is a mitochondrial disorder including, but not limited to, Leber's hereditary optic neuropathy.

[0386] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, an immune disease or disorder. In some embodiments, the immune disease or disorder is an immunodeficiency disease or disorder including, but not limited to, B-cell deficiency, T-cell deficiency, neutropenia, asplenia, complement deficiency, acquired immunodeficiency syndrome (AIDS) and immunodeficiency due to medical intervention (immunosuppression as an intended or adverse effect of a medical therapy). In some embodiments, the immune disease or disorder is an autoimmune disease or disorder including, but not limited to, Achalasia, Addison's disease, Adult Still's disease,

Agammaglobulinemia, Alopecia areata, Amyloidosis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome, Autoimmune angioedema, Autoimmune dysautonomia, Autoimmune encephalomyelitis, Autoimmune hepatitis, Autoimmune inner ear disease (AIED), Autoimmune myocarditis, Autoimmune oophoritis, Autoimmune orchitis, Autoimmune pancreatitis, Autoimmune retinopathy, Autoimmune urticaria, Axonal & neuronal neuropathy (AMAN), Baló disease, Behcet's disease, Benign mucosal pemphigoid, Bullous pemphigoid, Castleman disease (CD), Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic recurrent multifocal osteomyelitis (CRMO), Churg-Strauss Syndrome (CSS) or Eosinophilic Granulomatosis (EGPA), Cicatricial pemphigoid, Cogan's syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST syndrome, Crohn's disease, Dermatitis herpetiformis, Dermatomyositis, Devic's disease (neuromyelitis optica), Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic esophagitis (EoE), Eosinophilic fasciitis, Erythema nodosum, Essential mixed cryoglobulinemia, Evans syndrome, Fibromyalgia, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Giant cell myocarditis, Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, Hemolytic anemia, Henoch-Schonlein purpura (HSP), Herpes gestationis or pemphigoid gestationis (PG), Hidradenitis Suppurativa (HS) (Acne Inversa), Hypogammaglobulinemia, IgA Nephropathy, IgG4-related sclerosing disease, Immune thrombocytopenic purpura (ITP), Inclusion body myositis (IBM), Interstitial cystitis (IC), Juvenile arthritis, Juvenile diabetes (Type 1 diabetes), Juvenile myositis (JM), Kawasaki disease, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus, Lyme disease chronic, Meniere's disease, Microscopic polyangiitis (MPA), Mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Multifocal Motor Neuropathy (MMN) or MMNCB, Multiple sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neonatal Lupus, Neuromyelitis optica, Neutropenia, Ocular cicatricial pemphigoid, Optic neuritis, Palindromic rheumatism (PR), PANDAS, Paraneoplastic cerebellar degeneration (PCD), Paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Pars planitis (peripheral uveitis), Parsonnage-Turner syndrome, Pemphigus, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia (PA), POEMS syndrome, Polyarteritis nodosa, Polyglandular syndromes type I, II, III, Polymyalgia rheumatica, Polymyositis, Postmyocardial

infarction syndrome, Postpericardiotomy syndrome, Primary biliary cirrhosis, Primary sclerosing cholangitis, Progesterone dermatitis, Psoriasis, Psoriatic arthritis, Pure red cell aplasia (PRCA), Pyoderma gangrenosum, Raynaud's phenomenon, Reactive Arthritis, Reflex sympathetic dystrophy, Relapsing polychondritis, Restless legs syndrome (RLS), Retroperitoneal fibrosis, Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma, Sjögren's syndrome, Sperm & testicular autoimmunity, Stiff person syndrome (SPS), Subacute bacterial endocarditis (SBE), Susac's syndrome, Sympathetic ophthalmia (SO), Takayasu's arteritis, Temporal arteritis/Giant cell arteritis, Thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome (THS), Transverse myelitis, Type 1 diabetes, Ulcerative colitis (UC), Undifferentiated connective tissue disease (UCTD), Uveitis, Vasculitis, Vitiligo, Vogt-Koyanagi-Harada Disease, or Wegener's granulomatosis.

[0387] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, an inflammatory disease or disorder. In some embodiments, the inflammatory disease or disorder includes, but is not limited to, Alzheimer's disease, ankylosing spondylitis, arthritis, osteoarthritis, rheumatoid arthritis, psoriatic arthritis, asthma, atherosclerosis, Crohn's disease, colitis, dermatitis, diverticulitis, fibromyalgia, hepatitis, irritable bowel syndrome (IBS), systemic lupus erythematosus (SLE), nephritis, Parkinson's disease, ulcerative colitis, acute bronchitis, acute appendicitis, tonsillitis, infective meningitis, sinusitis, asthma, chronic peptic ulcer, tuberculosis, rheumatoid arthritis, periodontitis, gout, Scleroderma, vasculitis, and myositis.

[0388] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, a metabolic disease or disorder. In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, a degenerative or a progressive disease or disorder. In some embodiments, the degenerative or a progressive disease or disorder includes, but is not limited to, amyotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, and aging.

[0389] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, an infectious disease or disorder.

[0390] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, a pediatric or a developmental disease or disorder.

[0391] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, a cardiovascular disease or disorder.

[0392] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, a proliferative disease or disorder. In some embodiments, the proliferative disease or disorder is a cancer. In some embodiments, the cancer includes, but is not limited to, Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Adrenocortical Carcinoma, AIDS-Related Cancers, Kaposi Sarcoma (Soft Tissue Sarcoma), AIDS-Related Lymphoma (Lymphoma), Primary CNS Lymphoma (Lymphoma), Anal Cancer, Appendix Cancer, Gastrointestinal Carcinoid Tumors, Astrocytomas, Atypical Teratoid/Rhabdoid Tumor, Central Nervous System (Brain Cancer), Basal Cell Carcinoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Ewing Sarcoma, Osteosarcoma, Malignant Fibrous Histiocytoma, Brain Tumors, Breast Cancer, Burkitt Lymphoma, Carcinoid Tumor, Carcinoma, Cardiac (Heart) Tumors, Embryonal Tumors, Germ Cell Tumor, Primary CNS Lymphoma, Cervical Cancer, Cholangiocarcinoma, Chordoma, Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Chronic Myeloproliferative Neoplasms, Colorectal Cancer, Craniopharyngioma, Cutaneous T-Cell Lymphoma, Ductal Carcinoma In Situ, Embryonal Tumors, Endometrial Cancer (Uterine Cancer), Ependymoma, Esophageal Cancer, Esthesioneuroblastoma (Head and Neck Cancer), Ewing Sarcoma (Bone Cancer), Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Eye Cancer, Childhood Intraocular Melanoma, Intraocular Melanoma, Retinoblastoma, Fallopian Tube Cancer, Fibrous Histiocytoma of Bone, Malignant, and Osteosarcoma, Gallbladder Cancer, Gastric (Stomach) Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumors (GIST) (Soft Tissue Sarcoma), Childhood Gastrointestinal Stromal Tumors, Germ Cell Tumors, Childhood Extracranial Germ Cell Tumors, Extragonadal Germ Cell Tumors, Ovarian Germ Cell Tumors, Testicular Cancer, Gestational Trophoblastic Disease, Hairy Cell Leukemia, Head and Neck Cancer, Heart Tumors, Hepatocellular (Liver) Cancer, Histiocytosis, Hodgkin Lymphoma, Hypopharyngeal Cancer (Head and Neck Cancer), Intraocular Melanoma, Islet Cell Tumors, Pancreatic Neuroendocrine Tumors, Kaposi Sarcoma

(Soft Tissue Sarcoma), Kidney (Renal Cell) Cancer, Langerhans Cell Histiocytosis, Laryngeal Cancer (Head and Neck Cancer), Leukemia, Lip and Oral Cavity Cancer (Head and Neck Cancer), Liver Cancer, Lung Cancer (Non-Small Cell and Small Cell), Childhood Lung Cancer, Lymphoma, Male Breast Cancer, Malignant Fibrous Histiocytoma of Bone and Osteosarcoma, Melanoma, Merkel Cell Carcinoma (Skin Cancer), Mesothelioma, Metastatic Squamous Neck Cancer with Occult Primary (Head and Neck Cancer), Midline Tract Carcinoma With NUT Gene Changes, Mouth Cancer (Head and Neck Cancer), Multiple Endocrine Neoplasia Syndromes, Multiple Myeloma/Plasma Cell Neoplasms, Mycosis Fungoides (Lymphoma), Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Neoplasms, Nasal Cavity and Paranasal Sinus Cancer (Head and Neck Cancer), Nasopharyngeal Cancer (Head and Neck Cancer), Neuroblastoma, Non-Hodgkin Lymphoma, Non-Small Cell Lung Cancer, Oral Cancer, Lip and Oral Cavity Cancer and Oropharyngeal Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma of Bone, Ovarian Cancer, Pancreatic Cancer, Pancreatic Neuroendocrine Tumors (Islet Cell Tumors), Papillomatosis, Paraganglioma, Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer (Head and Neck Cancer), Pheochromocytoma, Plasma Cell Neoplasm/Multiple Myeloma, Pleuropulmonary Blastoma, Pregnancy and Breast Cancer, Primary Central Nervous System (CNS) Lymphoma, Primary Peritoneal Cancer, Prostate Cancer, Rectal Cancer, Recurrent Cancer, Renal Cell (Kidney) Cancer, Retinoblastoma, Rhabdomyosarcoma, Childhood (Soft Tissue Sarcoma), Salivary Gland Cancer (Head and Neck Cancer), Sarcoma, Childhood Rhabdomyosarcoma (Soft Tissue Sarcoma), Childhood Vascular Tumors (Soft Tissue Sarcoma), Ewing Sarcoma (Bone Cancer), Kaposi Sarcoma (Soft Tissue Sarcoma), Osteosarcoma (Bone Cancer), Uterine Sarcoma, Sézary Syndrome, Lymphoma, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Cell Carcinoma of the Skin, Squamous Neck Cancer, Stomach (Gastric) Cancer, T-Cell Lymphoma, Testicular Cancer, Throat Cancer (Head and Neck Cancer), Nasopharyngeal Cancer, Oropharyngeal Cancer, Hypopharyngeal Cancer, Thymoma and Thymic Carcinoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Renal Cell Cancer, Urethral Cancer, Uterine Sarcoma, Vaginal Cancer, Vascular Tumors (Soft Tissue Sarcoma), Vulvar Cancer, Wilms Tumor and Other Childhood Kidney Tumors.

[0393] In some embodiments of the methods of the disclosure, a subject of the disclosure has been diagnosed with the disease or disorder. In some embodiments, the subject of the disclosure

presents at least one sign or symptom of the disease or disorder. In some embodiments, the subject has a biomarker predictive of a risk of developing the disease or disorder. In some embodiments, the biomarker is a genetic mutation.

[0394] In some embodiments of the methods of the disclosure, a subject of the disclosure is female. In some embodiments of the methods of the disclosure, a subject of the disclosure is male. In some embodiments, a subject of the disclosure has two XX or XY chromosomes. In some embodiments, a subject of the disclosure has two XX or XY chromosomes and a third chromosome, either an X or a Y.

[0395] In some embodiments of the methods of the disclosure, a subject of the disclosure is a neonate, an infant, a child, an adult, a senior adult, or an elderly adult. In some embodiments of the methods of the disclosure, a subject of the disclosure is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or 31 days old. In some embodiments of the methods of the disclosure, a subject of the disclosure is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 months old. In some embodiments of the methods of the disclosure, a subject of the disclosure is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or any number of years or partial years in between of age.

[0396] In some embodiments of the methods of the disclosure, a subject of the disclosure is a mammal. In some embodiments, a subject of the disclosure is a non-human mammal.

[0397] In some embodiments of the methods of the disclosure, a subject of the disclosure is a human.

[0398] In some embodiments of the methods of the disclosure, a therapeutically effective amount comprises a single dose of a composition of the disclosure. In some embodiments, a therapeutically effective amount comprises a therapeutically effective amount comprises at least one dose of a composition of the disclosure. In some embodiments, a therapeutically effective amount comprises a therapeutically effective amount comprises one or more dose(s) of a composition of the disclosure.

[0399] In some embodiments of the methods of the disclosure, a therapeutically effective amount eliminates a sign or symptom of the disease or disorder. In some embodiments, a therapeutically effective amount reduces a severity of a sign or symptom of the disease or disorder.

[0400] In some embodiments of the methods of the disclosure, a therapeutically effective amount eliminates the disease or disorder.

[0401] In some embodiments of the methods of the disclosure, a therapeutically effective amount prevents an onset of a disease or disorder. In some embodiments, a therapeutically effective amount delays the onset of a disease or disorder. In some embodiments, a therapeutically effective amount reduces the severity of a sign or symptom of the disease or disorder. In some embodiments, a therapeutically effective amount improves a prognosis for the subject.

[0402] In some embodiments of the methods of the disclosure, a composition of the disclosure is administered to the subject systemically. In some embodiments, the composition of the disclosure is administered to the subject by an intravenous route. In some embodiments, the composition of the disclosure is administered to the subject by an injection or an infusion.

[0403] In some embodiments of the methods of the disclosure, a composition of the disclosure is administered to the subject locally. In some embodiments, the composition of the disclosure is administered to the subject by an intraosseous, intraocular, intracerebrospinal or intraspinal route. In some embodiments, the composition of the disclosure is administered directly to the cerebral spinal fluid of the central nervous system. In some embodiments, the composition of the disclosure is administered directly to a tissue or fluid of the eye and does not have bioavailability outside of ocular structures. In some embodiments, the composition of the disclosure is administered to the subject by an injection or an infusion.

[0404] In some embodiments, the compositions comprising the RNA-binding fusion proteins disclosed herein are formulated as pharmaceutical compositions. Briefly, pharmaceutical compositions for use as disclosed herein may comprise a fusion protein(s) or a polynucleotide encoding the fusion protein(s), optionally comprised in an AAV, which is optionally also immune orthogonal, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the disclosure may be formulated for oral, intravenous, topical, enteral, intraocular, and/or parenteral administration. In certain

embodiments, the compositions of the present disclosure are formulated for intravenous administration.

EXAMPLES

Example 1: RNA-guided cleavage of Viral RNA Molecules

[0405] A549 cells were cultured in DMEM with 10% FBS and 1% penicillin/streptomycin (GIBCO) and passaged at 90%–100% confluency. Cells were seeded at 1×10^5 cells per well of a 24-well plate for RNA isolation or $.5 \times 10^5$ cells per well. Cells were transfected with plasmids encoding *Campylobacter jejuni* Cas9 (CjeCas9) fused to the gene NTHL1 (residues 31-312, E43) or CPSF4L (full length, E67) with plasmids encoding one of four sites in Zika NS5 RNA. CjeCas9 was driven by an EFS promoter while the guide RNAs were driven by U6 promoter. The sequences of the sgRNAs are presented in Table 8. The sequences of the constructs used in this study are presented below (SEQ ID NO: 13656 and SEQ ID NO: 13657).

[0406] RNA isolations were carried out with RNAeasy columns (Qiagen) according to the manufacturer's protocol. RNA quality and concentrations were estimated using the Nanodrop spectrophotometer. cDNA preparation was done using Superscript III (Thermo) with random primers according to the manufacturer's protocol. qPCR was carried out with the following primers as listed in Table 7.

[0407] Figure 1 shows expression levels of Zika NS5 assessed in the presence of both E43 and E67 endonucleases with sgRNAs containing the various NS5-targeting spacer sequences as indicated in Table 8. Zika NS5 expression is displayed as fold change relative to the endonuclease loaded with an sgRNA containing a control (Lambda) spacer sequence.

[0408] Immunofluorescence microscopy was used to visualize Zika NS5 expression in the presence of E43 or E67 endonucleases fused to CjeCas9. Figure 2A shows a fluorescence microscopy image of cells transfected with CjeCas9-endonuclease fusions loaded with an sgRNA containing a Zika NS5-targeting spacer sequence. Expression of Zika NS5 is markedly decreased in the presence of CjeCas9-endonuclease fusions loaded with the appropriate Zika NS5-targeting sgRNA as compared to a CjeCas9-endonuclease fusion loaded with a non-Zika NS5 targeting sgRNA (Figures 2A and 2B). Figure 3 is a list of exemplary endonucleases for use in the compositions of the disclosure.

[0409] Table 7: qPCR primers

GAPDH_F	CAGCCTCAAGATCATCAGCAA (SEQ ID NO: 192)
GAPDH_R	TGTGGTCATGAGTCCTTCCA (SEQ ID NO: 193)
NS5_F	GAGGAGAGTGCCAGAGTTGT (SEQ ID NO: 194)
NS5_R	TCTCTCTCCCCATCCAGTGA (SEQ ID NO: 195)

[0410] Table 8: sgRNA sequences

NS5-targeting spacer 1	gcaatgatcttcatgttgggagc (SEQ ID NO: 196)
NS5-targeting spacer 2	gaacctgttgatgaactcttc (SEQ ID NO: 197)
NS5-targeting spacer 3	gttggtgattagagcttcattc (SEQ ID NO: 198)
NS5-targeting spacer 4	gagtgatcctcgttcaagaatcc (SEQ ID NO: 199)
Non-targeting control spacer (λ2)	GTGATAAGTGGAATGCCATG (SEQ ID NO: 200)
sgRNA scaffold (N's indicate spacer)	GNNNNNNNNNNNNNNNNNNNNNNNGUUUAAGAGCUAUG CUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGU CCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGU GCUUUUUUU (SEQ ID NO: 201)

[0411] A E43-CjeCas9 and sgRNA plasmid may comprise or consist of the sequence (U6:

N's=sgRNA spacer, E43, CjeCas9):

gtttattacagggacagcagagatccagtttggttaattaaggtaccgagggcctatttcccatgattccttcatatttgcataacgatacaagg
ctgttagagagataattagaattaatttgactgtaaacacaaagatatttagtacaataacgtgacgtagaaagtaataatttctgggtagttg
cagttttaaattatgttttaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggtttatatatcttGTGGAAAGG
 ACGAAACACCNNNNNNNNNNNNNNNNNNNNGTTTTAGTCCCTGAAGGGACTAAAAT
 AAAGAGTTTGCGGGACTCTGCGGGGTACAATCCCCTAAAACCGCTTTTTTTTCTGCTGC
 AGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAGCTT
 TTGTTCCCTTTAGTGAGGGTTAATTGCGCGAATTCGCTAGCTAGGTCTTGAAAGGAG
 TGGGAATTGGCTCCGGTGCCCGTCAAGTGGGCAGAGCGCACATCGCCACAGTCCCC
 GAGAAGTTGGGGGGAGGGGTGCGGCAATTGATCCGGTGCCTAGAGAAGGTGGCGCG
 GGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGG
 GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGGGTTTG
 CCGCCAGAACACAGGACCGGTTCTAGAGCGCTATTTAGAACCatgTGTTCTCCCCAA
 GAATCTGGCATGACCGCTCTTTCAGCGAGGATGTTGACGCGAAGCAGATCCCT
 GGGACCTGGGGCCGGGCCACGAGGGTGTGCGGAAGAACCAGGACCGTTGCGA
 CGGAGGGAAGCAGCAGCGGAAGCTCGGAAATCCCATTCTCCGGTTAAACGACC
 CCGCAAGGCACAACGGCTCAGGGTTGCTTACGAGGGGAGCGATTCCGAAAAGG
 GTGAAGGAGCAGAGCCCTTGAAGGTTCCAGTATGGGAACCCACAGGATTGGCAG
 CAGCAGCTTGTAACATCCGAGCAATGAGGAACAAAAAAGATGCACCTGTTGA
 TCACCTCGGAACCGAACATTGTTATGATTCTAGTGCGCCGCCAAAAGTCCGCC

GGTATCAGGTTCTGTTGAGTTTGATGCTGAGTAGTCAGACTAAGGACCAGGTT
 ACGGCCGGAGCAATGCAACGGCTTCGGGCACGGGGACTCACGGTCGATAGCAT
 TTTGCAGACCGATGACGCAACATTGGGTAACTCATATATCCAGTTGGCTTCTG
 GCGGAGCAAAGTGAAGTACATCAAGCAGACCTCAGCCATTCTCCAACAACATT
 ACGGAGGTGATATACCCGCAAGCGTAGCTGAACTGGTAGCACTGCCGGGCGTC
 GGTCCCAAAATGGCACATCTGGCTATGGCGGTTGCTTGGGGAACGGTGTCTGG
 TATCGCAGTTGATACGCATGTCCACCGCATCGCCAATCGGCTGAGGTGGACTA
 AAAAAGCCACTAAGTCTCCTGAAGAAACACGGGCTGCTCTGGAAGAGTGGCTT
 CCACGAGAGCTGTGGCATGAAATCAATGGATTGCTGGTTGGTTTCGGGCAGCA
 GACATGCTTGCCCGTGCACCCCCGGTGTCTATGCTTGCTTGAACCAGGCTTTGT
 GCCCAGCTGCCCAGGGCCTGAGTGGAAGTGAGACACCGGGAACATCTGAGTCTGC
 GACCCCGGAGAGCacaaacGCGCGAATCCTGGCCTTCGcgATTGGCATTAGCAGCAT
 CGGCTGGGCATTCTCTGAAAACGACGAACTGAAGGATTGCGGCGTGCGAATTT
 TCACTAAGGTCGAAAATCCCAAAACTGGTGAATCACTCGCTCTCCCTAGACGAC
 TGGCACGCTCCGCACGAAAGAGGCTTGCCCGCCGCAAGGCACGCTTGAACCAT
 CTTAAACACCTTATTGCAAATGAGTTTAACTGAATTATGAGGACTACCAATCC
 TTTGACGAGTCTCTTGCTAAAGCCTACAAAGGGAGCCTTATATCCCCGTATGAG
 CTCCGGTTCAGAGCACTCAACGAACTGCTGTCCAAACAGGATTTTGCTCGCGT
 GATTCTCCACATAGCGAAGAGGCGAGGATACGATGACATTA AAAACAGTGATG
 ATAAGGAAAAAGGGGCCATACTCAAAGCGATTAAGCAAAATGAAGAGAAGCTC
 GCTAACTATCAATCAGTAGGGGAGTATCTCTATAAAGAGTACTTCCAGAAGTTC
 AAAGAAAAATAGCAAGGAATTTACTAATGTCCGGAATAAAAAGGAGTCTTACGA
 AAGATGTATTGCGCAATCTTTCCTCAAGGACGAGCTCAAATTGATTTTCAAGAA
 ACAAGGGAATTTGGGTTTCAGCTTCTCAAAAAAATTTGAGGAAGAGGTTCTGA
 GCGTTGCCTTTTACAAACGCGCCCTTAAGGACTTCTCACATCTCGTAGGGAATT
 GTAGTTTCTTACCGATGAAAAACGGGCGCCAAAAAATAGCCCTTTGGCTTTTA
 TGTTTGTCGCTCTGACTCGCATCATTAACTGCTCAACAACCTTA AAAACACGG
 AAGGGATTCTGTACACAAAGGATGATCTGAACGCTCTGCTTAACGAAGTTTGA
 AGAACGGGACTTTGACCTACAAACAAACCAAAAAGCTTCTTGGTCTCAGTGATG
 ACTACGAATTCAAGGGAGAAAAAGGGACATATTTTCATCGAATTCAAGAAGTATA
 AGGAGTTCATCAAAGCCTTGGGCGAGCACAACCTTGTCTCAAGATGATCTCAAC
 GAAATTGCTAAGGATATCACTCTGATTAAAGACGAGATCAAGCTCAAAAAGGC
 GTTGGCGAAGTATGACCTTAACCAAAAACCAATAGATAGCCTCAGCAAGTTGG
 AATTTAAAGATCACTTGAATATAAGTTTCAAGGCCCTTAAGTTGGTCACCCCT
 TGATGCTTGAAGGAAAGAAATATGATGAGGCATGTAATGAGCTGAATCTCAAG
 GTTGCTATTAACGAAGACAAAAAAGATTTCTCCAGCTTTCAATGAGACTTAC
 TATAAGGACGAGGTTACCAATCCTGTGGTGCTCCGAGCCATCAAAGAGTATCG
 AAAGGTCCTGAATGCTTTGCTCAAAAAATACGGTAAGGTACACAAAAATAATAT
 TGAGCTCGCAAGGGAGGTCGGTAAGAACCACTCCAGCGCGCCAAAAATAGAAA
 AGGAACAGAATGAAAATTACAAAGCGAAAAAGGACGCCGAGCTCGAGTGCGAA
 AAGCTGGGCCTGAAAATAAACAGCAAGAACATTCTCAAACCTCCGCTCTTCAAA
 GAACAAAAAGAATTTTGTGCTTATAGTGGTGAGAAAAATAAAAATCTCCGATCTT
 CAAGACGAGAAGATGCTCGAAATAGACgcgATATATCCATATAGCAGGTCTTTTG
 ACGATTCTTACATGAATAAAGTGCTTGTTTTCACTAAGCAGAATCAGGAAAAGT
 TGAATCAGACCCCTTTGAGGCCTTTGGCAACGACTCAGCAAAGTGGCAGAAG
 ATCGAGGTCTTGGCTAAGAATCTTCCTACTAAGAAACAGAAAAAGGATATTGGAT

AAGAACTATAAAGACAAAGAACAAAAGAACTTTAAAGACCGCAACCTCAATGA
CACCAGATACATAGCAAGATTGGTTCTGAACTACACAAAAGATTATTTGGACTT
CTTGCCGCTGTCTGATGATGAGAACACGAACTCAACGACACGCAAAAGGGGT
CTAAAGTCCACGTCGAAGCTAAATCTGGGATGCTCACCTCAGCATTGAGGCAT
ACGTGGGGATTCTCAGCAAAGGACCGAAACAATCACCTGCACCATGCCATTGA
CGCAGTTATCATAGCGTATGCCAATAATTCAATAGTAAAAGCGTTTAGCGACTT
CAAGAAGGAACAAGAGTCCAACAGCGCCGAGCTCTACGCAAAAAGATTAGTG
AACTCGACTACAAAAACAAAAGAAAATTCTTTGAGCCGTTTCAGCGGATTTTCGAC
AGAAGGTATTGGATAAAATAGATGAAATTTTCGTGAGCAAACCCGAAAGGAAA
AAGCCCTCAGGCGCCTTGACGAAGAGACTTTCAGGAAGGAAGAGGAATTCTA
CCAAAGCTACGGCGGAAAAGAGGGGAGTTTTGAAGGCTCTCGAACTTGGAAGA
TTAGGAAGGTGAACGGCAAGATAGTGAAAAACGGCGATATGTTCCGGGTGAT
ATCTTCAAACATAAAAAACGAATAAATTTTATGCTGTGCCTATATACACTATG
GACTTCGCACTTAAGGTCCTGCCGAATAAGGCGGTAGCCCGATCTAAAAAAGG
CGAAATTAAGGACTGGATTTTGATGGATGAAAATTACGAGTTCTGCTTTTCTCT
CTACAAGGATTCCCTTATATTGATACAGACGAAAGATATGCAGGAACCGGAATT
CGTGTATTACAACGCTTTTACTTCCTCTACGGTATCTTTGATTGTCTCCAAACAT
GACAACAAATTCGAAACACTCAGTAAAAACCAAAGATTCTCTTTAAAAATGCG
AACGAGAAAGAAGTAATTGCAAAATCAATTGGCATCCAAAATTTGAAAGTTTTT
GAAAAATATATAGTATCTGCCCTCGGAGAGGTTACTAAAGCGGAATTTAGACA
GCGAGAGGACTTCAAAAAATCAGGTCCACCCAAGAAAAAACGCAAGGTGGAAGA
TCCGAAGAAAAAGCGAAAAGTGGATGTGtaaCGTTTTCCGGGACGCCGGCTGGATGA
TCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCACCCCCAACTTGTTTATTGC
AGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCAT
TTTTTTCATGCAATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTC
TGTATACCG (SEQ ID NO: 202).

[0412] A E67-CjeCas9 and sgRNA plasmid may comprise or consist of the sequence (U6:
N's=sgRNA spacer, E67, CjeCas9):

gtttattacagggacagcagagatccagtttggttaattaaggtaccgagggcctatttcccatgattccttcatatttgcataacgatacaagg
ctgttagagagataattagaattaatttgactgtaaacacaaagatatttagtacaataacgtgacgtagaaagtaataatttctgggtagtttg
cagttttaaattatgttttaaattggactatcatatgcttaccgtaacttgaaagtatttcgatttctggctttatatatcttGTGGAAAGG
ACGAAACACCNNNNNNNNNNNNNNNNNNNNGTTTTAGTCCCTGAAGGGACTAAAAT
AAAGAGTTTGCGGGACTCTGCGGGGTACAATCCCCTAAAACCGCTTTTTTTCCTGC
AGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAGCTT
TTGTTCCCTTTAGTGAGGGTTAATTGCGCGAATTCGCTAGCTAGGTCTTGAAAGGAG
TGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCC
GAGAAGTTGGGGGGAGGGGTCGGCAATTGATCCGGTGCCTAGAGAAGGTGGCGCG
GGGTAAACTGGGAAGTGATGTCGTGTAAGTGGCTCCGCCTTTTTCCCGAGGGTGGGG
GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGGGTTTG
CCGCCAGAACACAGGACCGGTTCTAGAGCGCTATTTAGAACCatgCAGGAGGTAATA
GCGGGGCTTGAGCGATTTACCTTTGCCTTCGAAAAAGACGTAGAGATGCAGAA
GGGAACCGGCCTGCTCCCATTTCAAGGTATGGACAAATCAGCATCTGCCGTGT
GCAATTTTTTACCAAGGGTCTGTGTGAAAAGGGGAAGCTCTGTCCATTTTCGCC
ATGATCGCGGAGAGAAGATGGTGGTGTGTAAGCACTGGCTGAGAGGGCTTTGC

AAAAAAGGCGACCACTGCAAATTTCTTCACCAATATGACCTGACTCGAATGCCT
GAGTGTTATTTTTACAGTAAGTTCGGTGACTGTAGCAACAAAGAATGCAGCTTC
TTGCATGTCAAACCAGCATTCAAGTCACAGGATTGCCCCGTGGTACGATCAGGG
TTTTTGCAAGGACGGTCCCCCTCTGCAAATATCGACACGTACCCAGAATTATGTG
CCTTAATTACCTGGTTCGGCTTCTGTCTGAAGGGCCAAAATGTCAGTTTGCTCA
AAAAATTCGCGAGTTCAAATTGCTCCCTGGGTCTAAAATTTGGGAACCCCAGGA
TTGGCAGCAGCAGCTTGTAACATCCGAGCAATGAGGAACAAAAAAGATGCAC
CTGTTGATCACCTCGGAACCGAACATTGTTATGATTCTAGTGCGCCGCCAAAAG
TCCGCCGGTATCAGGTTCTGTTGAGTTTGATGCTGAGTAGTCAGACTAAGGAC
CAGGTTACGGCCGGAGCAATGCAACGGCTTCGGGCACGGGGACTCACGGTCG
ATAGCATTTTGCAGACCGATGACGCAACATTGGGTAACTCATATATCCAGTTG
GCTTCTGGCGGAGCAAAGTGAAGTACATCAAGCAGACCTCAGCCATTCTCCAA
CAACATTACGGAGGTGATATACCCGCAAGCGTAGCTGAACTGGTAGCACTGCC
GGGCGTCGGTCCCAAAATGGCACATCTGGCTATGGCGGTTGCTTGGGGAACGG
TGTCTGGTATCGCAGTTGATACGCATGTCCACCGCATCGCCAATCGGCTGAGG
TGGACTAAAAAAGCCACTAAGTCTCCTGAAGAAACACGGGCTGCTCTGGAAGA
GTGGCTTCCACGAGAGCTGTGGCATGAAATCAATGGATTGCTGGTTGGTTTCG
GGCAGCAGACATGCTTGCCCGTGCACCCCCGGTGTCTGCTTGAACCAG
GCTTTGTGCCCAGCTGCCCAGGGCCTGAGTGGAAGTGAGACACCGGGAACATCT
GAGTCTGCGACCCCGGAGAGCacaaacGCGCGAATCCTGGCCTTCGcgATTGGCATT
AGCAGCATCGGCTGGGCATTCTCTGAAAACGACGAACTGAAGGATTGCGGCGT
GCGAATTTTCACTAAGGTCGAAAATCCCAAACTGGTGAATCACTCGCTCTCCC
TAGACGACTGGCAGCTCCGCACGAAAGAGGCTTGCCCGCCGCAAGGCACGCT
TGAACCATCTTAAACACCTTATTGCAAATGAGTTTAACTGAATTATGAGGACT
ACCAATCCTTTGACGAGTCTCTTGCTAAAGCCTACAAAGGGAGCCTTATATCCC
CGTATGAGCTCCGGTTCAGAGCACTCAACGAACTGCTGTCCAAACAGGATTTT
GCTCGCGTGATTCTCCACATAGCGAAGAGGGCGAGGATACGATGACATTAAAAA
CAGTGATGATAAGGAAAAAGGGGCCATACTCAAAGCGATTAAGCAAAATGAAG
AGAAGCTCGCTAACTATCAATCAGTAGGGGAGTATCTCTATAAAGAGTACTTCC
AGAAGTTCAAAGAAAAATAGCAAGGAATTTACTAATGTCCGGAATAAAAAGGAG
TCTTACGAAAGATGTATTGCGCAATCTTTCCTCAAGGACGAGCTCAAATTGATT
TTCAAGAAACAAAGGGAATTTGGGTTTCAGCTTCTCAAAAAAATTTGAGGAAGA
GGTTCTGAGCGTTGCCTTTTACAAACGCGCCCTTAAGGACTTCTCACATCTCGT
AGGGAATTGTAGTTTCTTCACCGATGAAAAACGGGCGCCAAAAAATAGCCCTTT
GGCTTTTATGTTTGTGCTCTGACTCGCATCATTAATCTGCTCAACAACCTTAA
AAACACGGAAGGGATTCTGTACACAAAGGATGATCTGAACGCTCTGCTTAACG
AAGTTTGAAGAACGGGACTTTGACCTACAAACAAACCAAAAAGCTTCTTGGTC
TCAGTGATGACTACGAATTCAAGGGAGAAAAAGGGACATATTTTCATCGAATTCA
AGAAGTATAAGGAGTTCATCAAAGCCTTGGGCGAGCACAACTTGTCTCAAGAT
GATCTCAACGAAATTGCTAAGGATATCACTCTGATTAAAGACGAGATCAAGCTC
AAAAAGGCGTTGGCGAAGTATGACCTTAACCAAAACCAAAATAGATAGCCTCAG
CAAGTTGGAATTTAAAGATCACTTGAATATAAGTTTCAAGGCCCTTAAGTTGGT
CACCCCTTGATGCTTGAAGGAAAGAAATATGATGAGGCATGTAATGAGCTGA
ATCTCAAGGTTGCTATTAACGAAGACAAAAAAGATTTCTCCAGCTTTCAATG
AGACTTACTATAAGGACGAGGTTACCAATCCTGTGGTGCTCCGAGCCATCAAA
GAGTATCGAAAGGTCCTGAATGCTTTGCTCAAAAAATACGGTAAGGTACACAA

AATAAATATTGAGCTCGCAAGGGAGGTTCGGTAAGAACCCTCCAGCGCGCCA
AAATAGAAAAGGAACAGAATGAAAATTACAAAGCGAAAAAGGACGCCGAGCTC
GAGTGCGAAAAGCTGGGCCTGAAAATAAACAGCAAGAACATTCTCAAACCTCCG
CCTCTTCAAAGAACAAAAAGAATTTTGTGCTTATAGTGGTGAGAAAATAAAAAAT
CTCCGATCTTCAAGACGAGAAGATGCTCGAAATAGACgcgATATATCCATATAGC
AGGTCTTTTGACGATTCTTACATGAATAAAGTGCTTGTTTTCTACTAAGCAGAAT
CAGGAAAAGTTGAATCAGACCCCCCTTTGAGGCCTTTGGCAACGACTCAGCAAA
GTGGCAGAAGATCGAGGTCTTGGCTAAGAATCTTCCTACTAAGAAACAGAAAA
GGATATTGGATAAGAAGCTATAAAGACAAAAGAACAAAAGAACTTTAAAGACCGC
AACCTCAATGACACCAGATACATAGCAAGATTGGTTCTGAACTACACAAAAGAT
TATTTGGACTTCTTGCCGCTGTCTGATGATGAGAACACGAAACTCAACGACACG
CAAAAGGGGTCTAAAGTCCACGTCGAAGCTAAATCTGGGATGCTCACCTCAGC
ATTGAGGCATACGTGGGGATTCTCAGCAAAGGACCGAAACAATCACCTGCACC
ATGCCATTGACGCAGTTATCATAGCGTATGCCAATAATTCAATAGTAAAAGCGT
TTAGCGACTTCAAGAAGGAACAAGAGTCCAACAGCGCCGAGCTCTACGCAAAA
AAGATTAGTGAAGTCTGACTACAAAAACAAAAGAAAATTCTTTGAGCCGTTTCAGC
GGATTTCGACAGAAGGTATTGGATAAAATAGATGAAATTTTCGTGAGCAAACCC
GAAAGGAAAAAGCCCTCAGGCGCCTTGCACGAAGAGACTTTCAGGAAGGAAGA
GGAATTCTACCAAAGCTACGGCGGAAAAGAGGGAGTTTTGAAGGCTCTCGAAC
TTGGAAGATTAGGAAGGTGAACGGCAAGATAGTGAAAAACGGCGATATGTTC
CGGGTTGATATCTTCAAACATAAAAAAACGAATAAATTTTATGCTGTGCCTATA
TACACTATGGACTTCGCACTTAAGGTCCTGCCGAATAAGGCGGTAGCCCGATC
TAAAAAAGGCGAAATTAAGGACTGGATTTTGATGGATGAAAATTACGAGTTCTG
CTTTTCTCTCTACAAGGATTCCCTTATATTGATACAGACGAAAGATATGCAGGA
ACCGGAATTCGTGTATTACAACGCTTTTACTTCCTCTACGGTATCTTTGATTGT
CTCCAAACATGACAACAAATTCGAAACACTCAGTAAAAACCAAAAGATTCTCTT
TAAAAATGCGAACGAGAAAGAAGTAATTGCAAAATCAATTGGCATCCAAAATTT
GAAAGTTTTTTGAAAAATATATAGTATCTGCCCTCGGAGAGGTTACTAAAGCGGA
ATTTAGACAGCGAGAGGACTTCAAAAAATCAGGTCCACCCAAGAAAAAACGCAA
GGTGGAAGATCCGAAGAAAAAGCGAAAAAGTGATGTGtaaCGTTTTCCGGGACGCCG
GCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCACCCCAACT
TGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTCACAA
ATAAAGCATTTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATC
TTATCATGTCTGTATACCG (SEQ ID NO: 203).

EXAMPLE EMBODIMENTS

Embodiment 1. A composition comprising:

(a) a first sequence comprising a first guide RNA (gRNA) that specifically binds a target sequence within an RNA molecule, wherein the target sequence comprises a sequence encoding a component of an adaptive immune response and

(b) a sequence encoding a fusion protein, the sequence comprising a sequence encoding a first RNA-binding polypeptide and a sequence encoding a second RNA-binding polypeptide,

wherein neither the first RNA-binding polypeptide nor the second RNA-binding polypeptide comprises a significant DNA-nuclease activity,

wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and

wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity.

Embodiment 2. A composition comprising: (a) a first sequence comprising a first guide RNA (gRNA) that specifically binds a first target sequence within a first RNA molecule, wherein the first target sequence comprises a sequence encoding a component of an adaptive immune response and

(b) a second sequence comprising a second guide RNA (gRNA) that specifically binds a second target sequence within a second RNA molecule and

(c) a sequence encoding a fusion protein, the sequence comprising a sequence encoding a first RNA-binding polypeptide and a sequence encoding a second RNA-binding polypeptide,

wherein neither the first RNA-binding polypeptide nor the second RNA-binding polypeptide comprises a significant DNA-nuclease activity,

wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and

wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity.

Embodiment 3. The composition of embodiment 2, wherein the first target sequence or the second target sequence comprises at least one repeated sequence.

Embodiment 4. The composition of embodiment 2, wherein the first sequence comprising the first gRNA further comprises a first promoter capable of expressing the gRNA in a eukaryotic cell and/or the second sequence comprising the second gRNA further comprises a second promoter capable of expressing the gRNA in a eukaryotic cell.

Embodiment 5. The composition of embodiment 2, wherein a sequence comprising the first sequence comprising the first gRNA and the second sequence comprising the second gRNA

comprises a promoter capable of expressing the first gRNA and the second gRNA in a eukaryotic cell.

Embodiment 6. The composition of embodiment 4, wherein the first promoter and the second promoter are identical.

Embodiment 7. The composition of embodiment 4, wherein the first promoter and the second promoter are not identical.

Embodiment 8. The composition of any one of embodiments 4-7, wherein the eukaryotic cell is an animal cell.

Embodiment 9. The composition of embodiment 8, wherein the animal cell is a mammalian cell.

Embodiment 10. The composition of embodiment 9, wherein the animal cell is a human cell.

Embodiment 11. The composition of any one of embodiments 5-10, wherein the promoter is a constitutively active promoter.

Embodiment 12. The composition of any one of embodiments 5-11, wherein the promoter comprises a sequence isolated or derived from a promoter capable of driving expression of an RNA polymerase.

Embodiment 13. The composition of embodiment 12, wherein the promoter comprises a sequence isolated or derived from a U6 promoter.

Embodiment 14. The composition of any one of embodiments 5-12, wherein the promoter comprises a sequence isolated or derived from a promoter capable of driving expression of a transfer RNA (tRNA).

Embodiment 15. The composition of embodiment 14, wherein the promoter comprises a sequence isolated or derived from an alanine tRNA promoter, an arginine tRNA promoter, an asparagine tRNA promoter, an aspartic acid tRNA promoter, a cysteine tRNA promoter, a glutamine tRNA promoter, a glutamic acid tRNA promoter, a glycine tRNA promoter, a histidine tRNA promoter, an isoleucine tRNA promoter, a leucine tRNA promoter, a lysine tRNA promoter, a methionine tRNA promoter, a phenylalanine tRNA promoter, a proline tRNA promoter, a serine tRNA promoter, a threonine tRNA promoter, a tryptophan tRNA promoter, a tyrosine tRNA promoter, or a valine tRNA promoter.

Embodiment 16. The composition of embodiment 14, wherein the promoter comprises a sequence isolated or derived from a valine tRNA promoter.

Embodiment 17. The composition of any one of embodiments 2-16, wherein the sequence comprising the first gRNA further comprises a first spacer sequence that specifically binds to the first target RNA sequence.

Embodiment 18. The composition of embodiment 17, wherein the first spacer sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 87%, 90%, 95%, 97%, 99% or any percentage in between of complementarity to the first target RNA sequence.

Embodiment 19. The composition of embodiment 17, wherein the first spacer sequence has 100% complementarity to the target RNA sequence.

Embodiment 20. The composition of any one of embodiments 17-19, wherein the first spacer sequence comprises or consists of 20 nucleotides.

Embodiment 21. The composition of any one of embodiments 17-19, wherein the first spacer sequence comprises or consists of 21 nucleotides.

Embodiment 22. The composition of embodiment 21, wherein the first spacer sequence comprises or consists of 20 nucleotides of an amino acid sequence encoding a Beta-2-microglobulin (β 2M) protein.

Embodiment 23. The composition of embodiment 22, wherein the first spacer sequence comprises or consists of 20 nucleotides of an amino acid sequence of
MSRSVALAVL ALLSLSGLEA IQRTPKIQVY SRHPADIEVD LLKNGERIEK
VEHSDLSFSK DWSFYLLYYT EFTPTEKDEY ACRVNHVTLS QPKIVKWDRD
M (SEQ ID NO: 88).

Embodiment 24. The composition of any one of embodiments 2-23, wherein the sequence comprising the first gRNA further comprises a first scaffold sequence that specifically binds to the first RNA binding protein.

Embodiment 25. The composition of embodiment 24, wherein the first scaffold sequence comprises a stem-loop structure.

Embodiment 26. The composition of embodiment 24 or 25, wherein the scaffold sequence comprises or consists of 90 nucleotides.

Embodiment 27. The composition of embodiment 24 or 25, wherein the scaffold sequence comprises or consists of 93 nucleotides.

Embodiment 28. The composition of embodiment 27, wherein the scaffold sequence comprises the sequence
GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUU
AUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 12) or
GUUUUAGAGCUAGAAAUAGCAAGUUAAAUAAGGCUAGUCCGUUAUCAACUUGA
AAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 13).

Embodiment 29. The composition of any one of embodiments 1-28, wherein the sequence comprising the second gRNA further comprises a second spacer sequence that specifically binds to the second target RNA sequence.

Embodiment 30. The composition of embodiment 29, wherein the second spacer sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 87%, 90%, 95%, 97%, 99% or any percentage in between of complementarity to the first target RNA sequence.

Embodiment 31. The composition of embodiment 29, wherein the second spacer sequence has 100% complementarity to the target RNA sequence.

Embodiment 32. The composition of any one of embodiments 29-31, wherein the second spacer sequence comprises or consists of 20 nucleotides.

Embodiment 33. The composition of any one of embodiments 29-31, wherein the second spacer sequence comprises or consists of 21 nucleotides.

Embodiment 34. The composition of any one of embodiments 2-34, wherein the second spacer sequence comprises or further comprises a sequence comprising at least 1, 2, 3, 4, 5, 6, or 7 repeats of the sequence CUG (SEQ ID NO: 18), CCUG (SEQ ID NO: 19), CAG (SEQ ID NO: 80), GGGGCC (SEQ ID NO: 81) or any combination thereof.

Embodiment 35. The composition of any one of embodiments 2-34, wherein the sequence comprising the second gRNA further comprises a second scaffold sequence that specifically binds to the first RNA binding protein.

Embodiment 36. The composition of embodiment 35, wherein the second scaffold sequence comprises a stem-loop structure.

Embodiment 37. The composition of embodiment 35 or 36, wherein the second scaffold sequence comprises or consists of 85 nucleotides.

Embodiment 38. The composition of embodiment 37, wherein the second scaffold sequence comprises the sequence

GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUU
AUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 12) or
GUUUUAGAGCUAGAAAUAGCAAGUUAAAUAAGGCUAGUCCGUUAUCAACUUGA
AAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 13).

Embodiment 39. The composition of embodiment 1, wherein the gRNA does not bind or does not selectively bind to a second sequence within the RNA molecule.

Embodiment 40. The composition of any one of embodiments 2-38, wherein the first gRNA does not bind or does not selectively bind to a second sequence within the first RNA molecule.

Embodiment 41. The composition of any one of embodiments 2-38, wherein the second gRNA does not bind or does not selectively bind to a second sequence within the second RNA molecule.

Embodiment 42. The composition of embodiment 39, wherein an RNA genome or an RNA transcriptome comprises the RNA molecule.

Embodiment 43. The composition of embodiment 40 or 41, wherein an RNA genome or an RNA transcriptome comprises the first RNA molecule or the second RNA molecule.

Embodiment 44. The composition of any one of embodiments 1-43, wherein the first RNA binding protein comprises a CRISPR-Cas protein.

Embodiment 45. The composition of embodiment 44, wherein the CRISPR-Cas protein is a Type II CRISPR-Cas protein.

Embodiment 46. The composition of embodiment 45, wherein the first RNA binding protein comprises a Cas9 polypeptide or an RNA-binding portion thereof.

Embodiment 47. The composition of embodiment 44, wherein the CRISPR-Cas protein is a Type V CRISPR-Cas protein.

Embodiment 48. The composition of embodiment 47, wherein the first RNA binding protein comprises a Cpf1 polypeptide or an RNA-binding portion thereof.

Embodiment 49. The composition of embodiment 44, wherein the CRISPR-Cas protein is a Type VI CRISPR-Cas protein.

Embodiment 50. The composition of embodiment 49, wherein the first RNA binding protein comprises a Cas13 polypeptide or an RNA-binding portion thereof.

Embodiment 51. The composition of any one of embodiments 44-50, wherein the CRISPR-Cas protein comprises a native RNA nuclease activity.

Embodiment 52. The composition of embodiment 51, wherein the native RNA nuclease activity is reduced or inhibited.

Embodiment 53. The composition of embodiment 52, wherein the native RNA nuclease activity is increased or induced.

Embodiment 54. The composition of any one of embodiments 44-53, wherein the CRISPR-Cas protein comprises a native DNA nuclease activity and wherein the native DNA nuclease activity is inhibited.

Embodiment 55. The composition of embodiment 54, wherein the CRISPR-Cas protein comprises a mutation.

Embodiment 56. The composition of embodiment 54 or 55, wherein a nuclease domain of the CRISPR-Cas protein comprises the mutation.

Embodiment 57. The composition of any one of embodiments 54-56, wherein the mutation occurs in a nucleic acid encoding the CRISPR-Cas protein.

Embodiment 58. The composition of any one of embodiments 54-56, wherein the mutation occurs in an amino acid encoding the CRISPR-Cas protein.

Embodiment 59. The composition of any one of embodiments 54-58, wherein the mutation comprises a substitution, an insertion, a deletion, a frameshift, an inversion, or a transposition.

Embodiment 60. The composition of embodiment 59, wherein the mutation comprises a deletion of a nuclease domain, a binding site within the nuclease domain, an active site within the nuclease domain, or at least one essential amino acid residue within the nuclease domain.

Embodiment 61. The composition of any one of embodiments 1-43, wherein the first RNA binding protein comprises a Pumilio and FBF (PUF) protein.

Embodiment 62. The composition of embodiment 61, wherein the first RNA binding protein comprises a Pumilio-based assembly (PUMBY) protein.

Embodiment 63. The composition of any one of embodiments 1-56, wherein the first RNA binding protein does not require multimerization for RNA-binding activity.

Embodiment 64. The composition of embodiment 63, wherein the first RNA binding protein is not a monomer of a multimer complex

Embodiment 65. The composition of embodiment 63, wherein a multimer protein complex does not comprise the first RNA binding protein.

Embodiment 66. The composition of any one of embodiments 1-65, wherein the first RNA binding protein selectively binds to a target sequence within the RNA molecule.

Embodiment 67. The composition of embodiment 66, wherein the first RNA binding protein does not comprise an affinity for a second sequence within the RNA molecule.

Embodiment 68. The composition of embodiment 66 or 67, wherein the first RNA binding protein does not comprise a high affinity for or selectively bind a second sequence within the RNA molecule.

Embodiment 69. The composition of embodiment 68, wherein an RNA genome or an RNA transcriptome comprises the RNA molecule.

Embodiment 70. The composition of any one of embodiments 1-69, wherein the first RNA binding protein comprises between 2 and 1300 amino acids, inclusive of the endpoints.

Embodiment 71. The composition of any one of embodiments 1-70, wherein the sequence encoding the first RNA binding protein further comprises a nuclear localization signal (NLS).

Embodiment 72. The composition of embodiment 71, wherein the sequence encoding a nuclear localization signal (NLS) is positioned 3' to the sequence encoding the first RNA binding protein.

Embodiment 73. The composition of embodiment 72, wherein the first RNA binding protein comprises an NLS at a C-terminus of the protein.

Embodiment 74. The composition of any one of embodiments 1-70, wherein the sequence encoding the first RNA binding protein further comprises a first sequence encoding a first NLS and a second sequence encoding a second NLS.

Embodiment 75. The composition of embodiment 74, wherein the sequence encoding the first NLS or the second NLS is positioned 3' to the sequence encoding the first RNA binding protein.

Embodiment 76. The composition of embodiment 75, wherein the first RNA binding protein comprises the first NLS or the second NLS at a C-terminus of the protein.

Embodiment 77. The composition of any one of embodiments 1-76, wherein the second RNA binding protein comprises or consists of a nuclease domain.

Embodiment 78. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of an RNase.

Embodiment 79. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNase1.

Embodiment 80. The composition of embodiment 79, wherein the RNase1 protein comprises or consists of SEQ ID NO: 20.

Embodiment 81. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNase4.

Embodiment 82. The composition of embodiment 81, wherein the RNase4 protein comprises or consists of SEQ ID NO: 21.

Embodiment 83. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNase6.

Embodiment 84. The composition of embodiment 83, wherein the RNase6 protein comprises or consists of SEQ ID NO: 22.

Embodiment 85. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNase7.

Embodiment 86. The composition of embodiment 85, wherein the RNase7 protein comprises or consists of SEQ ID NO: 23.

Embodiment 87. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNase8.

Embodiment 88. The composition of embodiment 87, wherein the RNase8 protein comprises or consists of SEQ ID NO: 24.

Embodiment 89. The composition of embodiment 88, wherein the second RNA binding protein comprises or consists of an RNase2.

Embodiment 90. The composition of embodiment 89, wherein the RNase2 protein comprises or consists of SEQ ID NO: 25.

Embodiment 91. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNase6PL.

Embodiment 92. The composition of embodiment 91, wherein the RNase6PL protein comprises or consists of SEQ ID NO: 26.

Embodiment 93. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNaseL.

Embodiment 94. The composition of embodiment 93, wherein the RNaseL protein comprises or consists of SEQ ID NO: 27.

Embodiment 95. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNaseT2.

Embodiment 96. The composition of embodiment 95, wherein the RNaseT2 protein comprises or consists of SEQ ID NO: 28.

Embodiment 97. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNase11.

Embodiment 98. The composition of embodiment 97, wherein the RNase11 protein comprises or consists of SEQ ID NO: 29.

Embodiment 99. The composition of embodiment 78, wherein the second RNA binding protein comprises or consists of an RNaseT2-like.

Embodiment 100. The composition of embodiment 99, wherein the RNaseT2-like protein comprises or consists of SEQ ID NO: 30.

Embodiment 101. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a NOB1 polypeptide.

Embodiment 102. The composition of embodiment 101, wherein the NOB1 polypeptide comprises or consists of SEQ ID NO: 31.

Embodiment 103. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of an endonuclease.

Embodiment 104. The composition of embodiment 103, wherein the second RNA binding protein comprises or consists of an endonuclease V (ENDOV) polypeptide.

Embodiment 105. The composition of embodiment 104, wherein the ENDOV protein comprises or consists of SEQ ID NO: 32.

Embodiment 106. The composition of embodiment 103, wherein the second RNA binding protein comprises or consists of an endonuclease G (ENDOG).

Embodiment 107. The composition of embodiment 106, wherein the ENDOG protein comprises or consists of SEQ ID NO: 33.

Embodiment 108. The composition of embodiment 103, wherein the second RNA binding protein comprises or consists of an endonuclease D1 (ENDOD1) polypeptide.

Embodiment 109. The composition of embodiment 108, wherein the ENDOD1 comprises or consists of SEQ ID NO: 34.

Embodiment 110. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a Human flap endonuclease-1 (hFEN1) polypeptide.

Embodiment 111. The composition of embodiment 110, wherein the hFEN1 protein comprises or consists of SEQ ID NO: 35.

Embodiment 112. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a human Schlafen 14 (hSLFN14) polypeptide.

Embodiment 113. The composition of embodiment 112, wherein the hSLFN14 polypeptide comprises or consists of SEQ ID NO: 36.

Embodiment 114. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a human beta-lactamase-like protein 2 (hLACTB2) polypeptide.

Embodiment 115. The composition of embodiment 114, wherein the hLACTB2 polypeptide comprises or consists of SEQ ID NO: 37.

Embodiment 116. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of an apurinic/apyrimidinic (AP) endodeoxyribonuclease (APEX2) polypeptide.

Embodiment 117. The composition of embodiment 116, wherein the APEX2 polypeptide comprises or consists of SEQ ID NO: 38.

Embodiment 118. The composition of embodiment 116, wherein the APEX2 polypeptide comprises or consists of SEQ ID NO: 39.

Embodiment 119. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of an angiogenin (ANG) polypeptide.

Embodiment 120. The composition of embodiment 119, wherein the ANG polypeptide comprises or consists of SEQ ID NO: 40.

Embodiment 121. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a heat responsive protein 12 (HRSP12) polypeptide.

Embodiment 122. The composition of embodiment 121, wherein the HRSP12 polypeptide comprises or consists of SEQ ID NO: 41.

Embodiment 123. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a Zinc Finger CCCH-Type Containing 12A (ZC3H12A) polypeptide.

Embodiment 124. The composition of embodiment 123, wherein the ZC3H12A polypeptide comprises or consists of SEQ ID NO: 42.

Embodiment 125. The composition of embodiment 124, wherein the ZC3H12A polypeptide comprises or consists of SEQ ID NO: 43.

Embodiment 126. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a Reactive Intermediate Imine Deaminase A (RIDA) polypeptide.

Embodiment 127. The composition of embodiment 126, wherein the RIDA polypeptide comprises or consists of SEQ ID NO: 44.

Embodiment 128. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a Phospholipase D Family Member 6 (PDL6) polypeptide.

Embodiment 129. The composition of embodiment 128, wherein the PDL6 polypeptide comprises or consists of SEQ ID NO: 126.

Embodiment 130. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a Endonuclease III-like protein 1 (NTHL) polypeptide.

Embodiment 131. The composition of embodiment 130, wherein the NTHL polypeptide comprises or consists of SEQ ID NO: 123.

Embodiment 132. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a Mitochondrial ribonuclease P catalytic subunit (KIAA0391) polypeptide.

Embodiment 133. The composition of embodiment 132, wherein the KIAA0391 polypeptide comprises or consists of SEQ ID NO: 127.

Embodiment 134. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of an apurinic or apyrimidinic site lyase (APEX1) polypeptide.

Embodiment 135. The composition of embodiment 134, wherein the APEX1 polypeptide comprises or consists of SEQ ID NO: 125.

Embodiment 136. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of an argonaute 2 (AGO2) polypeptide.

Embodiment 137. The composition of embodiment 136, wherein the AGO2 polypeptide comprises or consists of SEQ ID NO: 128.

Embodiment 138. The composition of embodiment 67, wherein the second RNA binding protein comprises or consists of a mitochondrial nuclease EXOG (EXOG) polypeptide.

Embodiment 139. The composition of embodiment 138, wherein the EXOG polypeptide comprises or consists of SEQ ID NO: 129.

Embodiment 140. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a Zinc Finger CCCH-Type Containing 12D (ZC3H12D) polypeptide.

Embodiment 141. The composition of embodiment 140, wherein the ZC3H12D polypeptide comprises or consists of SEQ ID NO: 130.

Embodiment 142. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of an endoplasmic reticulum to nucleus signaling 2 (ERN2) polypeptide.

Embodiment 143. The composition of embodiment 142, wherein the ERN2 polypeptide comprises or consists of SEQ ID NO: 131.

Embodiment 144. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a pelota mRNA surveillance and ribosome rescue factor (PELO) polypeptide.

Embodiment 145. The composition of embodiment 144, wherein the PELO polypeptide comprises or consists of SEQ ID NO: 132.

Embodiment 146. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a YBEY metallopeptidase (YBEY) polypeptide.

Embodiment 147. The composition of embodiment 146, wherein the YBEY polypeptide comprises or consists of SEQ ID NO: 133.

Embodiment 148. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a cleavage and polyadenylation specific factor 4 like (CPSF4L) polypeptide.

Embodiment 149. The composition of embodiment 148, wherein the CPSF4L polypeptide comprises or consists of SEQ ID NO: 134.

Embodiment 150. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of an hCG_2002731 polypeptide.

Embodiment 151. The composition of embodiment 150, wherein the hCG_2002731 polypeptide comprises or consists of SEQ ID NO: 135.

Embodiment 152. The composition of embodiment 150, wherein the hCG_2002731 polypeptide comprises or consists of SEQ ID NO: 136.

Embodiment 153. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of an Excision Repair Cross-Complementation Group 1 (ERCC1) polypeptide.

Embodiment 154. The composition of embodiment 153, wherein the ERCC1 polypeptide comprises or consists of SEQ ID NO: 137.

Embodiment 155. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a ras-related C3 botulinum toxin substrate 1 isoform (RAC1) polypeptide.

Embodiment 156. The composition of embodiment 155, wherein the RAC1 polypeptide comprises or consists of SEQ ID NO: 138.

Embodiment 157. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a Ribonuclease A A1 (RAA1) polypeptide.

Embodiment 158. The composition of embodiment 157, wherein the RAA1 polypeptide comprises or consists of SEQ ID NO: 139.

Embodiment 159. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a Ras Related Protein (RAB1) polypeptide.

Embodiment 160. The composition of embodiment 159, wherein the RAB1 polypeptide comprises or consists of SEQ ID NO: 140.

Embodiment 161. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a DNA Replication Helicase/Nuclease 2 (DNA2) polypeptide.

Embodiment 162. The composition of embodiment 161, wherein the DNA2 polypeptide comprises or consists of SEQ ID NO: 141.

Embodiment 163. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a FLJ35220 polypeptide.

Embodiment 164. The composition of embodiment 163, wherein the FLJ35220 polypeptide comprises or consists of SEQ ID NO: 142.

Embodiment 165. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a FLJ13173 polypeptide.

Embodiment 166. The composition of embodiment 165, wherein the FLJ13173 polypeptide comprises or consists of SEQ ID NO: 143.

Embodiment 167. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a DNA repair endonuclease XPF (ERCC4) polypeptide.

Embodiment 168. The composition of embodiment 167, wherein the ERCC4 polypeptide comprises or consists of SEQ ID NO: 124.

Embodiment 169. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R)) polypeptide.

Embodiment 170. The composition of embodiment 169, wherein the Rnase1(K41R) polypeptide comprises or consists of SEQ ID NO: 116.

Embodiment 171. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R, D121E)) polypeptide.

Embodiment 172. The composition of embodiment 171, wherein the Rnase1 (Rnase1(K41R, D121E)) polypeptide comprises or consists of SEQ ID NO: 117.

Embodiment 173. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R, D121E, H119N)) polypeptide.

Embodiment 174. The composition of embodiment 173, wherein the Rnase1 (Rnase1(K41R, D121E, H119N)) polypeptide comprises or consists of SEQ ID NO: 118.

Embodiment 175. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(H119N)) polypeptide.

Embodiment 166. The composition of embodiment 175, wherein the Rnase1 (Rnase1(H119N)) polypeptide comprises or consists of SEQ ID NO: 119.

Embodiment 177. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide.

Embodiment 178. The composition of embodiment 177, wherein the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide comprises or consists of SEQ ID NO: 120.

Embodiment 179. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide.

Embodiment 180. The composition of embodiment 179, wherein the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E)) polypeptide comprises or consists of SEQ ID NO: 121.

Embodiment 181. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide.

Embodiment 182. The composition of embodiment 181, wherein the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D)) polypeptide comprises or consists of SEQ ID NO: 122.

Embodiment 183. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of Teneurin Transmembrane Protein 1 (TENM1) polypeptide.

Embodiment 184. The composition of embodiment 173, wherein the TENM1 polypeptide comprises or consists of SEQ ID NO: 144.

Embodiment 185. The composition of embodiment 77, wherein the second RNA binding protein comprises or consists of Teneurin Transmembrane Protein 2 (TENM2) polypeptide.

Embodiment 186. The composition of embodiment 185, wherein the TENM2 polypeptide comprises or consists of SEQ ID NO: 145.

Embodiment 187. The composition of any one of embodiments 1-77, wherein the second RNA binding protein comprises or consists of a transcription activator-like effector nuclease (TALEN) polypeptide or a nuclease domain thereof.

Embodiment 188. The composition of embodiment 187, wherein the TALEN polypeptide comprises or consists of:

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1 MRIGKSSGWL NESVSLEYEH VSPPTPRDRT RRRPRAAGDG GLAHLHRRRLA VGYAEDTPRT
61 EARSPPARRP LPVAPASAPP APSLVPEPPM PVSLPAVSSP RFSAGSSAAI TDPFPSLPPT
121 PVLYAMAREL EALSDATWQP AVPLPAEPPT DARRGNTVFD EASASSPVIA SACPQAFASP
181 PRAPRSARAR RARTGGDAWP APTFLSRPSS SRIGRDVFGK LVALGYSREQ IRKLKQESLS
241 EIAKYHTTTLT GQGFTHADIC RISRRRQSLR VVARNYPELA AALPELTRAH IVDIARQSRG
301 DLALQALLPV ATALTAAPLR LSASQIATVA QYGERPAIQA LYRLRRKLTR APLHLTPQQV
361 VAIASNTGGK RALEAVCVQL PVLRAAPYRL STEQVVAIAS NKGGKQALEA VKAHLDDLGL
421 APYVLDTEQV VAIASHNGGK QALEAVKADL LDLRGAPYAL STEQVVAIAS HNGGKQALEA

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481 VKADLLELRG APYALSTEQV VAIASHNGGK QALEAVKAHL LDLRGVPYAL STEQVVAIAS
 541 HNGGKQALEA VKAQLLDLRG APYALSTAQV VAIASNGGGK QALEGIGEQL LKLRTAPYGL
 601 STEQVVAIAS HDGGKQALEA VGAQLVALRA APYALSTEQV VAIASNKGGK QALEAVKAQL
 661 LELRGAPYAL STAQVVAIAS HDGGNQALEA VGTQLVALRA APYALSTEQV VAIASHDGGK
 721 QALEAVGAQL VALRAAPYAL NTEQVVAIAS SHGGKQALEA VRALFPDLRA APYALSTAQL
 781 VAIASNPGGK QALEAVRALF RELRAAPYAL STEQVVAIAS NHGGKQALEA VRALFRGLRA
 841 APYGLSTAQV VAIASSNGGK QALEAVWALL PVLRAATPYDL NTAQIVAIAS HDGGKPALEA
 901 VWAKLPVLRG APYALSTAQV VAIACISGQQ ALEAIEAHMP TLRQASHLS PERVAIACI
 961 GGRSAVEAVR QGLPVKAIRR IRREKAPVAG PPPASLGPTP QELVAVLHFF RAHQQPRQAF
 1021 VDALAAFQAT RPALLRLSS VGVTEIEALG GTIPDATERW QRLGLRLGFR PATGAAAPSP
 1081 DSLQGFAQSL ERTLGSFGMA GQSACSPHRK RPAETAIAPR SIRRSPNNAG QPSEPWPDQL
 1141 AWLQRRKRRTA RSHIRADSAA SVPANLHLGT RAQFTPDRLR AEPGPIMQAH TSPASVSFGS
 1201 HVAFEPGLPD PGTPTSADLA SFEAEPFGVG PLDFHLDWLL QILET (SEQ ID NO: 205).

Embodiment 189. The composition of embodiment 187, wherein the TALEN polypeptide comprises or consists of:

1 mdpirsrtps parellpgpq pdrvqptadr ggappaggpl dglparrtms rtrlpsppap
 61 spafsagsfs dllrqfdpsl ldtsllldsm avgtphataa paecdevqsg lraaddpppt
 121 vrvavtaarp prakparr r aaqpsdaspa aqvdlrtlgy sqqqqekikp kvgstvaqhh
 181 ealvghgfth ahivalsrhp aalgtvavky qdmiaalpea thedivgvvk qwsgaralea
 241 lltvagelrg pplqltdgql vkiakrggv aveavhasrn altgaplnlt paqvvaianas
 301 nggkqaletv qrlpvlcqa hgltpaqvva iashdggkqa letmqrlpvl lcqahglppd
 361 qvvaianasni gkqaletvqr llpvlcqhah ltpdqvvaia shgggkqale tvqrlpvlc
 421 qahgltpdqv vaiashdggk qaletvqrl pvlcqhahgt pdqvvaianas gggkqaletv
 481 qrlpvlcqa hgltpdqvva iasnggkqal etvqrlpvl cqahgltpdq vvaishdgg
 541 kqaletvqrl lpvlcqhthgl tpaqvvaianas hdggkqalet vqqlpvlcqa ahgltpdqvv
 601 aiasniggkq alatvqrlp vlcqhahgtl pdqvvaianas gggkqaletv rllpvlcqhah
 661 gltpdqvvai asngggkqal etvqrlpvl cqahgltpqv vvaianasni gkqaletvqrl
 721 lpvlcqhahgl tpaqvvaianas hdggkqalet vqrlpvlcqa ahgltpdqvv aiasngggkq
 781 aletvqrlp vlcqhahgtl eqvvaianas nggkqaletv rllpvlcqhah gltpdqvvai
 841 asngggkqal etvqrlpvl cqahgltpaq vvaianasni gkqaletvqrl lpvlcqhahgl
 901 tlaqvvaianas niggkqalet vqrlpvlcqa ahgltpdqvv aiasniggkq aletvqrlp
 961 vlcqhahgtl pdqvvaianas nggkqaletv rllpvlcqhah gltpdqvvai asngggkqale
 1021 tvqrlpvlc qdhgltpdqv vaianasnggk qaletvqrl pvlcqhahgtl pnqvvaianas
 1081 gggkqaletv aqlsrpdpal aaltndhlva laclggrpam davkkgplpha pelirrnr
 1141 igertshrva dyaqvrvle ffqchshpay afdeamtqfg msrnlvqlf rrvgvtelea
 1201 rggtlppasq rwdrlqasg mkrakpspts aqtpdqaslh afadslerdl dapspmhegd
 1261 qtgassrkrs rsdravtgs aqhsfevrp egrdalhlpl swrvkrprtr igggldpdpgt
 1321 piaadlaass tvmweqdaap fagaaddfpa fneelawlm ellpqsgsvg gti (SEQ ID
 NO: 206).

Embodiment 190. The composition of any one of embodiments 1-77, wherein the second RNA binding protein comprises or consists of a zinc finger nuclease polypeptide or a nuclease domain thereof.

Embodiment 191. The composition of embodiment 190, wherein the zinc finger nuclease polypeptide comprises or consists of:

1 MSRPRFNPRG DFPLQRPRAP NPSGMRPPGP FMRPGSMGLP RFYPAGRARG IPHRFAGHES
 61 YQNMGPQRMN VQVTQHRTPD RLTKEKLDHFH EAQQKKGKPH GSRWDDEPHI SASVAVKQSS
 121 VTQVTEQSPK VQSRYTKESA SSILASFGLS NEDLEELSRYPDEQLTPENM PLILRDIRMR
 181 KMGRRLPNLP SQSRNKETLG SEAVSSNVID YGHASKYGYT EDPLEVRIYD PEIPTDEVEN
 241 EFQSQQNISA SVPNPNVICN SMFPVEDVFR QMDFPGESSN NRSFFSVESG TKMSGHLHISG
 301 GQSVLEPIKS VNQSINQTVS QTMSQSLIPP SMNQPPFSSE LISSVSQOER IPHEPVINSS
 361 NVHVGSRGSK KNYQSQADIP IRSPFGIVKA SWLPKFSHAD AQKMKRLPTP SMMNDYYAAS
 421 PRIFPHLCSL CNVECSHLKD WIQHQNSTH IESCRQLRQQ YPDWNPEILP SRRNEG NRKE
 481 NETPRRRSHS PSPRRRRSS SSHRFRRSRS PMHYMYRPRS RSPRICHRFI SRYRSRSRSR
 541 SPYRIRNPFR GSPKCFRSVS PERMSRRSVR SDRKKALED VVQRSGHGTE FNKQKHLEAA
 601 DKGHSPAQKP KTSSGTPKPSV KPTSATKSDS NLGGHSIRCK SKNLEDDTLS ECKQVSDKAV
 661 SLQRKLKEQ SLHYGSVLLI TELPEDGCTE EDVRKLFQPF GKVNDVLIVP YRKEAYLEME
 721 FKEAITAIMK YIETTPITIK GKSVKICVPG KKAQNKEVK KKTLESKKVS ASTLKRDATA
 781 SKAVEIVTST SAAKTGQAKA SVAKVNKSTG KSASSVKS VAVKGNKAS IKTA KSGGKK
 841 SLEAKKTGNV KNKDSNKPVT IPENSEIKTS IEVKATENCA KEAISDAALE ATENEPLNKE
 901 TEEMCVMLVS NLPNKGYSVE EVYDLAKPFG GLKDILILSS HKKAYIEINR KAAESMVKFY
 961 TCFPVIMDGN QLSISMAPEN MNIKDEEAI F ITLVKENDPE ANIDTIYDRF VHLDNLPEDG
 1021 LQCVLCVGLQ FGKVDHVFH SNRNKAILQL DSPESAQSMY SFLKQNPQNI GDHMLTCSLS
 1081 PKIDLPEVQI EHDPELEKES PGLKNSPIDE SEVQTATDSP SVKPNELEEE STPSIQTTETL
 1141 VQQEEPCEEE AEKATCDSD F AVETLELETQ GEEVKEEIP L VASASVSIEQ FTENAEECAL
 1201 NQQMFSNDLE KKGAEIINPK TALLPSDSVF AEERNLKGIL EESPSEAE D F ISGITQTMVE
 1261 AVAEVEKNET VSEILPSTCI VTLVPGIPTG DEKTVDKKNI SEKKGNMDEK EEKEFN TKET
 1321 RMDLQIGTEK AEKNEGRMDA EKVEKMAAMK EKPAENTL F K AYPNKGVGQA NKPDETSKTS
 1381 ILAVSDVSSS KPSIKAVIVS SPKAKATVSK TENQKSFPKS VPRDQINAEK KLSAKEFGLL
 1441 KPTSARSGLA ESSSKFKPTQ SSLTRGGSGR ISALQGKLSK LDYRDITKQS QETEARPSIM
 1501 KRDDSNNKTL AEQNTKNPKS TTGRSSKSKE EPLFPFNLDE FVTVDEVIEE VNPSQAKQNP
 1561 LKGKRKETLK NVPFSELNLK KKKGKTSTPR GVEGELSFTV LDEIGEEEDA AAHLAQALVT
 1621 VDEVIDEEEL NMEEMVKNSN SLFTLDELID QDDCISHSEP KDVTVLSVAE EQDLLKQERL
 1681 VTVDEIGEVE ELPLNESADI TFATLNTKGN EGDTV RDSIG FISSQVPEDP STLVTVDEIQ
 1741 DDSSDLHLVT LDEVTEEDED SLADFNNLKE ELNFVTVDEV GEEEDGDNDL KVELAQSKND
 1801 HPTDKKGNRK KRAVDTKKTK LESLSQVGPV NENVMEEDLK TMIERHLTAK TPTKRVIRIG
 1861 TLPSEKAVVT EPAKGEEAFQ MSEVDEESGL KDSEPERKRK KTEDSSSGKS VASDVPEELD
 1921 FLVPKAGFFC PICSLFYSGE KAMTNHCKST RHKQNTTEKFM AKQRKEKEQN EAEERSSR

(SEQ ID NO: 207).

Embodiment 192. The composition of any one of embodiments 1-191, wherein the composition further comprises (a) a sequence comprising a gRNA that specifically binds within an RNA molecule and

(b) a sequence encoding a nuclease.

Embodiment 193. The composition of embodiment 192, wherein the nuclease comprises a sequence isolated or derived from a CRISPR/Cas protein.

Embodiment 194. The composition of embodiment 193, wherein the CRISPR/Cas protein is isolated or derived from any one of a type I, a type IA, a type IB, a type IC, a type ID, a type IE, a type IF, a type IU, a type III, a type IIIA, a type IIIB, a type IIIC, a type IIID, a type IV, a type IVA, a type IVB, a type II, a type IIA, a type IIB, a type IIC, a type V, or a type VI CRISPR/Cas protein .

Embodiment 195. The composition of embodiment 192, wherein the nuclease comprises a sequence isolated or derived from a TALEN or a nuclease domain thereof.

Embodiment 196. The composition of embodiment 192, wherein the nuclease comprises a sequence isolated or derived from a zinc finger nuclease or a nuclease domain thereof.

Embodiment 197. The composition of any one of embodiments 191-196, wherein the target sequence comprises a sequence encoding a component of an adaptive immune response.

Embodiment 198. A vector comprising the composition of any one of embodiments 1-197.

Embodiment 199. The vector of embodiment 198, wherein the vector is a viral vector.

Embodiment 200. The vector of embodiment 199, wherein the vector comprises a sequence isolated or derived from a lentivirus, an adenovirus, an adeno-associated virus (AAV) vector, or a retrovirus.

Embodiment 201. The vector of embodiment 199 or 200, wherein the vector is replication incompetent.

Embodiment 202. The vector of embodiment any one of embodiments 100-201, wherein the vector comprises a sequence isolated or derived from an adeno-associated vector (AAV).

Embodiment 203. The vector of embodiment 202, wherein the adeno-associated virus (AAV) is an isolated AAV.

Embodiment 204. The vector of embodiment 202 or 203, wherein the adeno-associated virus (AAV) is a self-complementary adeno-associated virus (scAAV).

Embodiment 205. The vector of any one of embodiments 202-204, wherein the adeno-associated virus (AAV) is a recombinant adeno-associated virus (rAAV).

Embodiment 206. The vector of any one of embodiments 202-205, wherein the adeno-associated virus (AAV) comprises a sequence isolated or derived from an AAV of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or AAV12.

Embodiment 207. The vector of any one of embodiments 202-206, wherein the adeno-associated virus (AAV) comprises a sequence isolated or derived from an AAV of serotype AAV9.

Embodiment 208. The vector of any one of embodiments 202-206, wherein the adeno-associated virus (AAV) comprise a sequence isolated or derived from Anc80

Embodiment 209. The vector of any one of embodiments 100-201, wherein the vector is a retrovirus.

Embodiment 210. The vector of embodiment any one of claims 100-201, wherein the retrovirus is a lentivirus.

Embodiment 211. The vector of embodiment 198, wherein the vector is a non-viral vector.

Embodiment 212. The vector of embodiment 211, wherein the non-viral vector comprises a nanoparticle, a micelle, a liposome or lipoplex, a polymersome, a polyplex or a dendrimer.

Embodiment 213. A composition comprising the vector of any one of embodiments 198-212.

Embodiment 214. A cell comprising the vector of any one of embodiments 198-212.

Embodiment 215. A cell comprising the composition of embodiment 214.

Embodiment 216. The cell of embodiment 214 or 215, wherein the cell is a mammalian cell.

Embodiment 217. The cell of embodiment 216, wherein the cell is a human cell.

Embodiment 218. The cell of any one of embodiments 215-217, wherein the cell is an immune cell.

Embodiment 219. The cell of embodiment 218, wherein the immune cell is a T lymphocyte (T-cell).

Embodiment 220. The cell of embodiment 219, wherein the T-cell is an effector T-cell, a helper T-cell, a memory T-cell, a regulatory T-cell, a natural Killer T-cell, a mucosal-associated invariant T-cell, or a gamma delta T cell.

Embodiment 221. The cell of any one of embodiments 215-217, wherein the immune cell is an antigen presenting cell.

Embodiment 222. The cell of embodiment 221, wherein the antigen presenting cell is a dendritic cell, a macrophage, or a B cell.

Embodiment 223. The cell of embodiment 221, wherein the antigen presenting cell is a somatic cell.

Embodiment 224. The cell of any one of embodiments 215-223, wherein the cell is a healthy cell.

Embodiment 225. The cell of any one of embodiments 215-223, wherein the cell is not a healthy cell.

Embodiment 226. The cell of embodiment 225, where the cell is isolated or derived from a subject having a disease or disorder.

Embodiment 227. A composition comprising the cell of any one of embodiments 215-226.

Embodiment 228. A method of masking a cell from an adaptive immune response comprising contacting a composition of any one of embodiments 1-197, 213 or 227 to the cell to produce a modified cell, wherein the composition modifies a level of expression of an RNA molecule of the modified cell and wherein the RNA molecule encodes a component of an adaptive immune response.

Embodiment 229. The method of embodiment 228, wherein the cell is in vivo, in vitro, ex vivo or in situ.

Embodiment 230. The method of embodiment 228, wherein the cell is in vitro or ex vivo.

Embodiment 231. The method of any one of embodiments 228-230, wherein a plurality of cells comprises the cell.

Embodiment 232. The method of embodiment 231, wherein each cell of the plurality of cells contacts the composition, thereby producing a plurality of modified cells.

Embodiment 233. The method of any one of embodiments 228-230, wherein the method further comprises administering the modified cell to a subject.

Embodiment 234. The method of any one of embodiments 231-232, wherein the method further comprises administering the plurality of modified cells to a subject.

Embodiment 235. The method of embodiment 233, wherein the cell is autologous.

Embodiment 236. The method of embodiment 233, wherein the cell is allogeneic.

Embodiment 237. The method of embodiment 233, wherein the plurality of modified cells is autologous.

Embodiment 238. The method of embodiment 233, wherein the plurality of modified cells is allogeneic.

Embodiment 239. The method of any one of embodiments 228-238, wherein the component of an adaptive immune response comprises or consists of a component of a type I major histocompatibility complex (MHC I), a type II major histocompatibility complex (MHC II), a T-cell receptor (TCR), a costimulatory molecule or a combination thereof.

Embodiment 240. The method of embodiment 239, wherein the MHC I component comprises an $\alpha 1$ chain, an $\alpha 2$ chain, an $\alpha 3$ chain, or a $\beta 2M$ protein.

Embodiment 241. The method of any one of embodiments 228-238, wherein the component of an adaptive immune response comprises or consists of an MHC I $\beta 2M$ protein.

Embodiment 242. The method of embodiment 239, wherein the MHC II component comprises an α 1 chain, an α 2 chain, a β 1 chain, or a β 2 chain.

Embodiment 243. The method of embodiment 239, wherein the TCR component comprises an α -chain and a β -chain.

Embodiment 244. The method of embodiment 239, wherein the costimulatory molecule comprises a Cluster of Differentiation 28 (CD28), a Cluster of Differentiation 80 (CD80), a Cluster of Differentiation 86 (CD86), an Inducible T-cell COStimulator (ICOS), or an ICOS Ligand (ICOSLG) protein.

Embodiment 245. A method of preventing or reducing an adaptive immune response in a subject comprising administering a therapeutically effective amount of a composition of any one of embodiments 1-197, 213 or 227 to the subject, wherein the composition contacts at least one cell in the subject producing a modified cell, wherein the composition modifies a level of expression of an RNA molecule of the modified cell and wherein the RNA molecule encodes a component of an adaptive immune response.

Embodiment 246. A method of treating a disease or disorder in a subject comprising administering a therapeutically effective amount of a composition of any one of embodiments 1-197, 213 or 227 to the subject, wherein the composition contacts at least one cell in the subject producing a modified cell, wherein the composition modifies a level of expression of an RNA molecule of the modified cell and wherein the composition prevents or reduces an adaptive immune response to the modified cell.

Embodiment 247. The method of embodiment 246, wherein the component of an adaptive immune response comprises or consists of a component of a type I major histocompatibility complex (MHC I), a type II major histocompatibility complex (MHC II), a T-cell receptor (TCR), a costimulatory molecule or a combination thereof.

Embodiment 248. The method of embodiment 247, wherein the MHC I component comprises an $\alpha 1$ chain, an $\alpha 2$ chain, an $\alpha 3$ chain, or a $\beta 2M$ protein.

Embodiment 249. The method of embodiment 247 or 248, wherein the component of an adaptive immune response comprises or consists of an MHC I $\beta 2M$ protein.

Embodiment 250. The method of embodiment 249, wherein the MHC II component comprises an $\alpha 1$ chain, an $\alpha 2$ chain, a $\beta 1$ chain, or a $\beta 2$ chain.

Embodiment 251. The method of embodiment 247, wherein the TCR component comprises an α -chain and a β -chain.

Embodiment 252. The method of embodiment 247, wherein the costimulatory molecule comprises a Cluster of Differentiation 28 (CD28), a Cluster of Differentiation 80 (CD80), a Cluster of Differentiation 86 (CD86), an Inducible T-cell COStimulator (ICOS), or an ICOS Ligand (ICOSLG) protein.

Embodiment 253. The method of any one of embodiments 246-252, wherein the disease or disorder is a genetic disease or disorder.

Embodiment 254. The method of embodiment 253, wherein the disease or disorder is a single gene genetic disease or disorder.

Embodiment 255. The method of embodiment 254, wherein the disease or disorder results from microsatellite instability.

Embodiment 256. The method of embodiment 255, wherein the microsatellite instability occurs in a DNA sequence at least 1, 2, 3, 4, 5 or 6 repeated motifs.

Embodiment 257. The method of embodiment 256, wherein an RNA molecule comprises a transcript of the DNA sequence and wherein the composition binds to a target sequence of the RNA molecule comprising at least 1, 2, 3, 4, 5, or 6 repeated motifs.

Embodiment 258. The method of any one of embodiments 246-257, wherein the composition is administered systemically.

Embodiment 259. The method of embodiment 259, wherein the composition is administered intravenously.

Embodiment 260. The method of embodiment 258 or 259, wherein the composition is administered by an injection or an infusion.

Embodiment 261. The method of any one of embodiments 246-257, wherein the composition is administered locally.

Embodiment 262. The method of embodiment 261, wherein the composition is administered by an intraosseous, intraocular, intracerebral, or intraspinal route.

Embodiment 263. The method of embodiment 261 or 262, wherein the composition is administered by an injection or an infusion.

Embodiment 264. The method of any one of embodiments 265-263, wherein the therapeutically effective amount is a single dose.

Embodiment 265. The method of any one of embodiments 265-264, wherein the composition is non-genome integrating.

INCORPORATION BY REFERENCE

[0413] Every document cited herein, including any cross referenced or related patent or application is hereby incorporated herein by reference in its entirety unless expressly excluded or

otherwise limited. The citation of any document is not an admission that it is prior art with respect to any invention disclosed or claimed herein or that it alone, or in any combination with any other reference or references, teaches, suggests or discloses any such invention. Further, to the extent that any meaning or definition of a term in this document conflicts with any meaning or definition of the same term in a document incorporated by reference, the meaning or definition assigned to that term in this document shall govern.

OTHER EMBODIMENTS

[0414] While particular embodiments of the disclosure have been illustrated and described, various other changes and modifications can be made without departing from the spirit and scope of the disclosure. The scope of the appended claims includes all such changes and modifications that are within the scope of this disclosure.

CLAIMS

What is claimed is:

1. A composition comprising a nucleic acid sequence comprising a guide RNA (gRNA) sequence that specifically binds a target RNA sequence, wherein the target RNA sequence encodes a protein component of an adaptive immune response, and wherein the gRNA sequence comprises a spacer sequence comprising a portion of a nucleic acid sequence encoding the protein component, and wherein the protein component is selected from the group consisting of Beta-2-microglobulin (β 2M), Human Leukocyte Antigen A (HLA-A), Human Leukocyte Antigen B (HLA-B), Human Leukocyte Antigen C (HLA-C), Cluster of Differentiation 28 (CD28), Cluster of Differentiation 80 (CD80), Cluster of Differentiation 86 (CD86), Inducible T-cell Costimulator (ICOS), ICOS Ligand (ICOSLG), OX40L, Interleukin 12 (IL12), and CC Chemokine Receptor 7 (CCR7).
2. The composition of claim 1, wherein the adaptive immune response is selected from the group consisting of type I major histocompatibility complex (MHC I), type II major histocompatibility complex (MHC II), T-cell receptor (TCR), costimulatory molecule and a combination thereof.
3. The composition of claim 1, wherein the spacer sequence is about 20 or 21 nucleotides in length.
4. The composition of claim 1, wherein the spacer sequence and the target RNA sequence are reverse complements of one another.
5. The composition of claim 1, wherein the gRNA sequence comprises a scaffold sequence that specifically binds to a CRISPR/Cas polypeptide or portion thereof.
6. The composition of claim 5, wherein the CRISPR/Cas polypeptide or portion thereof is selected from the group consisting of Cas9, Cpf1, Cas13a, Cas13b, Cas13c and CasRX/Cas13d, wherein the CRISPR/Cas polypeptide has native, reduced or null activity.
7. The composition of claim 1, wherein the nucleic acid sequence comprises a promoter which drives expression of the gRNA sequence.
8. The composition of claim 7, wherein the promoter is selected from the group consisting of a polymerase III promoter and a tRNA promoter.

9. The composition of claim 8, wherein the polymerase III promoter is a U6 promoter.
10. The composition of claim 1, wherein the spacer sequence is a first spacer sequence that specifically binds a first target RNA sequence, and wherein the composition further comprises a second spacer sequence which specifically binds a second target RNA sequence, wherein the first spacer sequence and the second spacer sequence bind different target RNA sequences.
11. The composition of claim 10, wherein the gRNA sequence is a first gRNA sequence, and wherein the second spacer sequence is comprised within a second gRNA sequence.
12. The composition of claim 10, wherein the second target RNA sequence encodes a protein component of an adaptive immune response.
13. The composition of claim 10, wherein the second spacer sequence comprises a portion of a nucleic acid sequence encoding a protein component is selected from the group consisting of Beta-2-microglobulin (β 2M), Human Leukocyte Antigen A (HLA-A), Human Leukocyte Antigen B (HLA-B), Human Leukocyte Antigen C (HLA-C), Cluster of Differentiation 28 (CD28), Cluster of Differentiation 80 (CD80), Cluster of Differentiation 86 (CD86), Inducible T-cell Costimulator (ICOS), ICOS Ligand (ICOSLG), OX40L, Interleukin 12 (IL12), and CC Chemokine Receptor 7 (CCR7).
14. The composition of claim 10, wherein the second spacer sequence comprises at least 1, 2, 3, 4, 5, 6, or 7 repeats of a nucleic acid sequence selected from the group consisting of: CUG (SEQ ID NO: 18), CCUG (SEQ ID NO: 19), CAG (SEQ ID NO: 80), GGGGCC (SEQ ID NO: 81), and a combination thereof.
15. A composition comprising a nucleic acid sequence comprising: (a) a first guide RNA (gRNA) sequence that specifically binds a first target RNA sequence, and (b) a second gRNA that specifically binds a second target RNA sequence, wherein the first target RNA sequence encodes a protein component of an adaptive immune response, and wherein the first gRNA sequence comprises a spacer sequence comprising a portion of a nucleic acid sequence encoding the protein component, and wherein the protein component is selected from the group consisting of Beta-2-microglobulin (β 2M), Human Leukocyte Antigen A (HLA-A), Human Leukocyte Antigen B (HLA-B), Human Leukocyte Antigen C (HLA-C), Cluster of Differentiation 28 (CD28), Cluster of Differentiation 80 (CD80), Cluster of Differentiation 86 (CD86), Inducible T-cell

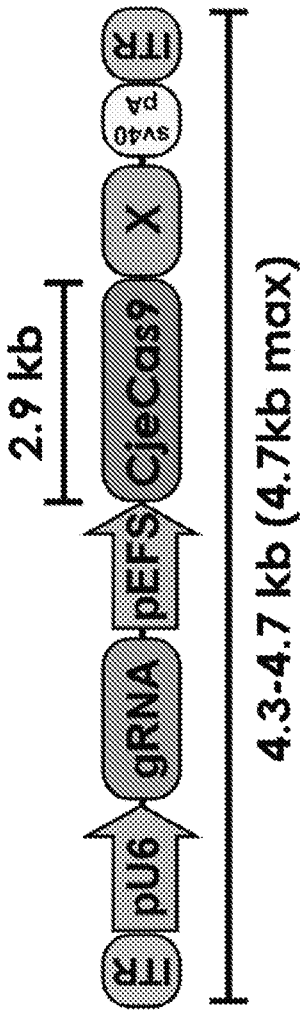
Costimulator (ICOS), ICOS Ligand (ICOSLG), OX40L, Interleukin 12 (IL12), and CC Chemokine Receptor 7 (CCR7).

- 16.** A composition comprising a nucleic acid sequence comprising: (a) the guide RNA (gRNA) sequence of claim 1 and (b) a nucleic acid sequence encoding a fusion protein, wherein the fusion protein comprises a first RNA-binding polypeptide and a second RNA-binding polypeptide, wherein neither the first RNA-binding polypeptide nor the second RNA-binding polypeptide comprises a significant DNA-nuclease activity, wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity.
- 17.** A composition comprising a nucleic acid sequence comprising: (a) the first and second guide RNA (gRNA) sequences of claim 11 and (b) a nucleic acid sequence encoding a fusion protein, wherein the fusion protein comprises a first RNA-binding polypeptide and a second RNA-binding polypeptide, wherein neither the first RNA-binding polypeptide nor the second RNA-binding polypeptide comprises a significant DNA-nuclease activity, wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity.
- 18.** A composition comprising a nucleic acid sequence comprising: (a) a first guide RNA (gRNA) that specifically binds a first target RNA sequence within a first RNA molecule, wherein the first target RNA sequence encodes a protein component of an adaptive immune response (b) a second guide RNA (gRNA) that specifically binds a second target RNA sequence within a second RNA molecule and (c) a nucleic acid sequence encoding a fusion protein, wherein the fusion protein comprises a first RNA-binding polypeptide a second RNA-binding polypeptide, wherein neither the first RNA-binding polypeptide nor the second RNA-binding polypeptide comprises a significant DNA-nuclease activity, wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity.
- 19.** The composition of claim 18, wherein the first gRNA sequence comprises a spacer sequence comprising a portion of a nucleic acid sequence encoding a protein selected

- from the group consisting of Beta-2-microglobulin (β 2M), HLA-A, HLA-B, HLA-C, CD28, CD80, CD86, ICOSLG, OX40L, IL12, and CCR7.
20. The composition of claim 18, wherein the first RNA-binding polypeptide or portion thereof is a CRISPR/Cas polypeptide or portion thereof.
 21. The composition of claim 20, wherein the CRISPR/Cas polypeptide or portion thereof is selected from the group consisting of Cas9, Cpf1, Cas13a, Cas13b, Cas13c and CasRX/Cas13d, wherein the CRISPR/Cas polypeptide has native, reduced or null activity.
 22. The composition of claim 18, wherein the second RNA-binding polypeptide binds RNA in a manner in which it associates with RNA.
 23. The composition of claim 22, wherein the second RNA-binding polypeptide associates with RNA in a manner in which it cleaves RNA.
 24. The composition of claim 18, wherein the nucleic acid sequence comprises a promoter.
 25. The composition of claim 18, wherein the second gRNA comprises a spacer sequence comprising at least 1, 2, 3, 4, 5, 6, or 7 repeats of a sequence selected from the group consisting of: CUG (SEQ ID NO: 18), CCUG (SEQ ID NO: 19), CAG (SEQ ID NO: 80), GGGGCC (SEQ ID NO: 81), and a combination thereof.
 26. The composition of claim 18, wherein the fusion protein comprises an NLS, NES or tag.
 27. A vector comprising the composition of claim 18.
 28. The vector of claim 27, wherein the vector is selected from the group consisting of: adeno-associated virus, retrovirus, lentivirus, adenovirus, nanoparticle, micelle, liposome, lipoplex, polymersome, polyplex, and dendrimer.
 29. A cell comprising the vector of claim 28.
 30. The composition of claim 18, wherein the second RNA-binding polypeptide is selected from the group consisting of: RNase1, RNase4, RNase6, RNase7, RNase8, RNase2, RNase6PL, RNaseL, RNaseT2, RNase11, RNaseT2-like, NOB1, ENDOV, ENDOG, ENDOD1, hFEN1, hSLFN14, hLACTB2, APEX2, ANG, HRSP12, ZC3H12A, RIDA, PDL6, NTHL, KIAA0391, APEX1, AGO2, EXOG, ZC3H12D, ERN2, PELO, YBEY, CPSF4L, hCG_2002731, ERCC1, RAC1, RAA1, RAB1, DNA2, FLJ35220, FLJ13173, ERCC4, RNase1(K41R), RNase1(K41R, D121E), RNase1(K41R, D121E, H119N), RNase1(H119N), RNase1(R39D, N67D, N88A, G89D, R91D, H119N),

RNAse1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E), RNAse1(R39D, N67D, N88A, G89D, R91D), TENM1, TENM2, RNAseK, TALEN, ZNF638, and hSMG6 PIN.

FIGURE 1A



Endonuclease-
C. jejuni Cas9

FIGURE 1B

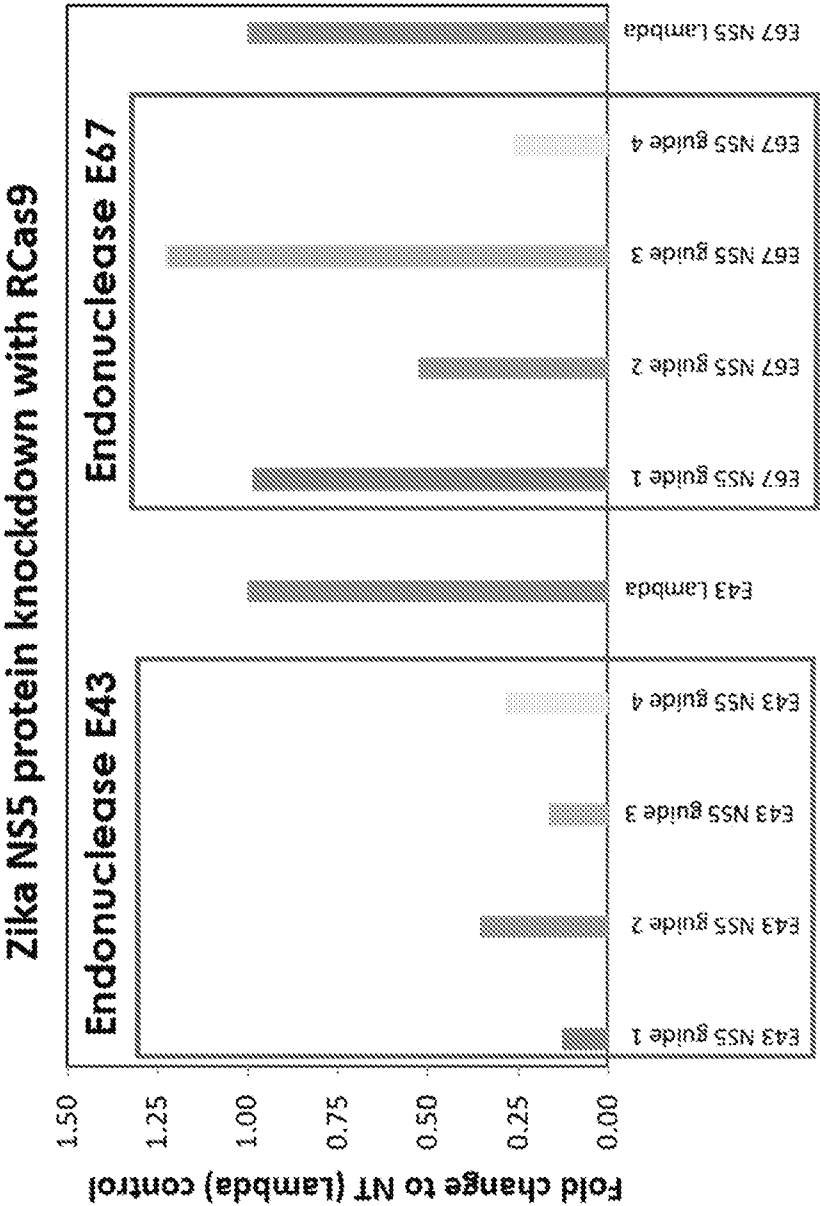


FIGURE 2A

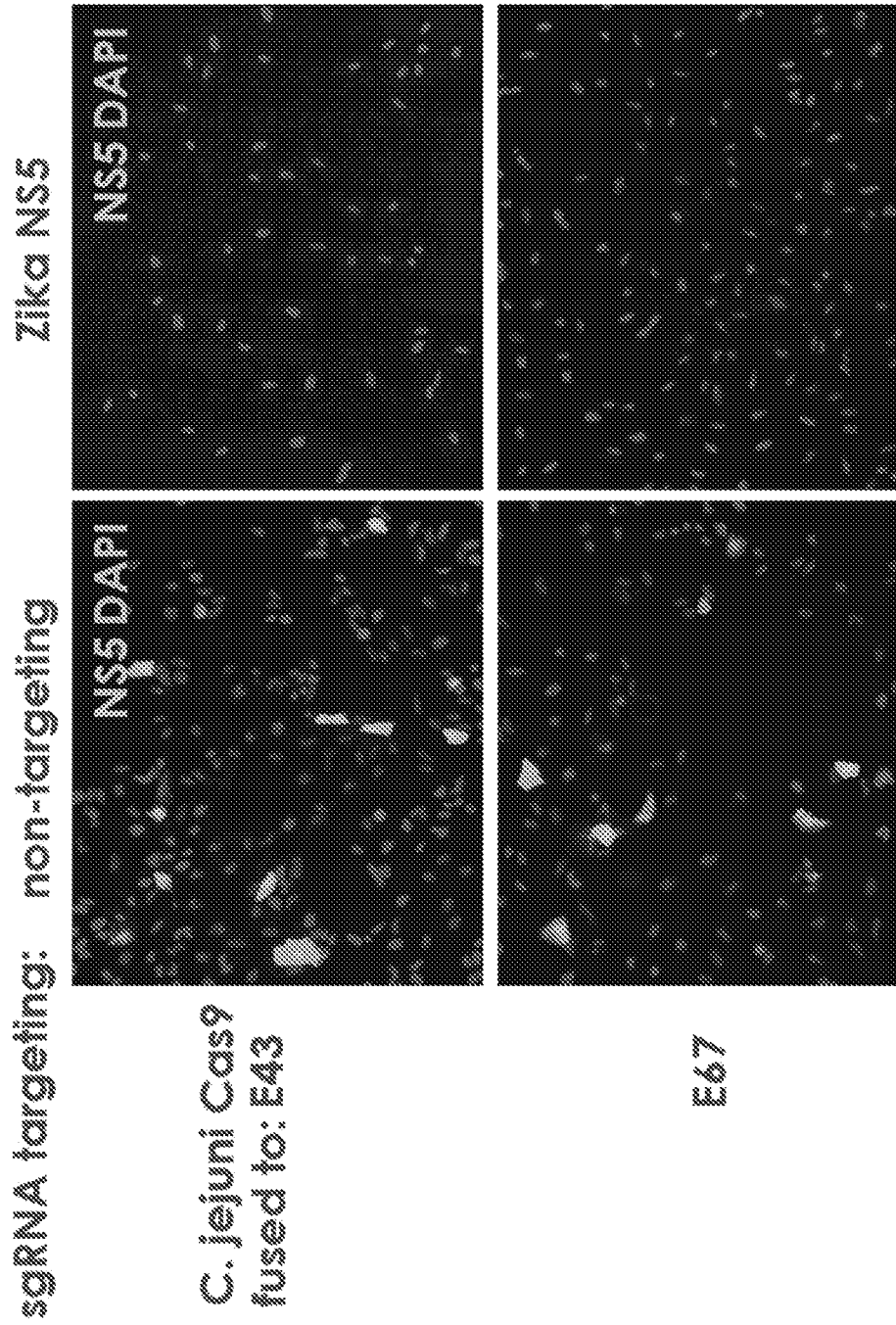


FIGURE 2B

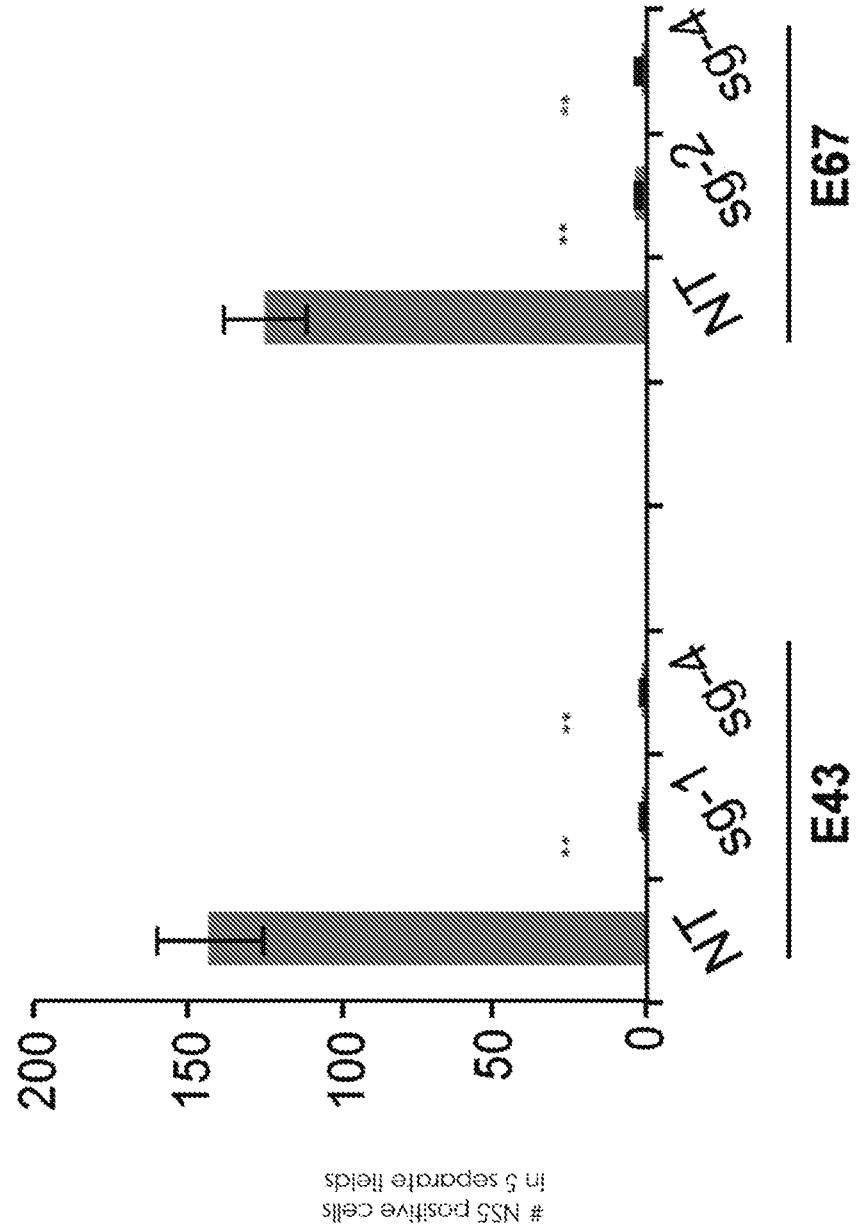


FIGURE 3

Endonuclease Name Key

E1	RNAseI	E53	EXOg
E2	RNAse4	E55	ZC3H12D
E3	RNAse6	E57	ERN2
E4	NOB1	E59	ENDOG
E5	EndoV	E61	PELO
E6	FEN1	E63	YBEY
E7	SLFN14	E65	ENDOD1
E8	LACTB2	E67	CPSF4L
E9	RNAse7	E69	hOG_2002731
E10	RNAse8	E71	hOG_2002731
E11	RNAse2	E73	ERCC1
E12	ANG	E75	RAC1
E13	HRSP12	E77	APEX2
E14	RNAse5P1	E79	APEX2_1-350
E15	RNAseL	E81	RAA1_25-156
E16	RNAseT2	E83	RAB1
E17	ZC3H12A	E85	RNAseK
E37	RIDA	E87	DNA2_FL
E39	RNAse11	E89	RNAse1(K41R)
E41	PDL6	E91	RNAse1(K41R, D121E)
E43	NTHL1	E93	RNAse1(K41R, D121E, H119N)
E45	KIAA0391	E95	RNAse1(H119N)
E47	APEX1	E97	RNAse1(R39D, M67D, N88A, G89D, R91D, H119N)
E49	Ago2	E99	RNAse1(R39D, M67D, N88A, G89D, R91D, H119N, K41R, D121E)
E51	ZC3H12A	E100	RNAse1 (R39D, M67D, N88A, G89D, R91D, H119N, K41R, D121E)
		E101	RNAse1(R39D, M67D, N89A, G89D, R91D)

FIGURE 4

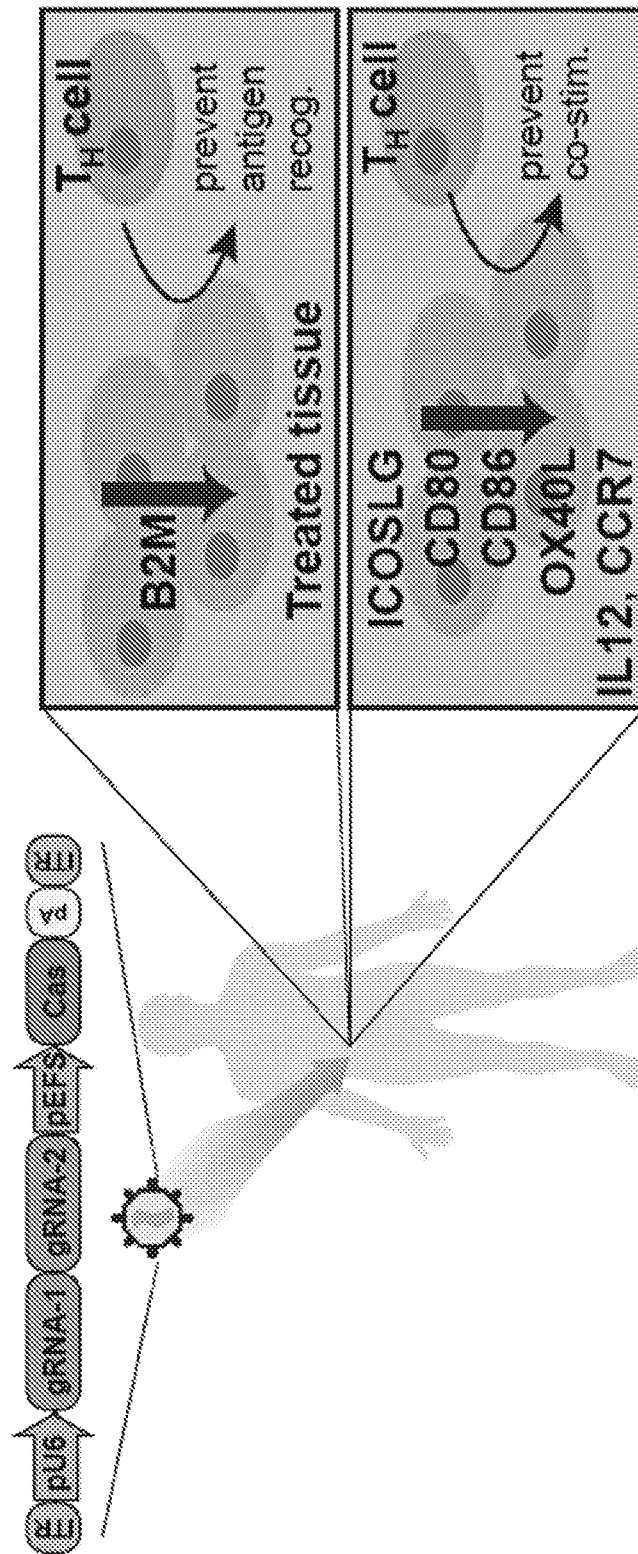


FIGURE 1A

Endonuclease-
C. jejuni Cas9

